Food and Agriculture Organization of the United Nations

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

BORGO EGNAZIA 29-31 OCTOBER 2018



Report Appendices

OPEN SESSION OF THE STANDING TECHNICAL COMMITTEE OF THE EUFMD

2018

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
European Commission for the control of Foot-and-Mouth disease (EuFMD)
Rome, 2018

Contents

Appendix 1
Meeting Agenda

29th October - DAY 1

PLENARY ROOM

	1: OPENING
9:00h	Opening
9:30h	*Keynote* **K. Sumption** - GLOBAL OVERVIEW
10h	*Keynote* **D. King** - GLOBAL STATUS REPORT FOR FMD: TRACKING THE EMERGENCE AND SPREAD OF NEW VIRAL LINEAGES
10:30h	*Coffee break*
	2: THE SCALE OF THE PROBLEM
11:00h	**M. de la Puente Arevalo** - MODELLING FMD VACCINE REQUIREMENTS FOR MULTI-COUNTRY FMD OUTBREAKS IN EUROPE
11:15h	**R. Sanson** - EVALUATING VACCINATION STRATEGIES TO CONTROL FMD: A COUNTRY COMPARISON STUDY
11:30h	**C. Miller** - UNDERSTANDING VACCINE DEMAND IN THE ENDEMIC SETTING
11:45h	**A. Railey** - HOUSEHOLD PERCEPTIONS OF RISK AS DRIVERS FOR ADOPTION OF FMD VACCINATION
12:00h	**Y. Qiu** - MASS FMD VACCINATION IN CENTRAL MYANMAR, 2015-2016
12:15h	**C. Bartels** - ASSESSMENT OF THE RISK OF INCURSION OF EXOTIC FMD VIRUSES INTO SOUTHEAST ASIA
12:30h	*Lunch*

	PLENARY ROOM	PARALLEL ROOM	
	3A: VACCINE SUPPLY	**3B: VIROLOGY**	
14:00h	**A. Dekker** - H.S. FRENKEL AND J. VAN BEKKUM, HOW THEY IMPROVED VACCINE AVAILABILITY AND QUALITY	**T. Nishi -** IDENTIFICATION OF GENES INVOLVED IN PATHOGENICITY OF FMD VIRUS USING TWO STRAINS ISOLATED IN JAPAN WITH DIFFERENT VIRAL FEATURES	14:00h
14:15h	**R. Bergevoet** - VACCINE BANKS: POLICY OPTIONS EVALUATED USING THE EU EVALUATION FRAMEWORK	**N. Knowles -** COMPLETE GENOME SEQUENCE ANALYSIS OF OVER 140 FMD VIRUSES ISOLATED FROM FREE-LIVING AFRICAN BUFFALO (SYNCERUS CAFFER) IN ZIMBABWE	14:15h
14:30h	**P. Hudelet** - THE CHALLENGES OF FMD VACCINE PRODUCTION	**K. Scott -** EVOLUTION AND COMPETITION OF SAT STRAINS DURING BUFFALO TRANSMISSION IN A CONTROLLED CHALLENGE EXPERIMENT	14:30h
14:45h	**N.M. Aineplan** - MUTUAL REGISTRATION OF VACCINES IN EAST AFRICA: PROGRESS AND ISSUES FOR BETTER ACCESS TO EFFECTIVE FMD VACCINES?	**L. Ferretti -** FMDV EVOLUTIONARY DYNAMICS WITHIN INFECTED BUFFALOES AND ITS LARGE-SCALE CONSEQUENCES	14:45h
15:00h	*Discussion*	**J. Biswal -** ANTIBODY RESPONSES TO THE MAJOR ANTIGENIC SITES OF FMD VIRUS SEROTYPE O AFTER PRIMO-VACCINATION, RE-VACCINATION AND AFTER NATURAL EXPOSURE	15:00h
		Discussion	15:15h
15:30h	*Coffee Break*		15:30h
	4A: BREAKING BARRIERS	**4B: IMMUNOPATHOLOGY**	
16:00 PM	**K. Mintiens -** ACCESS OF FARMERS AND CO-OPERATIVES TO VACCINES	**J. Valarcher -** GENE SIGNATURES ASSOCIATED WITH FMD VIRUS INFECTION AND PERSISTENCE PART I: PERSISTENT FMDV INFECTION IN AN AIR-LIQUID INTERFACE MODEL OF BOVINE SOFT PALATE	16:00 PM
16:15 PM	**B. Ahmadi -** EXPLORING PRIVATE AND PUBLIC SECTOR RIGHTS AND RESPONSIBILITIES IN PREVENTION AND CONTROL OF FMD: THE CASE OF RIGHT TO ACCESS VACCINES BY LIVESTOCK KEEPERS	**F. Pfaff** - GENE SIGNATURES ASSOCIATED WITH FMD VIRUS INFECTION AND PERSISTENCE PART II: TRANSCRIPTOMIC ANALYSIS OF ACUTE AND PERSISTENT FMDV INFECTION IN BOVINE SOFT PALATE	16:15 PM
16:30 PM	**M. Pérez-Filgueira -** FOOT-AND-MOUTH DISEASE RESEARCH GAP ANALYSIS WORKSHOP 2018	**J. Arzt -** TRANSMISION OF FMD FROM PERSISTENTLY INFECTED CARRIER CATTLE TO NAÏVE CATTLE VIA TRANSFER OF OROPHARYNGEAL FLUID	16:30 PM
16:45 PM	**K. Tjornhoj -** BIOSAFETY BARRIERS	*Discussion*	16:45 PM
17:00 PM	*Discussion*		
From 17:30h	POSTER SESSION		

30th October - DAY 2

	PLENARY ROOM	PARALLEL ROOM	
	5A: VACCINE SELECTION	**5B: MODELLING FREE & NON-FREE AREAS**	
8:30h	**A. Romey -** RAPID, ON SITE, DIAGNOSIS OF FMD AND SAFE AND COST-EFFECTIVE SHIPMENT OF SAMPLES USING LATERAL FLOW DEVICES FOR LABORATORY DIAGNOSTICS	**W. Probert -** REINFORCEMENT LEARNING FOR CONTEXT-DEPENDENT CONTROL OF EMERGENCY OUTBREAKS OF FMD	8:30h
8:45h	**B. Armson -** THE UTILITY OF POOLED MILK FOR FMD SURVEILLANCE IN NAKURU COUNTY, KENYA.	**R. Sanson -** INVESTIGATING THE BENEFITS OF AN ADAPTIVE MANAGEMENT APPROACH INVOLVING EMERGENCY VACCINATION USING SIMULATED FMD OUTBREAKS IN NEW ZEALAND	8:45h
9:00h	**C. Colenutt +** E. Brown **-** (Combined) EVALUATION OF ENVIRONMENTAL SAMPLING AS A LOW TECHNOLOGY METHOD FOR SURVEILLANCE OF FMD VIRUS IN AN ENDEMIC AREA **+** ENVIRONMENTAL SAMPLING: A SURVEILLANCE TOOL FOR FMD VIRUS IN MARKETPLACES	**S. Sellman -** EVALUATING OPTIMAL CONTROL STRATEGIES FOR FMDE WITH THE US DISEASE OUTBREAK SIMULATION	9:00h
9:15h	**P. Ribeca -** EFFECTIVE IN SILICO SEQUENCE-BASED PREDICTION OF FMDV VACCINE MATCHING	**K. VanderWaal -** BETWEEN-HERD TRANSMISSION DYNAMICS OF FMD IN KENYA RANGELANDS	9:15h
9:30h	**A. Capozzo + R. Reeve -** IN-VITRO CORRELATES OF HETEROLOGOUS PROTECTION USING AVIDITY AND IgG-SUBTYPING ELISAs + ASSESSMENT OF EXISTING AND FUTURE VACCINE SELECTION TECHNIQUES – MOVING FORWARD	**D. Ekwem -** USING NETWORKS OF LIVESTOCK MOBILITY TO IMPROVE CONTROL OF ENDEMIC FMD IN NORTHERN TANZANIA	9:30h
9:45h		**A. Appoloni (Via Adobe)** - LIVESTOCK MOBILITY IN WEST AFRICA: NETWORK ANALYSIS AND APPLICATIONS	9:45h
10:00h	*Discussion*	*Discussion*	10:00h
10:30h	*Coffee Break*		10:30h
	6A: GFRA - CONVENTIONAL VACCINES	**6B: MODELLING BUSINESS SECURITY DURING OUTBREAKS**	
11:00h	*Keynote* **T. Doel -** WHAT CAN THEY ACHIEVE, HOW STRAIGHTFORWARD WOULD IT BE TO REPLACE THEM GIVEN AN ALTERNATIVE?	**K. Mintiens -** MODELLING BIOSECURITY	11:00h
		M. Sanderson - SECURE BEEF SUPPLY IN THE U.S. – PLANNING FOR CONTROL AND CONTINUITY OF BUSINESS IN AN FMD OUTBREAK	11:15h
11:30h	*Keynote* **M. Perez-Filgueira -** QUALITATIVE ASPECTS OF THE IMMUNE RESPONSES RELATED TO PROTECTION AGAINST FMDV CHALLENGE IN CATTLE	**R. Bradhurst -** MODELLING MANAGEMENT STRATEGIES FOR VACCINATED ANIMALS AFTER AN OUTBEAK OF FMD AND THE IMPACT ON RETURN TO TRADE	11:30h
		M. Tildesley - MODELLING THE IMPACT OF REGIONAL MOVEMENT CONTROL POLICIES FOR FMD OUTBREAKS IN DISEASE FREE COUNTRIES	11:45h
12:00h	*Keynote* **W. Vosloo -** SPECIES SPECIFIC FMD VACCINES - WHAT IS THE EVIDENCE?	*Discussion*	12:00h
12:30h	*Lunch*		
	PLENARY ROOM		
	7: CHAMPIONING NEW VACCINES		
13:50h	**A. Capozzo + F. Maree** INTRODUCTION		
14:00h	*Keynote* **B. Charleston + E. van den Born -** Vaccine Efficacy (VACCINE EFFICACY OF FMD VIRUS-LIKE PARTICLES PRODUCED BY THE BACULOVIRUS EXPRESSION SYSTEM)		
14:30h	*Keynote* **T. de los Santos -** The GMO (Adenovirus) option (A CURRENT PERSPECTIVE ON ADENOVIRUS 5-VECTORED FMD VACCINES)		
15:00h	*Keynote* **M. Mourino + E. Rieder -** Attenuated FMD vaccines (FMD-LL3B3D VACCINE PLATFORM: SAFE, HIGHLY POTENT, FULLY DIVA COMPATIBLE, INACTIVATED FMD VIRUS VACCINES)		
15:30h	*Coffee break*		
	8A: THE FUTURE OF FMD VACCINES		
16:00h	**F. Maree -** AN OVERVIEW OF REVERSE GENETIC APPROACHES TO ENHANCED FMD VACCINES IN AFRICA		
16:15h	**M. Ryan -** RATIONAL DESIGN OF ATTENUATED FMDV VACCINES BY ELEVATION OF –CPG- AND –UPA- DINUCLEOTIDE FREQUENCIES		
16:30h	*Discussion*		
	8B: VACCINE EFFICACY & EFFECTIVENESS - IMPROVING THE USE OF FIELD STUDIES		
16:45h	**G. Ferrari + D. Paton** INTRODUCTION		
17:00h	**E. Brocchi -** FIELD TRIAL TO ESTIMATE THE EFFECTIVENESS OF THE VACCINATION PROGRAM IMPLEMENTED IN THE MAGHREB REGION		
17:15h	**M. Tildesley -** MODELLING THE IMPACT OF FARMING PRACTICES UPON VACCINE EFFECTIVENESS IN ENDEMIC SETTINGS – A CASE STUDY IN KENYA		
From 17:30h	POSTER SESSION		

31st October - DAY 3

BREAKOUTS

	ROOM 1	ROOM 2	ROOM 3	
	9A : AFRICA EPI-NET	9B: IMPROVING CONVENTIONAL VACCINES	9C: SIGN-UP DISCUSSIONS	
9:00	**D. Lefebvre + A. Vleeschauwer +** D. Ehizibolo - SEROTYPES IN NIGERIA	**H. Gaude -** A GVII-2015, A NEW HIGH POTENCY VACCINE WITH BROAD PROTECTION AGAINST A/ASIA/G-VII THREAT	Predicting Vaccine Demand	9:00
9:15	**K. Scott -** SEROTYPING SEROPREVALENCE OF FMD IN CATTLE FROM UGANDA SURVEILLANCE 2014-2018.	**J. Horsington -** INTRADERMAL APPLICATION OF FOOT-AND-MOUTH DISEASE VACCINES FOR PIGS		
9:30	**K. de Clercq -** FMD IN BURUNDI	**P. Eblé -** EFFICACY OF A/MAY/97 FMDV VACCINE AGAINST HETEROLOGOUS CHALLENGE WITH A FIELD VIRUS FROM THE EMERGING A/ASIA/G-VII LINEAGE IN CATTLE		
9:45	**W. Chikurunhe -** DOES THE AFRICAN BUFFALO REALLY SPREAD FMD? A SERO-SURVEY OF FMD IN CATTLE AROUND MANA POOLS CONSERVATION PARK OF NORTHERN ZIMBABWE	**T.Tuthill -** A SIMPLE UNIVERSAL TEST TO QUANTITATE 146S ANTIGEN DURING PRODUCTION OF FMD VACCINES	AESOP: A New Option for Emergency Reserves?	9:45
10:00	**E. Houndje -** CONTROL METHODS OF FMD IN BENIN BY TRIAL VACCINATION AND MEDICINAL PLANTS	**S. Parida -** IMPROVING THE DURATION OF IMMUNITY FOR FMD VACCINES		
10:15	**C. Kasanga -** MOLECULAR CHARACTERISATION OF FMD VIRUS DETECTED DURING 2015 – 2018 IN TANZANIA: INSIGHTS FOR VIRUS DIVERSITY AND EVOLUTION IN AFRICA	**S. Berryman -** THE USE OF REVERSE GENETICS TO FACILITATE THE GROWTH OF FMDV FOR THE PRODUCTION OF VACCINES		
10:30	**A. Diaoure -** FMD SURVEILLANCE AND CONTROL IN MALI			
10:30h - 11:00h	*Coffee / tea break*			10:30h - 11:00h

	ROOM 1	ROOM 2	ROOM 3	
	10A: DIAGNOSES & DIAGNOSTIC TOOLS	10B: VACCINE QUALITY ASSURANCE	10C: EuFMDiS DEMONSTRATION	
11:00h	**M. Eschbaumer -** MULTIPLEX REAL-TIME RT-PCR FOR DETECTION OF FMDV, RIFT VALLEY FEVER VIRUS AND BOVINE VIRAL DIARRHEA VIRUS IN BULK TANK MILK	**C. Stenfeldt -** THE INTERDEPENDENCE OF FMDV PATHOGENESIS, CHALLENGE SYSTEM, AND OUTCOME OF VACCINE STUDIES	**K. Mintiens.** Demonstration **+ Debate** on future work with S. Mortenson (EuFMD) and R. Bergevoet (EuFMD)	11:00h
11:15h	**L. Comtet -** EMERGENCY SUPPLY OF FMD DIAGNOSTIC KITS: REAGENT BANKS AND IDVET SOLUTIONS	**A. Dekker -** GOOD CORRELATION BETWEEN VACCINE MATCH IN POTENCY TESTS AND r1-VALUE		
11:30h	**C. Browning -** RESULTS FROM A INTER-LABORATORY EXERCISE TO EVALUATE NON-STRUCTURAL PROTEIN ELISA KITS	**S. Jamal -** POTENCY ASSESSMENT OF FMD VACCINES USING STANDARDISED SEROLOGICAL ASSAYS		
11:45h	**A. Ludi -** RESULTS OF THE 2016 AND 2017 PROFICIENCY TESTING SCHEMES FOR FMD DIAGNOSTIC METHODS	*Discussion*		
12:00h	*Discussion*			
12:30h - 14:00h	*Lunch break*			12:30h - 14:00h

	11A: MIDDLE EAST & ASIA EPI-NET	11B: VACCINE PERFORMANCE	
14:00 PM	**C. van Maanen** - DETECTION OF FMDV O/ME-SA/IND-2001E IN JORDAN	**S. Kerfua (Via Adobe)** - RETROSPECTIVE FMD OUTBREAK REPORTS FROM UGANDA AND TANZANIA BORDER DISTRICTS (2011-2016): IMPLICATIONS FOR FMD CONTROL BY VACCINATION	14:00 PM
14:15 PM	**I. Keskin -** THE ASSOCIATION BETWEEN BORDER PROVINCES OF TURKEY AND FMD OUTBREAKS	**S. El Azhari -** UPDATE OF FMD IN THE MAGHREB REGION: VACCINATION ISSUES	14:15 PM
14:30 PM	**M. McLaws -** RISK-BASED STRATEGIC PLANS FOR FIVE PRIORITY DISEASES IN THE PALESTINIAN AUTHORITY, INTEGRATED.	**G. Ilziibat** - ASSESSMENT OF FMD VACCINES IN MONGOLIA AND THE ROLE OF BACTRIAN CAMELS	14:30 PM
14:45 PM	**C. Bartels -** EMBEDDING PROGRESSIVE CONTROL FOR FMD IN THE POLICY AGENDA FOR LIVESTOCK PRODUCTION IN THREE COUNTRIES IN SOUTHEAST ASIA	*Discussion*	14:45 PM
15:00 PM	**K. Bankowska -** TRANS-POOL MOVMENT OF TWO FMDV SEROTYPE A LINEAGES: A/ASIA/G-VII AND A/AFRICA/G-IV		
15:15 PM	**G. Limon-Vega -** SOCIO-ECONOMIC IMPACT OF FMD OUTBREAKS AND CONTROL MEASURES AT DIFFERENT SCALES IN MONGOLIA: FROM NATIONAL LEVEL GROSS LOSSES TO HERDERS' FOOD SECURITY		
15:30h	*Coffee break*		

PLENARY ROOM

	12: CLOSURE AND AKNOWLEDGEMENTS
16:00h	TBD
17:00h	Closure

OS 18 - Posters (All to be shown on every day) - Draft

Size: max 90x120cm. No color or font restriction. No need to put the EuFMD logo. Posters will be visible for the entire meeting in the Foyer "Sala Colonnato" where coffee breaks are served.
Please send us a slide with the title and message of your poster. At the end of each plenary/parallel session, we will show a collated ppt of all the posters relevant to that session.
In addition to which, you are free to record an Instagram video or photo and upload it with the #OS18, and its title/session. It will be shown on the EuFMD Instagram on a monitor at the meeting and on our Instagram site.

Session 2	**Session 6a**	**D. Ehizibolo -** GENETIC CHARACTERIZATION OF FMD VIRUSES RESPONSIBLE FOR OUTBREAKS IN NIGERIA DURING 2016: RESURGENCE OF THE NOVEL FMD- SAT1 TOPOTYPE	**D. Paton -** USE OF SEROLOGICAL TESTS FOR CHECKING NSP PURITY OF FMD VACCINES	**J.S. Chahal** - A MODIFIED DENDRIMER-RNA VACCINE PLATFORM AGAINST FMD VIRUSES
A. Railey - HOUSEHOLD PREFERENCES FOR DIAGNOSTIC TESTING TO VACCINE MATCH IN AN ENDEMIC SETTING	**B. Jackson** - DEVELOPMENT OF MASTER VACCINE SEEDS FOR FOOT-AND-MOUTH DISEASE CONTROL IN SUB-SAHARAN AFRICA	**H. Ularamu** - CHARACTERIZATION OF FMDV ISOLATES CANDIDATE STRAINS FOR POLYVALENT VACCINE DEVELOPMENT IN NIGERIA	**P. Tuncer-Göktuna** - WHAT CAN WE SAY? HARMONY OR DISHARMONY BETWEEN VACCINE MATCHING AND CHALLENGE STUDY	**M. Chitray** - THE USE OF NOVEL SINGLE-CHAIN ANTIBODY FRAGMENTS AGAINST SAT SEROTYPE FMD VIRUSES IN DIAGNOSTICS
Session 3b	**Ah-Young Kim** - STABILIZING FACTORS ASSOCIATED WITH VACCINE ANTIGEN PRODUCTION USING KOREAN LOCAL STRAIN OF FMDV	**Session 10a**	**Session 11a**	**BK. Ku** - GENETIC CHARACTERIZATION OF THE 2018 FMD VIRUSES IN SOUTH KOREA
A. Dekker - VP1 IS IMPORTANT IN HEPARIN SULPHATE BINDING OF FMDV STRAIN O MANISA	**JH. Park** - ANTIGENIC PROPERTIES OF STABILIZED VIRUS PARTICLES FOR A FMD DISEASE VACCINE	**M. Eschbaumer** - FMD VIRUS ADSORBED TO GENOTUBE SWABS REMAINS INFECTIOUS AT HIGH TEMPERATURE	**F. Rosso -** REGIONAL COOPERATION BETWEEN TRANSCAUCASIA AND NEIGHBOURING COUNTRIES ON PREVENTION AND CONTROL OF FMD	**J. Maud.** HUMANS APPLY VACCINES: HOW CAN NEW TRAINING TOOLS BE USED TO BUILD CAPACITY FOR FMD CONTROL?
E. Foglia - REPLICATION DYNAMICS OF MIXED FMD VIRUSES IN VITRO	**J. Seago -** CHIMERIC SAT2 FMD VIRUS WITH INCREASED CAPSID THERMOSTABILITY FOR IMPROVED VACCINES	**L. Henry -** COMPARATIVE PERFORMANCE OF MONOCLONAL AND POLYCLONAL-BASED ANTIGEN ELISAS FOR FMDV DETECTION	**G. Pezzoni** - FMD OUTBREAKS DUE TO AN EXOTIC VIRUS SEROTYPE A LINEAGE (A/AFRICA/G-IV) IN ALGERIA IN 2017	**A. Morris** - INVESTIGATING CROSS REACTIVITY OF SEROLOGICAL ENZYME LINKED IMMUNOSORBENT ASSAYS
D. King - USING HIGH THROUGHPUT SEQUENCING TO CHARACTERISE LOW-FREQUENCY DIVERSITY OF FOOT-AND-MOUTH DISEASE VIRUS DURING VACCINE STRAIN ADAPTATION	**P. Zamorano** - NEW CAGE-LIKE PARTICLE ADJUVANT INCREASED THE IMMUNOGENICITY AND THE PROTECTION INDUCED BY A VACCINE AGAINST FMD VIRUS A/ARG/2001.	**J. Horsington** - INACTIVATION OF FMDV IN TISSUE SAMPLES TO ENSURE SAFE TRANSPORT FROM INFECTED PREMISES TO DIAGNOSTIC LABORATORIES	**N. Singanallur** - SERO-SURVEILLANCE FOR FMD IN SMALLHOLDER GOAT PRODUCTION IN LAO PDR, 2017–2018	**P. Opperman** - CONSTRUCTION OF A RECOMBINANT ANTIBODY PHAGE DISPLAY LIBRARY DERIVED FROM THE IMMUNE REPERTOIRE OF FMD–SAT IMMUNE BUFFALO. POTENTIALLY NEW DIAGNOSTIC REAGENTS?
M. Mahapatra - IDENTIFICATION OF NOVEL ANTIBODY BINDING DETERMINANTS OF SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUS	**Session 8b**	**S. Parida -** DETECTION OF EARLY FMD VIRUS INFECTION IN PIGS USING IgA AND IgM ASAAYS	**General**	**HM. Pyo** - INTER-LABORATORY PROFICIENCY TEST FOR SEROLOGICAL DIAGNOSIS OF FMD IN SOUTH KOREA
Session 5a	**JE. Park** - POST-VACCINATION MONITORING OF TRIVALENT FMD VACCINE CONTAINING O1 MANISA, O 3039, A22 IRAQ TO EVALUATE VACCINE EFFECTIVENESS IN SMALL SCALE FIELD TRIALS	**HM. Pyo** - PRODUCTION OF SWINE SEROLOGICAL PANEL FOR THE VALIDATION OF FMD ANTIBODY TESTS	**A. Asfor** - A NOVEL VP2 PEPTIDE ELISA FOR UNIVERSAL DETECTION OF ANTIBODIES FOR FMD SERO-SURVEILLANCE	**M. Saduakassova** - DEVELOPMENT AND EVALUATION OF LINEAGE-SPECIFIC REAL-TIME RT-PCR ASSAYS FOR THE DETECTION AND CHARACTERISATION OF FMD VIRUSES CIRCULATING IN ASIA
E. Brown - EVALUATING THE EFFICENCY OF ENVIRONMENTAL SAMPLING METHODS FOR THE DETECTION AND QUANTIFICATION FMD VIRUS	**Session 9a**	**Session 10b**	**SM. Barani** - CLINICAL SENSITIVITY OF CATTLE, SHEEP AND GOATS TO DIFFERENT SEROTYPES OF FMD VIRUS IN CENTRAL REGION OF IRAN	**A. Yasmin** - CHARACTERISATION OF BOVINE DENDRITIC CELLS FOLLOWING FMDV INFECTION
C. Colenutt + E. Brown - EVALUATION OF ENVIRONMENTAL SAMPLING AS A LOW TECHNOLOGY METHOD FOR SURVEILLANCE OF FOOT-AND-MOUTH DISEASE VIRUS IN AN ENDEMIC AREA	**F. Banda** - FMD OUTBREAK IN LUKULU DISTRICT , EVIDENCE OF VIRAL SPREAD OUTSIDE THE KNOWN ENDEMIC AREAS	**F. Feenstra** - INSIGHTS AND OPTIMISATION OF THE FMD VIRUS NEUTRALISATION TEST FOR R1 ANTIGENIC MATCHING	**S. Blaise-Bosseau** - DEVELOPMENT AND EVALUATION OF A MULTIPLEX CLASSICAL RT-PCR FOR SIMULTANEOUS DETECTION AND TYPING OF FMDV IN WEST AFRICA	

Appendix 2
List of Participants

APPENDIX 2: LIST OF PARTICIPANTS

EXECUTIVE COMMITTEE EuFMD

Dr ANGOT Jean-Luc
President of the EuFMD Committee
CGAAER (High Council for Food and Agriculture)
Ministère de L'agriculture et de l'alimentation
251 Rue de Vaugirard 75732 Paris cedex 15 Paris
France
jean-luc.angot@agriculture.gouv.fr

Dr SUMPTION Keith
Executive Secretary of the EuFMD
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
Keith.Sumption@fao.org

Dr BORRELLO Silvio
Direttore Generale
Direzione Generale della Sanita' Animale e dei Farmaci Veterinari
Ministero della Salute
Viale Giorgio Ribotta 5 - 00144 - Roma Rome
Italy
b.cappelletti@sanita.it

STANDING TECHNICAL COMMITTEE EuFMD

Dr RYAN Eoin
Chair of Standing Technical Committee
Ruminant Animal Health Division
Department of Agriculture Food and the Marine
Backweston Campus Celbridge
Ireland
eoin.ryan@agriculture.gov.ie

Dr BERGEVOET Ron
Senior Reseacher
Wageningen Economic Research
Wageningen University
Hollandseweg 1 6700 AA Wageningen
The Netherlands
ron.bergevoet@outlook.com

Dr MORTENSEN Sten
Veterinary R&D Manager
Animal Health Unit
Danish Veterinary and Food Administration
Stationsparken 31-33 DK-2600 Glostrup
Denmark
stm@fvst.dk

Dr SCHWABENBAUER Karin
Member of the STC
Federal Ministry for Food and Agriculture
Germany
Bienenweg 19 Tegernheim
Germany
karin.schwabenbauer@gmail.com

Dr ZIENTARA Stephan
Head of Unit
Virology
Anses
14 Rue Pierre et Marie Curie Maisons-Alfort
France
stephan.zientara@vet-alfort.fr

SPECIAL COMMITTEE ON RESEARCH AND PROGRAMME DEVELOPMENT EuFMD

Dr ALEXANDROV Tsviatko
Head of Department
Animal Health
Bulgarian Food Safety Agency
Pencho Slaveikov 15A Sofia
Bulgaria
tsv.alexandrov@yahoo.com

Dr BAKKALI-KASSIMI Labib
Deputy Head of Virology Unit
UMR 1161
ANSES - Animal Health Laboratory
14 rue Pierre et Marie Curie Maisons-Alfort
France
labib.bakkali-kassimi@anses.fr

Dr BELLAICHE Michel
CVO
Israeli Veterinary Services
Ministry of Agriculture
SHACHAM 7 Shoham
Israel
Michelb@moag.gov.il

Dr BROCCHI Emiliana
Head of National Reference Laboratory for Vesicular Diseases – FAO/OIE Reference Centre for FMD
Biotechnology Department
IZSLER
Via bianchi 9 Brescia
Italy
emiliana.brocchi@izsler.it

Dr BULUT Abdulnaci
FMD Expert Diagnosis
FMD Institute
Dumlupinar Bulvard 35 Cukurambar Çankaya
ANKARA
Turkey
abdulnaci.bulut@tarim.gov.tr

Dr DE CLERCQ Kris
Head of Unit
Infectious Diseases in Animals
Sciensano
Groeselenberg 99 1180 Ukkel
Belgium
kris.declercq@sciensano.be

Dr DEKKER Aldo
Senior Scientist
Virology Department
WBVR-Lelystad
Houtribweg 39 Lelystad
The Netherlands
Aldo.Dekker@wur.nl

Dr ESCHBAUMER Michael
Head of Laboratory
Institute of Diagnostic Virology
Friedrich-Loeffler-Institut
Suedufer 10 Greifswald
Germany
michael.eschbaumer@fli.de

Dr KING Donald
Group Leader
WRLFMD
The Pirbright Institute
Ash Road Pirbright
United Kingdom
donald.king@pirbright.ac.uk

Dr LYONS Nick
Veterinary Epidemiologist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
nicholas.lyons@fao.org

Dr VALARCHER Jean Francois
Prof Ruminant Medicine
Department of Clinical Sciences
Swedish University of Agricultural Sciences
Box 7054 Uppsala
Sweden
jean-francois.valarcher@slu.se

SPECIAL COMMITTEE ON BIORISK

Dr TJØRNEHØJ Kirsten
Senior Adviser Biosafety Officer
Division for Diagnostics and Scientific Advice
National Veterinary Institute Technical University of Denmark
Lindholm Island Dk-4771 Kalvehave
Denmark
kitj@vet.dtu.dk

OBSERVERS - DG-SANTE

Dr CAMARA Ewa
Veterinary Legislative Officer
Unit G2 Animal Health and Welfare
DG SANTE European Commission
Rue Froissart 101 1049 Brussels
Belgium
ewa.camara@ec.europa.eu

OIE

Dr QIU Yu
OIE SEACFMD Project Officer
Sub-Regional Representation for South-East Asia
OIE
C/o DLD 69/1 Phaya Thai Road Bangkok
Thailand
y.qiu@oie.int

FAO

Dr LUBROTH Juan
Chief Veterinary Officer Animal Health Service FAO
AGAH (Animal Health Service)
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla Rome
Italy
juan.lubroth@fao.org

Reference laboratories

ANSES

Dr BLAISE-BOISSEAU Sandra
Research Scientist
Animal Health Laboratory Virology Unit
ANSES
14 rue Pierre et Marie Curie Maisons-Alfort
France
sandra.blaise-boisseau@anses.fr

Prof LALOY Eve
Assistant Professor
BIOPIC Team UMR 1161 Virologie Laboratory of Animal Health
ANSES
France
eve.laloy@vet-alfort.fr

Dr ROMEY Aurore
Assitant Ingenior
Animal Health Laboratory Virology Unit
ANSES
14 rue Pierre et Marie Curie Maisons-Alfort
France
aurore.romey@anses.fr

ISTITUTI ZOOPROFILATTICI SPERIMENTALI ITALY IZSLER (Lombardia and Emilia Romagna)

Dr FOGLIA Efrem Alessandro
Fellow Researcher
Agenti ad Alta Diffusione e Biotecnologie Diagnostiche IZSLER
Via bianchi 9 brescia
Italy
e.foglia@izsler.it

Dr GRAZIOLI Santina
Biologist
Biotechnology Departement
IZSLER
Via Bianchi 9 Brescia
Italy
Santina.Grazioli@Izsler.It

Dr PEZZONI Giulia
Biologist
National Reference Laboratory for Vesicular Diseases and OIE/FAO Reference Laboratory for FMDV, IZSLER
via Bianchi, 9 Brescia 25124
Italy
giulia.pezzoni@izsler.it

Dr VARISCO Giorgio
Technical Director
Public Health Entity
IZSLER
Via Bianchi 9 Brescia
Italy
DIREZIONESANITARIA@IZSLER.IT

IZSPB (Puglia and Basilicat

Dr BEVERELLI Matteo
Dirigente Veterinario Sanita' Animale
IZSPB
Via Giovanni Leonardo Marugi 38 Lecce
Italy
matteo.beverelli@izspb.it

Dr CONSENTI Barbara
Direttore Sanitario IZSPB
via Manfredonia, n. 20 - Foggia
Italy
Barbara.consenti@izspb.it

Dr FASANELLA Antonio
Direttore Generale
IZSPB
via Manfredonia, n. 20 - Foggia
Italy
antonio.fasanella@izspb.it

Dr SEVI Agostino
Presidente CDA
IZSPB
Via Manfredonia 20 71121 Foggia
Italy
Agostino.sevi@izspb.it

Dr TANTALO Pietro
Direttore Amministrativo
IZSPB
Via Manfredonia 20 71121 Foggia
Italy
pietro.tantalo@izspb.it

The Pirbright Institute

Dr AMINA Yasmin
PhD Student
Molecular Virology
The Pirbright Institute
Ash Road Pirbright
United Kingdom
amina.yasmin@pirbright.ac.uk

Dr ARMSON Bryony
PhD Student
Vesicular Disease Reference Laboratory
The Pirbright Institute
Ash Road Woking
United Kingdom
bryony.armson@pirbright.ac.uk

Dr ASFOR Amin
Postdoctpral-Research Scientist
Virus Programme
The Pirbright Institute
Ash Road Woking
United Kingdom
amin.asfor@pirbright.ac.uk

Dr BACHANEK Bankowska-Kasia
Senior Scientist
WRLFMD
The Pirbright Institute
Ash Road Pirbright
United Kingdom
kasia.bankowska@pirbright.ac.uk

Dr BERRYMAN Stephen
Senior Postdoctoral Scientist
Picornavirus Molecular Biology Group
Pirbright Institute
Ash Road Woking
Afghanistan
stephen.berryman@pirbright.ac.uk

Dr BROWN Emma
Research Assistant
Transmission Biology
The Pirbright Institute
Ash Road Woking
United Kingdom
emma.brown@pirbright.ac.uk

Dr BROWNING Clare
Diagnostic Scientist
World Reference Laboratory
The Pirbright Institute
Ash Road Pirbright Woking Surrey GU24 0NF
United Kingdom
clare.browning@pirbright.ac.uk

Dr CARR Veronica
Research Scientist
Viral Immunology
The Pirbright Institute
Ash Road Woking Surrey
United Kingdom
veronica.carr@pirbright.ac.uk

Dr CHARLESTON Bryan
CEO
Director
The Pirbright Institute
Ash Road Pirbright
United Kingdom
bryan.charleston@pirbright.ac.uk

Dr COLENUTT Claire
Postdoctoral Scientist
Transmission Biology
The Pirbright Institute
Ash Road Pirbright **United Kingdom**
claire.colenutt@pirbright.ac.uk

Dr DI NARDO Antonello
Molecular Epidemiologist
Vesicular Disease Reference Laboratory
The Pirbright Institute
Ash Road Pirbright Woking Surrey
United Kingdom
antonello.di-nardo@pirbright.ac.uk

Dr FERRETTI Luca
Research Scientist
Integrative Biology
The Pirbright Institute
Ash Road Woking
United Kingdom
luca.ferretti@pirbright.ac.uk

Dr GUBBINS Simon
Senior Epidemiologist
Transmission Biology
The Pirbright Institute
Ash Road Pirbright
United Kingdom
simon.gubbins@pirbright.ac.uk

Dr HARVEY Yongjie
Research Associate
Molecular Virology
The Pirbright Institute
Ash road Woking
United Kingdom
kitty.harvey@pirbright.ac.uk

Dr HENRY Elisabeth
Research Assistant
Vesicular Disease Reference Laboratories
The Pirbright Institute
Ash Road Woking
United Kingdom
lissie.henry@pirbright.ac.uk

Dr JACKSON Ben
Post Doctoral Scientist
Molecular Virology
The Pirbright Institute
Ash Rd Pirbright Woking
United Kingdom
ben.jackson@pirbright.ac.uk

Dr KNOWLES Nick
Head of Molecular Epidemiology
Vesicular Disease Reference Laboartory Group
The Pirbright Institute
Ash Road Pirbright
United Kingdom
nick.knowles@pirbright.ac.uk

Dr KRUPALI Parekh
Research Scientist
Vaccine Differentiation Group
The Pirbright Institute
Ash road Woking
United Kingdom
krupali.parekh@pirbright.ac.uk

Dr LIMON-VEGA Georgina
Postdoctoral researcher
Vesicular Disease Reference Laboratory
The Pirbright Institute
Ash Road Surrey
United Kingdom
georgina.limon-vega@pirbright.ac.uk

Dr LUDI Anna
Head of Serology
World Reference Laboratory
The Pirbright Institute
Ash Road Pirbright
United Kingdom
Anna.Ludi@pirbright.ac.uk

Dr MAHAPATRA Mana
Senior Scientist
Vaccine Differentiation
The Pirbright Institute
Ash Road Woking
United Kingdom
mana.mahapatra@pirbright.ac.uk

Dr MORRIS Alison
Manager of Diagnostic Development Team
World Reference Laboratory
The Pirbright Institute
Ash Road Pirbright
United Kingdom
Alison.Morris@pirbright.ac.uk

Dr NELSON Noel
Dispersion Scientist
Transmission Biology
The Pirbright Institute
Ash Road Pirbright Nr Woking Surrey
United Kingdom
noel.nelson@pirbright.ac.uk

Dr PARIDA Satya
Head of the Group
Vaccine Differentiation
The Pirbright Institute
Ash Road Woking
United Kingdom
satya.parida@pirbright.ac.uk

Dr PATON David
Senior Veterinary Advisor
World Reference Laboratory
The Pirbright Institute
Ash Road Pirbright
United Kingdom
dajapaton@gmail.com

Dr PEREZ-MARTIN Eva
Scientist
Viral Immunology
The Pribright Institute
Ash Road Woking
United Kingdom
eva.perez@pirbright.ac.uk

Dr PORTA Claudine
Post-Doc
Viral Immunology
The Pirbright Institute
Ash Road Pirbright
United Kingdom
claudine.porta@pirbright.ac.uk

Dr RIBECA Paolo
Head of Integrative Biology and Bioinformatics
Integrative Biology and Bioinformatics
The Pirbright Institute
Ash Road Woking
United Kingdom
paolo.ribeca@pirbright.ac.uk

Dr SEAGO Julian
Group Leader
Plowright Building
The Pirbright Institute
Ash Road Pirbright
United Kingdom
julian.seago@pirbright.ac.uk

Dr TUTHILL Tobias
Head of Programme Virus
Picornavirus Structure
The Pirbright Institute
Ash Road Pirbright
United Kingdom
toby.tuthill@pirbright.ac.uk

Dr WATERS Ryan
Named Veterinary Surgeon
Animal Services
The Pirbright Institute
Ash Road Pirbright Guildford
United Kingdom
ryan.waters@pirbright.ac.uk

EuFMD Team - Technical

Dr ANOWER A.K.M. Mostafa
Short Term Professional
EuFMD Pillar III
FAO
Viale delle Terme di Caracalla Rome
Italy
Akm.Anower@fao.org

Dr BAKKOURI Abdenacer
DVM Program Specialist (Pillar II 2-3 Component Manager)
EuFMD
Viale delle Terme di Caracalla 00153 Roma
Italy
abdenacer.bakkouri@fao.org

Dr BESSONG OJONG Willington
Animal Health Officer
EUFMD
FAO
Viale della Terme di Caracalla Rome
Italy
willington.bessongojong@fao.org

Dr CHEVANNE Etienne
Risk Management Specialist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
etienne.chevanne@gmail.com

Dr KRSTEVSKI Kiril
Animal Health Officer Pillar I
EuFMD Commission
EuFMD Commission
Viale delle Terme di Caracalla Rome
Italy
kiril.krstevski@fao.org

Dr MAUD Jenny
Training Programmes Manager
EuFMD FAO
Viale delle Terme di Caracalla Rome
Italy
jenny.maud@fao.org

Dr MCLAWS Melissa
Veterinary Epidemiologist Consultant
EuFMD
571 Windermere Ave Ottawa
Canada
melissa.mclaws@gmail.com

Dr MILLER Corissa
Support Officer Global Vaccine Security (STP)
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
corissa.miller@fao.org

Dr NOVA Rodrigo
Animal Health Officer
Animal Health FAO
EuFMD
Via di Terme di Caracalla Rome
Italy
rodrigo.nova@fao.org

Dr PÖTZSCH Carsten
EuFMD Consultant
EuFMD
FAO
Fontanestr. 12 Tramnitz
Germany
carsten@potzsch.eu

Dr ROSSO Fabrizio
EuFMD-Risk Reduction Programme Manager
AGAH
FAO
Viale Terme di Caracalla Rome
Italy
fabrizio.rosso@fao.org

EuFMD Team - Operational

Ms ADDARI Chiara
Programme Specialist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
chiara.addari@fao.org

Ms ALVITI Alessandra
Intern
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
alessandra.alviti@fao.org

Mr BRYAN Ross
Intern
EuFMD
FAO
Viale delle Terme di caracalla Rome
Italy
ross.bryan@fao.org

Ms CARRAZ Cécile
Workplan Coordinator
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
Cecile.Carraz@fao.org

Ms CRICHTON Philippa
EuFMD Programme Support
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
philippa.crichton@fao.org

Ms EPPS Silvia
Programme Specialist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
silvia.epps@fao.org

Mr LICASTRO Maurizio
Programme Specialist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
maurizio.licastro@fao.org

Ms RENZETTI Francesca
Programme Specialist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
francesca.renzetti@fao.org

Ms RUMICH Nadia
EuFMD Communications and Networks
AGAH
FAO
Viale Terme di Caracalla Rome
Italy
nadia.rumich@fao.org

Ms TOMAT Erica
Travel Training and Operations Specialist
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Italy
Erica.Tomat@fao.org

DELEGATES A-Z by Country

ALGERIA

Dr MADANI Hafsa
Head of Virology Department
Central Veterinary Medecine
National Institute of Animal Health
B.P 204 Hacen Badi Belfort El Harrach 16051
Algeria
hafsasfr@yahoo.fr

Dr OUAGUENI Nassim
Marketing & Technical Manager North Africa
Animal Health
Boehringer Ingelheim
Coop. El Yasmine, 29 route du Stade
Algeria
nassim.ouagueni@merial.com

Dr OUALI Karima
Dr. Vétérinaire experte FMD
Direction des Services Vétérinaires
Ministère de l'Agriculture du
Développement Rural et de la Pêche
12 Avenue Colonel Amirouche.
Algeria
oualikary@gmail.com

ARGENTINA

Dr CAPOZZO Alejandra
Inependent Researcher (CONICET)
Institute of Virology
National Institute of Agriculture of Argentina
N. Repetto and De Los Reseros S/N
Hurlingham Buenos Aires
Argentina
capozzo.alejandra@inta.gob.ar

Dr PEREZ-FILGUEIRA Mariano
Lead Researcher
Institute of Virology
National Institute of Agricultural Technology
N Repetto y De Los Reseros s/n Hurlingham
Buenos Aires
Argentina
perez.mariano@inta.gob.ar

Dr SMITSAART Eliana
Head of FMD R&D
Research & Development
Biogenesis Bago S.A.
Ruta Panamericana km 385 Garin
Argentina
gabriela.kleber@biogenesisbago.com

AUSTRALIA

Dr ATKINSON Robert
Veterinarian
1/75 Torrens Street Braddon 2612
Australia
r.atk@icloud.com

Dr BRADHURST Richard
Research Fellow
School of Biosciences
University of Melbourne
Parkville Melbourne
Australia
richard.bradhurst@unimelb.edu.au

Dr GIBSON Katherine
Senior Manager Special Projects
Animal Health Australia
95 Northbourne Avenue Turner Canberra
Australia
kgibson@animalhealthaustralia.com.au

Dr HORSINGTON Jacquelyn
Research Scientist
Health and Biosecurity
CSIRO-AAHL
5 Portarlington Rd Geelong
Australia
jacquelyn.horsington@csiro.au

Dr SENEQUE Sacha
Veterinary Public Health Managing Director
Ceva Global
Ceva
134 Point Walter Rd Bicton WA. 6157
Australia
sacha.seneque@ceva.com

Dr SINGANALLUR Nagendrakumar
Research Scientist
Transboundary Animal Diseases Mitigation
Australian Animal Health Laboratory Csiro-Health And Biosecurity
5 Portarlington Road Geelong
Australia
nagendra.singanallur@csiro.au

Dr THOMSON Sally
Senior Veterinary Officer
Deparment of Agriculture and Water Resources
Australian Government Department of Agriculture and Water Resources
7 London Circuit Canberra
Australia
sal.thomson@agriculture.gov.au

Dr VOSLOO Wilna
Principal Research Scientist
Australian Animal Health Lab
CSIRO
5 Portarlington Road Geelong
Australia
wilna.vosloo@csiro.au

BELGIUM

Dr GOOVAERTS Daniel
Owner
Consulting
DGVAC Consulting
Langenberg 3 2460 Lichtaart
Belgium
Dannygoovaerts@skynet.be

Dr LEFEBVRE David
Scientist
Department of Infectious Diseases in Animals
Sciensano
Groeselenberg 99 Ukkel
Belgium
david.lefebvre@sciensano.be

BRAZIL

Dr COSIVI Ottorino
Director
Centro Panamericano de Fiebre Aftosa
Pan American Health Organization (PAHO)
Av. Governador Leonel de Moura Brizola 7778
Duque de Caxias (Rio de Janeiro) **Brazil**
cosivio@paho.org

Dr MOZZER Otto
Director
R&D Biologicals
CEVA Santé Animale
Rodovia MG 050 Km 188 2002 - Distrito Industrial - MG 35675-000 Juatuba
Brazil
otto.mozzer@ceva.com

BULGARIA

Dr TCHAKAROVA Simona
Head of NRL
Exotic and Emerging Diseases
NDRVMI
Pencho Slaveikov 15 Sofia
Bulgaria
agrhhh@gmail.com

CAMEROON

Dr DICKMU JUMBO Simon
Director
Diagnostic and Animal Health
Laboratoire National Vétérinaire (LANAVET)
Bokle Garoua
Cameroon
drsimondickmu@yahoo.fr

CHINA

Dr DAI Chunming
Manager of Dairy Farm
Junlebao Dairy Co., Ltd
Junlebao Dairy Co., Ltd
Shijiazhuang Shijiazhuang
China
gaodp2007@sina.com

Dr EARANKY Mohan Venkata Subramanya
Vice - President - Operations
Manufacturing Operations
Shanghai Shenlian Biomedical Corporation
48 Jiangchuan East Road Minhang District Shanghai
China
Mohan@slbio.com.cn

Dr GAO Dianping
Technical Director
Division Of Biological Products
China Animal Husbandry Industry Co. Ltd
Building No.16part Eight No.188 Southwest Fourth Ring Fengtai Beijing
China
gaodp2007@sina.com

Dr GUAN Ming Xu
Consultant
R&D department
Jinyu Baoling Biopharmaceutical Co. Ltd.
No 58 Ordors Street Hohhot City Inner Mongolia Hohhot City
China
liuyanxia@jinyubaoling.com.cn

Dr GUOLIANG Chen
Veterinary Director
Haid Group
Guangzhou Guangzhou
China
gaodp2007@sina.com

Dr HOU Xinfeng
General Manager
JUNLEBAO Dairy Group
No.36Luquan Shijiazhuang
China
gaodp2007@sina.com

Dr JINGSHAN Xue
Chief Scientist
Research institute
China Animal Husbandry Industry Co. ltd Beijing
China
gaodp2007@sina.com

Dr LAN Yun
Veterinary Director
Guangxi State Farms Animal Husbandry Group Co., LTD Nanning
China
gaodp2007@sina.com

Dr LIU Yanxia
Manager
Production Department
Jinyu Baoling Biopharmaceutical Co. Ltd.
No 58 Ordors Street Hohhot City Inner Mongolia Hohhot City
China
liuyanxia@jinyubaoling.com.cn

Dr RODRIGUEZ PERSICO Juan Manuel
Manager
Asia Marketing And Technical
Biogenesis Bago
828-838 Zhangyang Road Huadu Mansion Pudong New District Shanghai
China
juan.persico@biogenesisbago.com

Dr WANG Chen Ching
Manager
R&D
CAHG ZhiHe(BeiJing) Biotechnology Co. Ltd.
5F Building 13 Yard 7 ZEEP No.156 BeiQing Rd. Haidian District BeiJing
China
alecwang@hotmail.com

Dr WEI Xuefeng
Vice President
R&D department
Jinyu Baoling Biopharmaceutical Co. Ltd.
No 58 Ordors Street Hohhot City Inner Mongolia Hohhot City
China
liuyanxia@jinyubaoling.com.cn

Dr XIE Wenqiang
Manager
Marketing Department
Jinyu Baoling Biopharmaceutical Co. Ltd.
No 58 Ordors Street Hohhot City Inner Mongolia Hohhot City
China
liuyanxia@jinyubaoling.com.cn

Dr YIN Zhifeng
Deputy Manager of Production Technology
Junlebao Dairy Co., Ltd
Shijiazhuang
China
gaodp2007@sina.com

Dr ZHANG Yan
Assistant of Vice President
Shanghai Shen Lian Biomedical Corporation
No.48 Jiangchuan East Road Minhang District Shanghai
China
yanzhang@slbio.com.cn

Dr ZHIYING Chen
Technical Director
Shanghai Shenlian Biomedical Corporation
48 Jiangchuan East Road Minhang District Shanghai
China
yanzhang@slbio.com.cn

CZECH REPUBLIC

Dr KRIVDA Vlastimil
Head od NRL for FMD
Serology and Virology
State Veterinary Institute in Prague
Sidlistni 136/24 Prague 6
Czech Republic
krivda@svupraha.cz

Dr MIKULCOVÁ Helena
Deputy Head of the NRL for FMD
Serology and Virology State Veterinary Institute in Prague
Síslištní 136/24 Prague 6
Czech Republic
helena.mikulcova@svupraha.cz

DENMARK

Prof BELSHAM Graham
Professor
National Veterinary Institute
Technical University of Denmark (DTU)
Lindholm Kalvehave
Denmark
grbe@vet.dtu.dk

Dr LOHSE Louise
Senior Advisor
National Veterinary Institute
Technical University of Denmark
Lindholm DK-4771 Kalvehave
Denmark
loloh@vet.dtu.dk

Dr WESTERGAARD Jorgen
Consultant
International
ADC-Consult
Mikkelborg Alle 7 Horsholm
Denmark
adc-consult@youmail.dk

EGYPT

Dr ABDELAZIM Moustafa
Area Technical & Marketing Manager
Companion Animals
North East Africa
Cairo
Egypt
mostafa.abdelazim@boehringer-ingelheim.com

Prof AFFASH Mahmoud
University Professor
Reproduction
Faculty Of Veterinary Medicine - Benisuef University
Benisuef Al Shamlah، 62511 Benisuff
Egypt
mahmoudaffash@yahoo.com

Prof AHMED Hussein
University Professor
Virology
Faculty Of Veterinary Medicine Benisuef Univ.
El-Shaheed Gamal El-Deen Afify Oula Giza
Giza Governorate Cairo
Egypt
husseinhussein502@yahoo.com

Dr DUDNIKOV Leonid
Site Director
Vaccines
Middle East for Veterinary Vaccines - MEVAC
20 Joseph Titi El Nozhaa elGedea Cairo
Egypt
officemanager@me-vac.com

Dr IBRAHIM Magdy
CEO
Vaccines
Middle East for Veterinary Vaccines - MEVAC
20 joseph tito ElNozhaa elGededa Cairo
Egypt
officemanager@me-vac.com

Dr KASSEM Ahmed
TMVS North East Africe Boehringer-Ingelheim
Animal Haelth
Boehringer Ingelheim
78 elmoltaka Elaraby Sheraton Cairo
Egypt
ahmed.kassem@boehringer-ingelheim.com

Dr RASHED Bayoumy
Physician
Sales and Marketing
Egypt
6103street no 9 mokattam Cairo
Egypt
rashedbayoumy@ift-online.com

Dr WAEER Mohamed
Veterinaire
Veterinary Care
Dina Farms
80 Alex cairo road Cairo
Egypt
rodinaamin@ift-online.com

FRANCE

Dr BONIN Jacques
Head of the Veterinay Public Health Center
Animal Health Division
Merial / Boehringer-Ingelheim
29 Avenue Tony Garnier 69007 Lyon
France
jacques.bonin@boehringer-ingelheim.com

Dr COMTET Loic
R&D Manager
R&D
IDvet
310 Rue Louis Pasteur Montpellier
France
loic.comtet@id-vet.com

Dr COURTAY Bruno
Director
Business Operations Development
Boehringer Ingelheim
29 Avenue Tony Garnier Lyon
France
Bruno.courtay@merial.com

Dr DENORMANDIE Nicolas
Director of the Technical Service / Middle East Africa & LATAM support
VPH (Veterinary Public Health)
Boehringer Ingelheim
29 Avenue Tony Garnier Lyon
France
nicolas.denormandie@boehringer-ingelheim.com

Dr DEZIER Cedric
Technical Director
The Veterinary Public Health Center
Boehringer Ingelheim
29 Avenue Tony Garnier Lyon
France
cedric.dezier@boehringer-ingelheim.com

Dr ENCHÉRY François
Clinical Manager
R&D
Boehringer Ingelheim
29 avenue Tony Garnier Lyon
France
francois.enchery@boehringer-ingelheim.com

Dr EVANS Amanda
Manager Vph Marketing Communications
Veterinary Public Health
Boehringer Ingelheim
29 avenue Tony Garnier Lyon
France
amanda.evans-verfay@boehringer-ingelheim.com

Dr GAUDE Hélène
Global Project Leader
R&D
Boehringer Ingelheim
813 cours du 3ème millenaire Saint Priest
France
helene.gaude@boehringer.com

Dr GUIBAL Audrey
Strategic Reserves Manager
Veterinary Public Health
Boehringer Ingelheim
29 Avenue Tony Garnier Lyon
France
audrey.guibal@merial.com

Dr HAMERS Claude
Dir. Scientific Support and Trial Management
The Veterinary Public Health Center
Boehringer Ingelheim
29 Avenue Tony Garnier Lyon
France
Claude.HAMERS@boehringer-ingelheim.com

Dr HUDELET Pascal
Head Technical Services
The Veterinary Public Health Center
Boehringer Ingelheim
29 avenue Tony Garnier Lyon
France
pascal.hudelet@merial.com

Dr IMBERT Stephane
Regional Director Europe Africa & Middle East The Veterinary Public Health Center BI
The Veterinary Public Health Center
Boehringer Ingelheim
29 Avaenue Tony Garnier Lyon
France
stephane.imbert@boehringer-ingelheim.com

Dr MEZHOUD Irina
Communications Assistant
Veterinary Public Health
Boehringer Ingelheim
29 ave Tony Garnier 69007 Lyon
France
irina.mezhoud@boehringer-ingelheim.com

Dr POLLET-OGIER Marie-France
Head of Bio New Product Transfer
R&D
Boehringer Ingelheim
813 cours du 3eme Millénaire 69800 Saint Priest
France
marie-france.pollet-ogier@boehringer-ingelheim.com

Dr ROMEY Aurore
Assitant Ingenior
Animal Health Laboratory Virology Unit
ANSES
14 rue Pierre et Marie Curie Maisons-Alfort
France
aurore.romey@anses.fr

Dr ROTSZTAJN Nathalie
Global FMD Marketing Director
Veterinary Public Health
Boehringer Ingelheim
29 av. Tony Garnier Lyon
France
nathalie.rotsztajn@boehringer-ingelheim.com

Dr TANO Lazare
Technical Manager META
Boehringer Ingelheim
29 avenue Tony Garnier Lyon
France
lazare.tano@boehringer-ingelheim.com

GERMANY

Dr CARLSON Jolene
Post Doctoral Researcher
Friedrich-Loeffler-Institut
Dr.
Suedufer 10 Greifswald
Germany
jolene.carlson@fli.de

Dr PFAFF Florian
Post Doctoral Researcher
Institute of Diagnostic Virology
Friedrich-Loeffler-Institut - German Federal Research Institute for Animal Health
Südufer 10 Greifswald - Insel Riems
Germany
florian.pfaff@fli.de

HUNGARY

Dr PENZES Zoltan
Global Bio R&D Director
Bio R&D
Ceva-Phylaxia Co. Ltd.
Szallas u. 5. Budapest
Hungary
andrea.sandor@ceva.com

IRELAND

Dr SAMMIN Donal
Head of Laboratories
DAFM Laboratories
Department of Agriculture Food and the Marine IRELAND
Backweston Celbridge Co Kildare
Ireland
donal.sammin@agriculture.gov.ie

Dr SHERIDAN Hazel
Senior Superintending Veterinary Inspector
NDCC Veterinary International
Department of Agriculture, Fisheries and Food
Ireland
Hazel.Sheridan@agriculture.gov.ie

ISRAEL

Prof KLEMENT Eyal
Associate Professor
Koret School of Veterinary Medicine
Hebrew University Jerusalem Israel
Pob 12 Rehovot
Israel
eyal.klement@gmail.com

Dr VAN STRATEN Michael
Head
Herd Health & Epidemiology
Hachaklait Veterinary Services
Qelahim 10 Qelahim
Israel
vanstraten@hachaklait.co.il

ITALY

Dr BALDI Loredana
Veterinarian Head of Departemnt
Epidemiologic Obervatories of Biostatistic
Zooprofilaxis Institute of South Italy
Via Salute 2 Portici Napoli Portici
Italy
loredana.baldi@cert.izsmportici.it

Dr CAPPELLETTI Benedetta
General Directorate for Animal Health and Veterinary Medicines
Export Of Animals and Products Thereof
Ministero della Salute
Viale Giorgio Ribotta 5 Rome
Italy
b.cappelletti@sanita.it

Dr DE FELICE Alessandra
Veterinarian
Observatory Veterinary Health
Zooprofilaxis Institute of South Italy
Via Salute 2 Portici Napoli Portici
Italy
alessandra.defelice@izsmportici.it

Dr FERRARI Giancarlo
Veterinarian
Animal Health
Istituto Zooprofilattico del Lazio e Toscana
Via Appia Nuova n. 1411 Rome
Italy
giancarlo.ferrari@izslt.it

Dr KRIZ Nikolaus
Head of Unit Animal Health and Welfare
Risk Assessment and Scientific Advice
EFSA
Via Carlo Magno 1/A Parma
Italy
Nikolaus.Kriz@efsa.europa.eu

Dr MINTIENS Koen
FMD Quantitative Risk Assessor
European Commission for the Control of Foot-and-Mouth Disease
FAO
Viale delle Terme di Caracalla Rome
Italy
koen.mintiens@fao.org

Dr POZZATO Nicola
Veterinarian
Verona Diagnostic Laboratory
Istituto Zooprofilattico Sperimentale delle Venezie
Via San Giacomo 5 Verona
Italy
npozzato@izsvenezie.it

Dr SCICLUNA Maria Teresa
Schouten
Virology Unit
Istituto Zooprofillatico Sperimentale Lazio e Toscana
Via appia Nuova 1411 Rome
Italy
teresa.scicluna@izslt.it

JAPAN

Dr NISHI Tatuya
Researcher
Exotic Disease Research Station
National Institute of Animal Health National Agriculture and Food Research Organization
6-20-1 Josui-honcho Kodaira Tokyo
Japan
ultra1124@affrc.go.jp

KAZAKHSTAN

Dr SADUAKASSOVA Meruyert
PhD Student
Department for Epizootological Monitoring and Risks Assessment of Animal Viral Diseases
Kazakh Scientific Research Veterinary Institute
Raiymbeck Ave. 223 Almaty
Kazakhstan
sadumeru_87@mail.ru

MALI

Dr DIAOURÉ Abdoulaye
Directeur VSF-Suisse Mali et Reoprésentant pour l'Afrique de l'Ouest
Perogramme Mali/Afrique de l'Ouest
VSF-Suisse
Rue 432 porte 898 Hamdallaye ACI-2000 Bamako
Mali
abdoulaye.diaoure@vsf-suisse.org

MALTA

Dr BONNICI Sabrina
Veterinarian
Veterinary Regulation Division
Ministry of Environment Sustainable Development and Climate Change
'Peridot Court' Flt 7 Triq l'Immakulata Marsascala
Malta
sabrina-grace.bonnici@gov.mt

MONGOLIA

Dr DAVAANYAM Odonzul
Virologist
General Authorithy For Veterinary Service
State Central Veterinary Laboratory
Zaisan Khan-Uul District 63-2-37 Ulaanbaatar
Mongolia
odonzul.1121@yahoo.com

Dr ULZIIBAT Gerelmaa
FMD Research
General Agency of Veterinary Services under the Ministry of Agriculture in Mongolia
State Central Veterinary Laboratory
Khan-uul District Zaisan 53/03 17024-Ulaanbaatar Mongolia Ulaanbaatar
Mongolia
gerelmaa@scvl.gov.mn

MOROCCO

Dr DOUK Abdelwahed
Head of the Service of Prophylactic Actions
Animal Health Departement
ONSSA
Street Haj Ahmed Cherkaoui Agdal RABAT
Morocco
abdelwahed.douk@gmail.com

Dr EL AZHARI Safae
Veterinarian + PhD Student
Quality Controle + Département De Pathologie Et De Santé Publique Vétérinaires Biopharma + Institut Agronomique Et Veterinaire Hassan Ii
N°3318 Lot Wifaq Temara
Morocco
safaeelazhari@gmail.com

Dr EL MRINI Meryem
Veterinarian
Animal Health
Onssa
Av Hadj Ahmed Cherkaoui Agdal Rabat
Morocco
meryem.pathologiste@gmail.com

NETHERLANDS (The)

Dr BARTELS Chris
Consultant Veterinary Epidemiology and PCP-FMD Management
Director
Animal Health Works
St. Odulphusstraat Bakhuizen
The Netherlands
chrisb@animalhealth.works

Dr BROKS Venice
Research Assistant
VPH
Boehringer Ingelheim
Houtribweg 39 Lelystad
The Netherlands
venice.broks@boehringer-ingelheim.com

Dr COCO MARTIN Jose
R&D Site Head
Vaccines R&D
Boehringer Ingelheim
Houtribweg 39
The Netherlands
jose.coco-martin@boehringer-ingelheim.com

Dr EBLÉ Phaedra
Researcher
Department of Virology
Wageningen Bioveterinary Research
Houtribweg 39 Lelystad
The Netherlands
phaedra.eble@wur.nl

Dr FEENSTRA Femke
Scientist
Vaccines and VPH Lelystad
Boehringer Ingelheim
Houtribweg Lelystad
The Netherlands
femke.feenstra@boehringer-ingelheim.com

Dr SCHOUTEN Matthijs
Policy Officer Animal Health
Animal Supply Chain and Animal Welfare Department
Ministry of Agriculture Nature & Food Quality
P.O. Box 20401 2500 EK The Hague
The Netherlands
m.c.w.schouten@minez.nl

Dr VAN DEN BORN Erwin
Project Leader R&D
R&D Swine Biologicals
MSD Animal Health
Wim de Körverstraat 35 Boxmeer
The Netherlands
erwin.van.den.born@merck.com

Dr VAN MAANEN Cornelis
Virologist/Senior Scientist
Department of Small Ruminants Horses and Companion Animals
GD Animal Health
Arnsbergstraat 7 Deventer
The Netherlands
c.v.maanen@gddeventer.com

Dr VAN SCHAIJK Ben
Senior Scientist
R&D Vaccines – Veterinary Public Health
Boehringer Ingelheim
Houtribweg 39 Lelystad
The Netherlands
Ben.van_Schaijk@boehringer.com

NEW ZEALAND

Dr SANSON Robert
Veterinary Epidemiologist
Digital Products and Services
AsureQuality Limited
Batchelar Centre Tennent Drive Palmerston North
New Zealand
robert.sanson@asurequality.com

PAKISTAN

Prof JAMAL Syed
Associate Professor
Biotechnology
University of Malakand
Chakdara Khyber Pakhtunkhwa
Pakistan
jamal115@yahoo.com

SLOVAKIA

Dr JADUD Peter
NRL
Virology
SVFI Veterinary Institute in Zvolen
Pod Drahami 918 Zvolen
Slovakia
jadud@svuzv.sk

Dr MOJZIS Miroslav
Director
Virology
SVFI Veterinary Institute in Zvolen
Pod Drahami 918 Zvolen
Slovakia
molbiology@svuzv.sk

SLOVENIA

Dr HROVATIN Breda
Head of Animal Health and Welfare Division
Animal Health And Animal Welfare Division
The Administration of the Republic of Slovenia for Food Safety Veterinary Sector and Plant Protection
Dunajska 22 Ljubljana
Slovenia
breda.hrovatin@gov.si

SOUTH AFRICA

Dr CHITRAY Melanie
Reasearcher
Agricultural Research Council
South Africa
Chitraym@arc.agric.za

Dr MAREE Francois Frederick
Specialist Researcher
Vaccine Diagnostic Development
Agricultural Research Council
100 Old Soutpan rd Onderstepoort
South Africa
mareef@arc.agric.za

Dr OPPERMAN Pamela
Senior Researcher
Vaccine and Diagnostic Development
Agricultural Research Council Onderstepoort Veterinary institute
100 Old Soutpans road Pretoria
South Africa
storeyp@arc.agric.za

Dr SCOTT Katherine
Researcher
Vaccine Diagnostic Development Programme
Agricultural Research Council
100 Old Soutpan rd Onderstepoort Pretoria
South Africa
scottka@arc.agric.za

SOUTH KOREA

Dr KANG Bokyn
South Korea
bkkang@mediandx.com

Dr KIM Ahyoung
Research Scientist
Center for FMD Vaccine Research
Animal and Plant Quarantine Agency
177 Hyeoksin 8-ro Gimcheon-Si
South Korea
mochsha@korea.kr

Dr KO Mikyeong
Researcher
Center of FMD Vaccine Research
Animal and Plant Quarantine Agency
177 Hyeonsin 8-ro Gimcheon-si
South Korea
rose-corea@hanmail.net

Dr KU Bok kyung
Senior Veterinary Researcher
Animal and Plant Quarantine Agency
Foot and Mouth Disease Division
177 hyeoksin 8 ro 39660 Gimcheonsi
South Korea
kubk@korea.kr

Dr NA Keunsok
Deputy Manager
Sales
MEDIAN Diagnostics Inc.
Beobwon-ro 128 Songpa-gu Seoul
South Korea
ksna@mediandx.com

Dr PARK Jong Hyun
Senior Researcher
Center for FMD Vaccine Research
Animal and Plant Quarantine Agency
177 Hyeoksin 8-ro Gimcheon
South Korea
parkjhvet@korea.kr

Dr PYO Hyun Mi
Veterinary Research Officer
Foot-and-Mouth Disease Division
Animal and Plant Quarantine Agency
177 Hyeoksin 8-ro Gimcheon
South Korea
hmpyo@korea.kr

SPAIN

Dr CACERES GARRIDO German
National Focal Point Eufmd Spain
Epidemiology Unit Animal Health Department
Ministry of Agriculture Fisheries and Food
Calle Almagro 33 despacho 2A15 Madrid
Spain
gcaceres@mapama.es

Dr DE LA PUENTE AREVALO Maria
FMD Emergency Preparedness Officer
EuFMD
FAO
Viale delle Terme di Caracalla Rome
Spain

Dr MOURIÑO Mercedes
Laboratory and Emerging Diseases Senior Manager
Veterinary Medicine Research and Development
Zoetis Manufacturing & Research Spain S.L.
Ctra. Camprodon s/n- La Riba 17813 Vall de Bianya
Spain
mercedes.mourino@zoetis.com

SUDAN

Dr ABDELRAHMAN MOHAMED Ahmed Hussien
Sudan

Dr BRIMA ISMAEL Yassir Abakar
Sudan

Dr MOHAMED AHMED HABIBALLA Hanan Yousif
Sudan

Dr OSMAN IDRIS Salah Eldein
Sudan

SWEDEN

Dr GRANT Malin
Epidemiologist
Department of Disease Control and Epidemiology
National Veterinary Institute
SE-751 89 Uppsala
Sweden
malin.grant@sva.se

Dr HALLGREN Gunilla
Veterinary Epidemiologist
Department of Epidemiology and Disease Control
National Veterinary Institute
75189 Uppsala
Sweden
gunilla.hallgren@sva.se

Dr JUREMALM Mikael
Head of R&D
Boehringer Ingelheim Svanova
Boehringer Ingelheim
Box 1545 SE-75145 Upsala
Sweden
mikael.juremalm@boehringer-ingelheim.com

Dr SELLMAN Stefan
Postdoc
IFM
Linköping University
Linköping
Sweden
stefan.sellman@liu.se

SWITZERLAND

Dr SAUTTER Carmen
Scientific Staff
Biosafety
Institute of Virology and Immunology (IVI)
Sensemattstr. 293 3147 Mittelhäusern
Switzerland
carmen.sautter@ivi.admin.ch

Dr SCHRODER Bjoern
Global Product Placement
Thermo Fisher Scientific
Switzerland
bjoern.schroeder@thermofisher.com

TUNISIA

Dr BROUR Emna
Sanitary Veterinary Doctor
Veterinary Services Department
Ministry of Agriculture and Water Resources
30 Avenue Alain Savary Tunis
Tunisia
emnabrour@gmail.com

Dr HAJ AMMAR Heni
Veterinarian
DGSV
DGSV-Tunisia
30 Rue Alain SAVARY Tunis
Tunisia
hajammar.vet@gmail.com

TURKEY

Dr KESKIN Ipek
Veterinarian
Epidemiology
Veterinary Control Central Research Institute of the Ministry of Agriculture and Forestry
Ahmet Sefik Kolayli Caddesi 21/21A Ankara
Turkey
ipekkeskin@hotmail.com

Dr TUNCER GÖKTUNA Pelin
Chief
Typing Laboratory
SAP Institute/Government
Dumlupınar Blv. No:35 Çankaya 06510 Ankara
Turkey
pelin.tuncergoktuna@tarimorman.gov.tr

UGANDA

Dr AINEPLAN Noel
Ag. Manager Harmonisation and International Linkages
Office of the Secretary to the Authority
Uganda National Drug Authority
Rumee towers Plot 19 Lumumba Avenue Kampala
Uganda
amnoel@nda.or.ug

Dr ATIM Stella
National FMD Focal Point Person for Uganda
Animal Health
Ministry of Agriculture Animal Industry and Fisheries
Lugard Street Entebbe
Uganda
stellatim93@gmail.com

UNITED KINGDOM

Dr ATKINSON John
Associate Director
Intergovernmental Veterinary Health
MSD Animal Health
8 Acres Close Helmsley
United Kingdom
john.atkinson@merck.com

Dr DOEL Timothy
Retired Consultant
Office
Tdcd Ltd
1 Penrose Way Alton
United Kingdom
timandclaudia.doel@gmail.com

Dr EKWEM Divine
Student
Biodiversity
University Of Glasgow
University Avenue Glasgow
United Kingdom
divine.ekwem@glasgow.ac.uk;
d.ekwem.1@research.gla.ac.uk

Dr FAITHFULL Mark
Technical Manager
Animal Health
Merial Animal Health Limited
Ash Road Guildford
United Kingdom
jo.barbour@merial.com

Dr GAUNTLETT Francesca
Scientific Adviser
Advice Services – Exotics and Risk
Animal and Plant Health Agency (APHA)
Nobel House 17 Smith Sq SW1P 3 JR London
United Kingdom
francesca.gauntlett@apha.gsi.gov.uk

Dr GOLDE William
Principal Scientist
Vaccines Pillar
Moredun Research Institute
Pentlands Science Park Bush Loan Penicuik
United Kingdom
william.golde@moredun.ac.uk

Dr GUYVER-FLETCHER Glen
PhD Student
Department of Life Sciences
University of Warwick
University Road Coventry
United Kingdom
g.guyver-fletcher@warwick.ac.uk

Dr LEMBO Tiziana
Senior Lecturer
Institute of Biodiversity Animal Health and Comparative Medicine
University of Glasgow
464 Bearsden Rd Glasgow
United Kingdom
tiziana.lembo@glasgow.ac.uk

Dr MOIR Ruth
Scientific Advisor (Epidemiology)
APHA
DEFRA
Nobel House 17 Smith Sq SW1P 3JR London
United Kingdom
ruth.moir@defra.gsi.gov.uk

Dr PROBERT William
Postdoctoral Researcher
Nuffield Department of Health
University of Oxford
Big Data Institute Li Ka Shing Centre for Health Information and Discovery Oxford
United Kingdom
william.probert@bdi.ox.ac.uk

Dr REEVE Richard
Reader
Boyd Orr Centre for Population and Ecosystem Health
University of Glasgow
Graham Kerr Building Glasgow
United Kingdom
Richard.Reeve@glasgow.ac.uk

Prof RYAN Martin
Professor
Biomedical Sciences Research Complex School of Biology
University of St Andrews
North Haugh St Andrews
United Kingdom
mdr1@st-andrews.ac.uk

Dr SALT Jeremy
Chief Scientific Officer
R&D
GALVmed
Doherty Building Pentlands Science Park Bush Loan
United Kingdom
jeremy.salt@galvmed.org

Dr SMITH Andy
Foot And Mouth Disease Policy Adviser
Department Environment Food and Rural Affairs
Uk
Nobel House London
United Kingdom
Andy.C.Smith@defra.gsi.gov.uk

Dr STEPHAN Lévon
Government Veterinary Advisor
Veterinary Exotic Notifiable Disease Unit
Animal and Plant Health Agency
Nobel House 17 Smith Sq SW1P 3 JR London
United Kingdom
levon.stephan@apha.gsi.gov.uk

Prof TILDESLEY Michael
Associate Professor
Systems Biology and Infectious Disease Epidemiology Research Centre
University of Warwick
Gibbet Hill Road Coventry
United Kingdom
M.J.Tildesley@warwick.ac.uk

Dr VOSOUGH AHMADI Bouda
Research Economist
Land Economy Environment and Society Research Group
Scotland's Rurual College (SRUC)
West Mains Road Edinburgh
United Kingdom
bouda.v.ahmadi@sruc.ac.uk

Dr WILD Claire
AS - APHA
DEFRA
Nobel House 17 Smith Sq SW1P 3 JR London
United Kingdom
Lesley.columbine@apha.gsi.gov.uk

Dr YALCINDAG Erhan
PostDoc
Genetics & Genomics
Roslin Institute
University of Edinburgh Easter Bush Midlothian Edinburgh
United Kingdom
eyalcind@exseed.ed.ac.uk

Dr ZHANG Guo
FMD Vaccine Matching Specialist
Veterinary Public Health Center
Boehringer Ingelheim
Ash Road Pirbright Woking Surrey
United Kingdom
guo.zhang@merial.com

Dr ZIOLKOWSKA Francesca
Strategic Communications
International Veterinary Vaccinology Network (Ivvn)
Ivvn
Roslin Stitute Edinburgh
United Kingdom
FRANCESCA.ZIOLKOWSKA@ED.AC.UK

UNITED STATES

Dr ARZT Jonathan
Veterinary Medical Officer
Foreign Animal Disease Research
40550 Route 25 Orient Point , Ny 11957
United States
Jonathan.Arzt@ARS.USDA.GOV

Dr BARNABEI Jamie
Veterinary Medical Officer
APHIS
USDA
1920 Dayton Avenue Ames
United States
daniel.j.grause@aphis.usda.gov

Dr CHAHAL Jasdave
Chief Scientist
Immuno
Tiba Biotech LLC
1 Broadway 14th floor Cambridge
United States
chahal@tiba.bio

Dr DE LOS SANTOS Teresa
Research Microbiologist
Piadc
Usda Ars
40550 Route 25 Orient Ny 11957
United States
paula.disabella@ars.usda.gov

Dr DELGADO Amy
Veterinarian
VS
USDA APHIS
2150 Centre Ave Bldg B Fort Collins
United States
amy.h.delgado@aphis.usda.gov

Dr DUQUE Hernando
Veterinary Medical Officer
APHIS
USDA
1920 Dayton Avenue Ames
United States
Hernando.Duque@aphis.usda.gov

Dr PARKER Elizabeth
International and Strategic Partnerships Specialist
AgriLife Research
Texas A&M University System
578 John Kimbrough Blvd Suite 201 College Station Texas 77843-2477
United States
Elizabeth.Parker@ag.tamu.edu

Dr RAILEY Ashley Flynn
PhD Student
Paul G. Allen School for Global Animal Health
Washington State University
240 SE Ott Rd Pullman
United States
ashley.railey@wsu.edu

Dr RIEDER Aida Elizabeth
Research Computational Biologist
Piadc
Usda Ars
40550 Route 25 Orient Ny 119578
United States
paula.disabella@ars.usda.gov

Dr RODRIGUEZ Luis L.
Research Leader
Foreign Animal Disease Research
Usda Ars Plum Island Animal Disease Center
40550 Route 25 Orient New York 11957
United States
Luis.Rodriguez@ars.usda.gov

Prof SANDERSON Michael
Professor
Diagnostic Medicine/Pathobiology
Kansas State University
306 Coles Hall Manhattan
United States
sandersn@vet.k-state.edu

Dr STENFELDT Carolina
Research Associate
Department of Veterinary Population Biology
University of Minnesota
Plum Island Animal Disease Center Greenport
United States
Carolina.Stenfeldt@ars.usda.gov

Prof VANDERWAAL Kim
Assistant Professor
Veterinary Population Medicine
University of Minnesota
1988 Fitch Avenue 385A ASVM Saint Paul
United States
kvw@umn.edu

VIETNAM

Dr DUNG Do Huu
Director of Planning
Department of Animal Health
Department of Animal Health Ministry of Agriculture and Rural Development Vietnam
No 15 Lane 78 Giai Phong Road Hanoi
Vietnam
ACortez@tti-corp.com

ZAMBIA

Dr BANDA Frank
Research Scientist
Veterinary
Central Veterinary Research Institute
33980 Lusaka Lusaka
Zambia
frankbanda2001@yahoo.co.uk

ZIMBABWE

Dr CHIKURUNHE Wilmot
Provincial Veterinary Officer
Veterinary Services
Zimbabwe
305 Hillview Park Bindura
Zimbabwe
wchikuru@gmail.com

Appendix 3
Poster Presentations

APPENDIX 3: POSTER PRESENTATIONS

POSTER

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

A.F. Raileya[a] and T.L. Marsha[b] [a]Paul G. Allen School for Global Animal Health, Washington State University, USA; [b]School of Economic Sciences, Washington State University, USA

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

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

A. Dekker, C.H. Boonstra-Leendertse*

Virology department, Wageningen Bioveterinary Research, Houtribweg 39, 8221 RA Lelystad, the Netherlands.

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

REPLICATION DYNAMICS OF MIXED FMD VIRUSES IN VITRO

E. A. Foglia, G. Pezzoni, S. Grazioli, A. Bregoli, E. Brocchi
IZSLER, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Introduction

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

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

Materials and Methods

Three cell lines, BHK-21, IBRS-2 and LFBK$\alpha_{\alpha v\beta 6}$, were co-infected with two FMDV serotypes (O and A) at different ratios; samples were collected sequentially up to 48 hours after in- fection and analysed by ELISA and a serotype-specific rRT-PCR, that enabled identification and quantification of the grown viruses. To investigate the possible impact of virus strains, experiments were repeated using two different topotypes per each serotype, namely O/ME- SA/Ind-2001d with A/ASIA/Iran-05 and O/EA-3 with A/AFRICA/G-IV.

Results

The results of both serotype-specific Ag-ELISA and rRT-PCR showed that FMD viruses of se- rotype A have a better fitness than type O viruses when cultured in BHK-21 and IBRS-2 cell lines, while LFBK$_{\alpha v\beta 6}$ cells allowed replication of the various co-infecting viruses without pro- moting one specific serotype. In this cell line the selection was only oriented versus one virus when its concentration was 100X compared to the other virus, suggesting that LFBK$_{\alpha v\beta 6}$ cells supports the growth of both serotypes with similar efficiency.

Discussion

Our results corroborate previous observations that LFBK$_{\alpha v\beta 6}$ are the preferable cell line for VI from field suspect samples, thanks to the speed of viral replication and to a wider susceptibility to various FMD viruses, with no predilection for a specific serotype. Conversely, BHK-21 and IBRS-2 cells are more susceptible to FMDV serotype A compared to O.

POSTER

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

D. King[1], F. Feenstra[2], L. Lasecka-Dykes[1], G. Freimanis[1], E. Laing[3], D.P. King[1], J. Coco-Martin[2]

[1] The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK; [2] Boehringer Ingelheim Animal Health Netherlands BV, Lelystad, Houtribweg 39, 8221 RA, The Netherlands; [3]Department of Microbial and Cellular Sciences, School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK

Introduction

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

Materials and Methods

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

Results

FMDV RNA yields for all samples were between 2.82x107 and 2.94x104 RNA copies/ µl. Analysis of the viruses derived from the O1Kaufbeuren clone revealed an expansion of low-frequency non-synonymous variants over the passage series. In contrast, for wildtype derived viruses, the overall low-frequency diversity of TUR/44/2012 was high but remained stable following each passage, whilst the TUR/05/2012 dataset saw a moderate increase in diversity, until a consensus change in passage 3 resulted in stability of diversity in the subsequent passages.

Discussion

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

POSTER

IDENTIFICATION OF NOVEL ANTIBODY BINDING DETERMINANTS OF SEROTYPE O FMDV

M. Mahapatra, S. Upadhyaya, A. Asfor and S. Parida*
The Pirbright Institute, Ash Road, Woking, Surrey, GU24 0NF, UK

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 mo- noclonal antibodies. In addition, there is evidence of the existence of other, yet unidentified epitopes, which are believed to play a role in antibody-mediated protection. However, the relative importance of different epitopes in FMD vaccine induced-protection has not been ascertained to date in great details. Attempts were made in this study to identify such epi- topes using a reverse genetics approach.

Materials and methods

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

Results

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

Discussion

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

POSTER

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

C. Colenutt[1], E. Brown[1], N. Nelson[2], J. Wadsworth[1], J. Maud[3], B. Adhikari[3,4], S.C. Kafle[5], M. Upadhyaya[6], S.K. Pandey[7], D.J. Paton[1], K. Sumption[3], S. Gubbins[1] [1] The Pirbright Institute, Ash Road, Pirbright, Surrey, GU24 0NF, United Kingdom; [2] The Met Offi- ce, FitzRoy Road, Exeter, Devon, EX1 3PB, UK; [3] European Commission for the Control of Foot- and-Mouth disease (EuFMD), Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy; [4] Food and Agriculture Organisation of the United Nations, Nepal Country Office; [5] National FMD and TADs Laboratory, Department of Livestock Services, Ministry of Livestock Deve- lopment, Nepal; [6]Veterinary Epidemiology Centre, Department of Livestock Services, Ministry of Livestock Development, Nepal; [7]Directorate of Animal Health, Department of Livestock Services, Ministry of Livestock Development, Nepal.

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

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

E. Brown[1], N. Nelson[2], S. Gubbins[1], C. Colenutt[1]

The Pirbright Institute, Ash Road, Pirbright, Surrey, GU24 0NF. United Kingdom; Met Office, FitzRoy Road, Exeter, Devon, EX1 3PB. United Kingdom

Introduction

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

Materials and methods

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

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

Results

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

Discussion

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

POSTER

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

B. Jackson[1], Y. Harvey[1], E. Perez-Martin[1], V. Carr[1], M. M Harmsen[2], N. Knowles[1], V. Mioulet[1], D. King[1], B. Charleston[1] and J. Seago[1]

[1] The Pirbright Institute, Woking, Surrey, GU24 0NF, United Kingdom; [2] Wageningen Bioveteri- nary Research, Division Virology, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

Introduction

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

Materials and methods

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

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

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

POSTER

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

*A-Y. Kim, H. Kim, S.H. Park, S.Y. Park, J-M. Lee, J-S. Kim, K-S. Cho, B. Kim, and Y-J. Ko**

Center for Foot-and-Mouth Disease Vaccine Research, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon City, Gyeongsangbuk-do, 39660, Republic of Korea

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Materials and methods

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

Results

Among combinations of media that resulted in high titers more than $5x10^7$ $TCID_{50}$/*ml*, three combinations exhibited high stability when the O/SKR/JC/2014 seed viruses were stored at -70^0C. In particular, purified 146S particles was the least degraded when they were suspended in potassium-based solution not only for 4^0C maintenance, but also for -70^0C storage, which should expose vaccine antigen to the degradation due to freeze-thaw cycle.

Discussion

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

POSTER

ANTIGENIC PROPERTIES OF STABILIZED VIRUS PARTICLES FOR A FMD VACCINE

*M-K. Ko, S-Y. Lee, J-H. Choi, S-H. You, S-H. Shin, H-E. Cho, M-J. Lee, S-M. Kim, B. Kim, J-H. Park**
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Materials and methods

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

Results

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

Discussion

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

POSTER

CHIMERIC SAT2 FMDV WITH INCREASED CAPSID THERMOSTABILITY FOR IMPROVED VACCINES

A. Kotecha[3], E. Perez-Martin[1], Y. Harvey[1], F. Zhang[1], S. Ilca[3], E.E. Fry[3], B. Jackson[1], F. Maree[2], K. Scott[2], C.W. Hecksel[5], M.M Harmsen[4], V. Mioulet[1], B. Wood[1], N. Juleff[1], D.I. Stuart[3,5],B. Charleston[1] and J. Seago[1] [1] The Pirbright Institute, Woking, Surrey, GU24 0NF, United Kingdom; [2] Transboundary Animal Disease Programme, ARC-Oderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 0110, South Africa; [3] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive Oxford OX3 7BN, United Kingdom; [4] Wageningen Biove- terinary Research, Division Virology, P.O. Box 65, 8200 AB Lelystad, The Netherlands; [5] Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK.

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Materials and methods

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

Results

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

Discussion

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

POSTER

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

J. Bidart[1,2], C. Kornuta[1,2], M. Gammella[1], C. Langellotti[1,2], R. Galarza[3], L. Calvinho[4], V. Quattrocchi[1], I. Marcipar[2,5], P. Zamorano[1,2,6]

[1]Instituto de Virología-CICVyA, INTA, Hurlingham, Argentina; [2]CONICET, CABA, Argentina; [3]AER Chascomus, INTA, Chascomus, Argentina; [4]EEA Rafaela, INTA, Rafaela, Argentina; [5]Facultad de Bioquímica y Ciencias Biológicas - Universidad Nacional del Litoral, Santa Fe, Argentina; [6]Universidad del Salvador, CABA, Argentina.

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

Materials and Methods

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

Results

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

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

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

POSTER

POST-VACCINATION MONITORING OF TRIVALENT FMD VACCINE CONTAI- NING O1 MANISA, O 3039, A22 IRAQ TO EVALUATE VACCINE EFFECTIVE- NESS IN SMALL SCALE FIELD TRIALS

JE. Park, HJ. Lyuk, HM. Pyo, JW. Byun, SH. Wee, MY. Park*
Foot-and-Mouth Disease Division, Animal and Plant Quarantine Agency, 177, Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do, Republic of Korea.

Introduction

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

Materials and methods

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

Results

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

Discussion

Previously, vaccine matching studies carried out by the World Reference Laboratory, Pirbright indicated that the r1 value was 0.43 based on VNT. In this study, we showed that emergency vaccination using inactivated vaccine with high r1(>0.3) value with the field strain provided high VN-titers, which may be justifiable as protection in pigs. In addition, monitoring post-vac- cination serology is an important component of evaluation for FMD vaccination programs.

POSTER

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

F. Banda 1, P. Lebea2, E. Fana3, T. Sinkombe1, G. Dautu1 , Y. Sinkala1

1Department of Veterinary Services, Lusaka, Zambia; 2Councial for Scientific and Industrial Research, Pretoria, South Africa; 3Botswana Vaccine Institute, Gaborone, Botswana.

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

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

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

POSTER

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

D.O. Ehizibolo[1], I. Fish[2], B. Brito[3], S. Pauszek[2], C. Stenfeldt[2], M. Bertram[2], G.H. Ularamu[1], Y.S. Wungak[1], D.D. Lazarus1, A.G. Ardo[4], C.I. Nwosuh1, and J. Arzt2

FMD Laboratory, Viral Research Division, National Veterinary Research Institute, Vom, Nigeria; Foreign Animal Disease Research Unit, ARS/USDA-Plum Island Animal Disease Center, 40550 Route 25, 11957, Orient Point, NY USA; The ithree institute, University of Technology Sydney, 15 Broadway, Ultimo NSW 2007, Australia; Extension Unit, National Veterinary Research Institute, Vom, Nigeria.

Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

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

H.G. Ularamu, Y.S. Wungak, D. D. Lazarus, D.O. Ehizibolo, A.A.Chukwuedo, C.I. Nwosuh Virology Research Division, National Veterinary Research Institute, Vom, Nigeria.

Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

FMDV ADSORBED TO GENOTUBE SWABS REMAINS INFECTIOUS AT HIGH TEMPERATURE

M. Eschbaumer

National Reference Laboratory for FMD, Institute of Diagnostic Virology, Friedrich-Loeffler-Ins- titut, Federal Research Institute for Animal Health, Suedufer 10, 17493 Greifswald-Insel Riems, Germany

Introduction

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

Materials and Methods

GenoTubes were dipped in FMDV A IRN/8/2015, dried and then incubated for 2 hours in a forced-air oven preheated to 100°C. A second set was kept at room temperature. To assess the time required for heat transfer to the swab, a thermocouple was placed inside a sealed swab tube that was also placed in the oven. After heating, the foam tip of each swab was washed with culture media. The eluate was titrated on LFBK-αVα6 cells. Cytopathic effect on the titration plates was evaluated microscopically and confirmed by antigen ELISA.

Results

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

Discussion

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

POSTER

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

L. Henry1, A. Morris1, V. Mioulet1, B. A. Wood1, A. Gray1, D. P. King1, S. Grazioli2, G. Pezzo- ni2, E. Brocchi2

1World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD), The Pirbright Insti- tute, Ash Road, Pirbright, Woking GU24 0NF, UK; 2Instituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy.

Introduction

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

Methods

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

Results

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

Discussion

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

POSTER

INACTIVATION OF FOMDV IN TISSUE SAMPLES TO ENSURE SAFE TRANS- PORT FROM INFECTED PREMISES TO DIAGNOSTIC LABORATORIES

J. Horsington1, M. Eschbaumer2, N. Singanallur1, W. Vosloo1

1CSIRO-Australian Animal Health Laboratory, Private Bag 24, Geelong, Victoria 3220, Australia; 2Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Suedufer 10, 17493 Greifswald, Germany.

Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

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

*K. Parekh, S. Parida**

The Pirbright Institute, Ash Road, Woking, Surrey GU24 0NF, UK.

Introduction

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

Materials and Methods

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

Results and discussion

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

POSTER

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

HY. Kim, JW. Byun, MY. Park, C.S. Kim, K. Roque, H.J. Lyuk, J.E. Park, S.H. Wee, H.M. Pyo
Foot-and-mouth disease Division, Animal and Plant Quarantine Agency, Gimcheon, Gyeong-sang buk-do 39660, Republic of Korea

Introduction

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

Materials and methods

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

Results

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

Conclusion

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

POSTER

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

F. Feenstra1, V. Broks2, P. Giskus2, C. Markopoulou1, D. Pialot3, JP. Cambon3, G. Zhang[4], H. Gaude[5], C. Hamer[6], J. Coco-Martin1, P. Hudelet[6]

Boehringer Ingelheim Animal Health; [1] Ruminant and VPH Vaccine R&D, Lelystad, the Nether- lands; 2 VPH technical services, Lelystad, the Netherlands; 3 Bioprocess Development and Industrialisation, Lyon, France; 4 VPH technical services, Pirbright, UK; 5 Ruminant and VPH Vaccines R&D, Lyon, France; 6 VPH technical services, Lyon, France.

Introduction

Vaccination is one of the most important interventions in foot-and-mouth disease virus (FMDV) outbreak prevention and control. Antigenic matching between vaccine and outbreak virus is critical for vaccination effectiveness and is usually determined using serological assays, such as virus neutralisation tests (VNTs). The ratio between the titer of vaccine serum against a field virus and the titer of vaccine serum against the vaccine virus (r1) is calculated. An r1 value <0.3 is supposed to be predictive of a vaccine mismatch. However, while the r1 value is generally used as indicator for protection, its reliability is limited, it has high variance and substantial variation between laboratories exists. Currently, there is no reliable in vitro alter- native for r1. Therefore, there is a need to optimize the current assay as well as develop novel assays.

Materials and methods

At Boehringer Ingelheim Animal Health an r1 VNT protocol had been developed using publi- cly available IBRS-2 cells (FLI), and reagents and disposables which are commercially available to enable assay harmonisation between laboratories. This assay has been validated and the intrinsic test variance was determined. Thereby the effect of repeating the assay and perfor- ming the assay in 1D or 2D was examined.

Results

This VNT has a low variance, but r1 variance is still high. Repeating the assay increased con- fidence and use of strict cell culture protocols did reduce the assay variance. Performing the assay in 1D instead of 2D gives similar variance, while enabling the performance of repeats.

Discussion

These studies have led to more insight in r1 variability and to what extend it can be reduced. A VNT protocol for FMDV antigenic matching has been created, enabling assay harmonisation between laboratories. This assay could contribute to more reliable vaccine matching, howe- ver, research on novel assays enabling more predictable vaccine matching is still required.

POSTER

USE OF SEROLOGICAL TESTS FOR CHECKING NSP PURITY OF FMD VACCINES

S. Duffy1, D. Paton2

[1] Centre of Quantitative Studies in Animal Health. National University of Rosario, Argentina; [2] The Pirbright Institute, Ash Road, Woking, Surrey, GU24 0NF, UK.

Introduction

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

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

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

P. Tuncer-Göktuna *, *C. Çokçalışkan, N. Taşçene, E. Aras Uzun, C. Gündüzalp, A. Arslan, Y. Gültekin, G.N. Balci, O. Kara, V. Gülyaz*
Foot-and-Mouth Disease Institute, 06044, Ankara, Turkey

Introduction

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

Materials and methods

In this study, the protection levels (r1 value) of four vaccine strains of the serotype A produ- ced by FMD Institute were investigated. The vaccine strains were belonging to A/Asia/GVII lineage (ANep84, ATUR16, ATUR17) and A/Asia/IRN05 (ATUR06). Results were obtained by using in vitro and in vivo methods; VNT for r1 value and challenge studies, respectively. In challenge studies 42 cattle were used.

Results

There were poor antigenic relationship detected between A/Asia/IRN05ATUR06-A/Asia/GVI-IANep84 and A/Asia/GVIIATUR17-A/Asia/GVIIATUR16 in vitro. A/Asia/GVIIATUR16-A/Asia/ GVIIATUR17 r1 value was detected as almost 0,3 (0,27) and the rest of four tests were showed protection (r1-values ≥0,3). After challenge, lesions were occurred on tongue (ino- culation site) in all animals. Most severe clinical signs including back hoof were seen in the challenge study with A/Asia/IRN05ATUR06. Lesions on the palate were seen on the A/Asia/ GVIIATUR17 challenge study, finally there were no lesions occurred except inoculation si- tes on the other three challenge studies which A/Asia/GVIIANep84 and A/Asia/GVIIATUR16 were used.

Discussion

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

POSTER

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

F. Rosso1, K. Sumption1, C. Potzsch1, I. Keskin2, G. Ferrari3, A. Skrypnyk, N. Bulut[5], L. Avaliani[6], Z. Rukhadze[7], T. Aliyeva[8] , S. Kharatyan [9]

[1] European Commission for the Control of FMD; [2] Etlik Veterinary Control Central Research Institute, Ministry of Agriculture and Forestry of Turkey; [3]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Italy; [5] National focal point for EuFMD activities in Turkey; [6] Veterinary Department of National Food Agency of Georgia; [7]National focal point for EuFMD activities in Georgia; [8] National focal point and coordinator for EuFMD activities in Azerbaijan ; [9] National focal point for EuFMD activities in Armenia.

Introduction

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

Material and methods

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

Results

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

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

Discussion

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

POSTER

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

G. Pezzoni1, A. Bregoli1, S. Grazioli1, I. Barbieri1, E.A. Foglia1, H. Madani2, A. Omani2, J. Wadsworth3, K. Bachanek-Bankowska3, N. J. Knowles3, D. P. King3, E. Brocchi1

1 Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy;
2 Institut National de la Médecine Vétérinaire, El Harrach, Algiers, Algeria;
3 The Pirbright Institute (TPI, Pirbright, Woking, Surrey, GU24 0NF, United Kingdom.

Introduction

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

Methods and Results

A total of six samples originated from three different outbreaks in Algeria were analyzed, FMDV serotype A was detected by Ag-ELISA. Virus isolates from all three outbreaks were obtained after the first passage in LFBK-αVα6 cells, which showed a better performance for virus isolation compared to BHK-21 and IB-RS2 cells. The phylogenetic analysis of the VP1 coding sequences grouped them within the A/AFRICA/G-IV lineage, most closely related to sequences originating from Nigeria in 2015, sharing more than 98% nt identity; older FMDV sequences (2009-2013) from Nigeria had lower nt identities (86.3-94.2%). FMDV sequences from countries in West Africa (Cameroon, Togo, Mali) and North-East Africa (Egypt, Eritrea, Sudan) were found to be more distantly related with nt identities ranging between 84.2- 90.2% and 82.8-87.8%, respectively. One complete full genome sequence (8119 nt) and two near-complete sequences (7616 and 7627 nt) were obtained from the three isolates by Miseq Illumina platform. Sequences differed at only 27 nt sites, 19/24 located within the polyprotein-coding region were synonymous and five non-synonymous.

Discussion

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

POSTER

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

N.B. Singanallur1, S. Nampanya2, V. Soukvilay2, C. Keokhamphet2, S. Khounsy2, P. Windsor3, W. Vosloo1

1Transboundary Diseases Mitigation, Australian Animal Health Laboratory, CSIRO Health and Biosecurity, 5 Portarlington Road, Geelong, Vic 3220, Australia; 2National Animal Health Laboratory, Vientiane, Lao People's Democratic Republic; 3Sydney School of Veterinary Science, The University of Sydney, Camden, NSW 2570, Australia

Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

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

A. Asfor 1, N. Howe1, S. Grazioli 2, G. Wilsden1, A. Ludi1, E. Brocchi 2 , D. King [1], S. Parida 1, T. Tuthill[1]

[1]The Pirbright Institute, Ash Road, Woking, GU24 0NF, UK; 2Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy.

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

POSTER

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

SM. Barani[*1], *K. Mirzaie2, J. Emami2, CJM. Bartels3*

1 Iran Veterinary Organization, Iranian Scientific Society of Large Animal Internal Medicine, Iran; [2] *Iran Veterinary Organization, DVM, Ph.D. Candidate of Epidemiology University of Tehran, Iran;* [3] *Animal Health Works, Bakhuizen, the Netherlands.*

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

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

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

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

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

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

POSTER

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

K. Gorna1, GL. Diop2 , M. Diop2, E. Laloy1, M.M. Lo2, A. Relmy1, A. Romey1, M.T. Seck 2, H.G. Ularamu3, S. Zientara1, S. Blaise-Boisseau1 and L. Bakkali Kassimi1

1 Laboratoire de Santé Animale de Maisons- Alfort, Laboratoire de référence Nationale et OIE pour la Fièvre Aphteuse, UMR Virologie 1161, Université Paris-Est, Anses, Maisons-Alfort, France; 2 Institut sénégalais de recherches agricoles (ISRA) / Laboratoire national de l'élevage et de recherches veterinaires (LNERV) Route du Front de Terre, BP. 2057 Dakar Hann – Sénégal; 3 FMD Research Centre, Nat. Vet. Res. Inst. (NVRI), PMB 01 Vom. Nigeria.

Introduction

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

Materials-methods

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

Results

The 6-plex prototype detected all FMDV strains tested and identified the four serotypes of interest (O/A/SA1/SAT2) without any improper amplification. Using this multiplex protocol, 37 samples from Benin were positive for the 3D target and were correctly serotyped by 6-plex (33) or by 3-plex and simplex RT-PCR (4). 39/40 isolates from Nigeria were properly serotyped using 6-plex (30) or simplex (9). The corresponding field samples as well 39 clinical positive samples from Senegal are under investigation.

Discussion

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

POSTER

A MODIFIED DENDRIMER-RNA VACCINE PLATFORM AGAINST FMD

J.S. Chahal Tiba Biotech LLC

Abstract

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

POSTER

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

M. Chitray[1, 2], P. Opperman1, W. Van Wyngaardt1, J. Fehrsen1, J. Frischmuth3 and F. Maree[1, 2]
1Agricultural Research Council, Onderstepoort Veterinary Research, Vaccines and Diagnostic Development, Private Bag X05, Onderstepoort 0110, South Africa; 2University of Pretoria, Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, Pretoria 0002, South Africa; 3National Bioproducts Institute, Biotechnology division, Private Bag X9043, Pinetown 3600, South Africa.

Introduction

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

Material and methods

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

Results

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

Discussion

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

POSTER

GENETIC CHARACTERIZATION OF THE 2018 FMD VIRUSES IN SOUTH KOREA

B.K. Ku, J.J Nah, S. Ryoo1, T.S. Kim,H.J.Lee, J.W.Lee, B.S.Ha, S.M. Lee, S.M. Jung, M.K Shin, S.H. Wee*
Foot-and-Mouth Disease Division, Animal and Plant Quarantine Agency, 177 Hyeoksin8-ro, Gim-cheon-si, Gyeongsangbuk-do, 39660 Republic of Korea.

Introduction

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

Materials and methods

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

Results

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

Discussion

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

POSTER

INVESTIGATING CROSS REACTIVITY OF SEROLOGICAL ENZYME LINKED IMMUNOSORBENT ASSAYS

A. Morris1, S. Grazioli2, G. Pezzoni, G. Wilsden1, C. Browning1, S. Gubbins1, A. Ludi1, D. King1, E. Brocchi2

1 The Pirbright Institute, Ash Road, Pirbright, Surrey, United Kingdom; 2Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy.

Introduction

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

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

Method and Results

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

Discussion

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

POSTER

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

P. Opperman1, M. Chitray[1, 2], T. Nefefe1, J. Fehrsen1 and F. Maree[1,2]

1Agricultural Research Council, Onderstepoort Veterinary Research, Vaccines and Diagnostic Development, Private Bag X05, Onderstepoort 0110, South Africa; 2University of Pretoria, Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, Pretoria 0002, South Africa.

Introduction

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

Materials and methods

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

Results

This is the first time a recombinant antibody phage display library derived from the immune repertoire of FMD–SAT immune buffalo has been constructed. The total library size was 3.84 x107 cfu. Virus-specific binders will be selected and characterised and their use in the deve- lopment of improved diagnostic assays investigated.

Discussion

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

POSTER

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

HJ. Lyuk, JE. Park, JW. Byun, MY. Park, C.S. Kim, K. Roque, HY. Kim, S.H. Wee, H.M. Pyo
Foot-and-mouth disease Division, Animal and Plant Quarantine Agency, Gimcheon, Gyeong-sang buk-do 39660, Republic of Korea

Introduction

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

Materials and methods

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

Results

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

Conclusion

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

POSTER

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

M.A. Saduakassova[*,1], *A.A. Sultanov1, L.B. Kutumbetov1, J. Wadsworth2, B.A. Wood2, N.J. Knowles2, D.P. King2, K. Bachanek-Bankowska2*

1Kazakh Scientific Research Veterinary Institute, 223 Raimbek Avenue, Almaty, Kazakhstan 050016; 2The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, United Kingdom.

Introduction

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

Methods

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

Results

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

Discussion

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

POSTER

CHARACTERISATION OF BOVINE DENDRITIC CELLS FOLLOWING FMDV INFECTION

A. Yasmin1, K. Moffat1, E. Reid1, B. Charleston1, S. Milling2 and J. Seago1

1The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK; 2 Institute of Infec- tion, Immunity & Inflammation College of Medical, Veterinary & Life Science Glasgow Biomedical Research Centre Room B6-12 The Sir Graeme Davies Building University of Glasgow 120 Universi- ty Place G12 8TA Glasgow.

Introduction

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

Material and methods

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

Results

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

Discussion

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

POSTER

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

J. Maud, K. Sumption, N. Rumich, M. Hovari, R. Nova Chavez, F. Rosso and N. Lyons
European Commission for the Control of Foot-and-Mouth Disease, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00153, Rome, Italy.

Introduction

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

Materials and Methods

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

Appendix 4

Global Status Report for FMD: Tracking the Emergence and Spread of New Viral Lineages

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

D.P. King, V. Mioulet, A. B. Ludi, N. Knowles, B. Wood, A. Gray, B. Thapa, L. Henry, M. Azhar, H. Baker, G. Wilsden, C. Browning, M. Henstock, B. Statham, A. Bin-Tarif, K.Bachanek-Bankowska, J. Wadsworth, A. Di Nardo, DJ. Paton, N. Lyons, A. Morris, D. Wiseman, B. Johns, S. Belgrave and J. Maryan, on behalf of the OIE/FAO FMD Laboratory Network WRLFMD, Vesicular Disease Reference Laboratory, The Pirbright Institute, Ash Road, Pirbright, GU24 0NF, UK

Introduction

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

Discussion

In this presentation we discuss lessons learned to date from the EuFMD training program, with a focus on areas where we have aimed to build capacity to enable effective use of FMD vaccines. We discuss key target audiences identified and the appropriateness of the training methodologies that have been applied. We project forwards, considering new future au- diences for FMD related training and prioritizing critical capacities which will need to built to enable application of new tools for disease control most effectively. Data from the OIE/FAO FMD Laboratory Network (www.foot-and-mouth.org/) are used to monitor the transboundary movements of FMDV, and to provide recommendations about the suitability of vaccine strains that can be used to control outbreaks. In the past twelve months, particular attention has focussed on the emergence of new FMDV lineages into a number of countries in the European neighbourhood. Recent outbreaks in Algeria have been caused by two different FMDV topotypes (A/AFRICA/ G-IV in 2017 and O/EA-3 in 2018). In contrast to previous cases in North Africa due to the O/ME-SA/Ind-2001 lineage originating from South Asia, phylogenetic analyses place West Africa as the source of both of lineages (closest viral sequences are from Nigeria); however, without obvious direct epidemiological connections, we should be cautious in attributing specific sources since there are many countries in West and Central Africa that do not submit samples for analyses. New emerging FMD lineages have also been detected in Israel and Palestine, where O/EA-3 has also been detected, as well as cases in Israel due to the A/ ASIA/G-VII lineage (previously found in Iran, Turkey, Saudi Arabia and Armenia). Elsewhere, key epidemiological events highlighted during 2017-18 by the Network include (i) detection of a new FMDV lineage (within the O/ME-SA topotype) causing outbreaks in Bashkortostan, Russia, (ii) tracking of the spread of the A/ASIA/Sea-97 and O/ME-SA/Ind-2001 lineages in East Asian countries (including South Korea), and (iii) characterisation of outbreaks due to serotype Asia 1 in Myanmar; the first cases due to this serotype anywhere in Southeast Asia since 2008.

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

Presentation available HERE.

Appendix 5
Modelling FMD Vaccine Requirements for Multi-Country FMD Outbreaks in Europe

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

G. Garner[1], M. Hovari[1] and K. Sumption[1]
[1]European Commission for the Control of Foot-and-Mouth Disease – FAO Rome

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

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

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

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

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

Presentation available HERE.

Appendix 6

Evaluating Vaccination Strategies to Control FMD: A Country Comparison Study

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

T. Rawdon [1], G. Garner [2] , R. Sanson [3], M. Stevenson [4], C. Cook[5], C. Birch [5], S. Roche [2], K. Patyk [6] , K. Forde Folle [6], C. Dubé [7] , T. Smylie[8], Z. Yu [9] [1] Diagnostics and Surveillance Services Directorate, Ministry for Primary Industries, Upper Hutt 5140, New Zealand; [2] Epidemiology and One Health Program, Department of Agriculture and Water Resources, Canberra City ACT 2016, Australia; [3] AsureQuality Limited, Palmerston North 4440, New Zealand; [4] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville Victoria 3010, Australia ; [5] Animal and Plant Health Agency (APHA), Weybridge, United Kingdom; [6] Science Technology and Analysis Services, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, Colorado, United States; [7] Animal Health Risk Assessment Unit, Canadian Food Inspection Agency, Ontario, Canada; [8] Foreign Animal Disease Section, Canadian Food Inspection Agency, Ontario, Canada; [9] Readiness and Response Services Directorate, Ministry for Primary Industries, Wellington 6140, New Zealand.

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 7
Understanding Vaccine Demand in the Endemic Setting

APPENDIX 7: UNDERSTANDING VACCINE DEMAND IN THE ENDEMIC SETTING

K. Sumption[1], C. Miller[1], N. Lyons[1,2], C. Bartels[1] and T. Knight-Jones[3]

[1] European Commission for the Control of Foot-and-Mouth Disease – FAO Rome, [2] The Pirbright Institute, UK, [3] Department of Environment, States of Jersey

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 8
Household Perceptions of Risk As Drivers for Adoption of FMD Vaccination

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

A.F. Railey[a], T. Lembo[b], G.H. Palmera, G.M. Shirimac, and T.L. Marsha[b]
[a] Paul G. Allen School for Global Animal Health, Washington State University, USA;
[b]College of Medical, Veterinary and Life Sciences, University of Glasgow, Scotland; [c]Nelson Mandela African Institution of Science and Technology, Tanzania. d School of Economic Sciences, Washington State University, USA.

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

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

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

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

Presentation available HERE.

Appendix 9
Mass FMD Vaccination in Central Myanmar, 2015-2016

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

Y. Qiu[1], R. Abila[1], H.H.Win[2]
[1]OIE Sub-Regional Representation for South-East Asia, c/o DLD, 69/1 Phaya Thai Road, Ratchathewi 10400, Bangkok, Thailand; [2]Livestock Breeding and Veterinary Department, Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar

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

Materials and Methods
18 townships in Central Myanmar were included in the mass vaccination campaign, based on the susceptible animal estimates, animal movement information, and the socioeconomic importance. Three rounds of vaccination were carried out in February and March of 2015, and February 2016, respectively, using a high potency (≥6PD50) vaccine comprising O1/ Manisa and O/3039. Serum samples were collected from a cohort of initially FMD naïve cattle at 0, 30 and 180 days post the 2nd vaccination and tested by LP ELISA to evaluate the magnitude and longevity of the vaccine-induced immunity.

Results
Approximately 210,000 animals in up to 1,100 villages, belonging to more than 54,000 owners, have received 3 injections of FMD vaccine during the 2015-2016 vaccination campaign in Central Myanmar. A vaccination coverage rate of 78% in the project areas was estimated through a participatory approach.
The PVM study shows that at 30 days post the 2nd vaccination, 86% of cattle had protective antibodies (defined as titer>1:100) against a viral strain that is antigenically equivalent to O/3039. The protective percentage declined to 44% at 180 days post the 2nd vaccination. Field surveillance shows that despite extensive outbreaks occurred in Myanmar from August to October of 2015, few outbreaks occurred in the vaccinated villages.

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

Presentation available HERE.

Appendix 10

Assessment of the Risk of Incursion Of Exotic FMD Viruses into Southeast Asia

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

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

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

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

Results
Ten release and six exposure pathways were characterized. Overall, the likelihood of future incursions was assessed as high. The pathways involving imports of live animals and animal products from FMD-endemic countries in neighbouring regions had the highest likelihood. Surprisingly, even FMD-free countries allow these types of imports.
An incursion would likely have a negative impact on animal health and welfare and, in some cases, valuable trading markets would be jeopardised. An exotic FMDV would likely spread extensively within SEA due to intense intra-regional livestock trade, weak surveillance and lack of well-integrated and risk-based national FMD strategies.

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

Key words
Risk assessment, exotic FMD viruses, risk pathways
Acknowledgements
Southeast Asian sub-regional office of the World Organisation for Animal Health and Welfare, Australia's Department of Foreign Affairs and Trade, Australia's Department of Agriculture and Water Resources.

Presentation available HERE.

Appendix 11
Vaccine Banks: Policy Options Evaluated Using the EU Evaluation Framework

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

R. Bergevoet[1]

[1] *Wageningen Economic Research, Postbox 35, 6700 AA Wageningen*

Introduction

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

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

Methods

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

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

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

Results and discussion

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

Presentation available HERE.

Appendix 12
The Challenges of FMD Vaccine Production

APPENDIX 12: THE CHALLENGES OF FMD VACCINE PRODUCTION

P. Hudelet[1]

[1]Head, Technical Service, The Veterinary Public Health Center, Boehringer-Ingelheim. 29 Av Tony Garnier, Lyon, France

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

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

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

Appendix 13

Mutual Registration of Vaccines in East Africa: Progress and Issues for Better Access to Effective FMD Vaccines?

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

Noel M. Aineplan Uganda National Drug Authority

Most countries in the world have a system of assessment and approval of medicines to ensure that they meet high standards of safety, quality and efficacy before they granted a Marketing Authorisation (MA) by a national regulatory authority (NRA) and are authorized for sale. A mutual recognition procedure (MRP) was developed in the East African Community (EAC) for this purpose.
The first step in developing an MRP is to ensure that each one of a group of countries is working to the same standards. This can be achieved by developing harmonized guidelines, which each of the countries agrees to follow. In 2012 an EAC Technical Working Group (TWG) was inaugurated and it has successfully developed a harmonized process for the registration of veterinary vaccines.

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

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

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

Presentation available HERE.

Appendix 14

Identification of Genes Involved in Pathogenicity of FMD Virus Using Two Strains Isolated in Japan with Different Viral Features

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

T. Nishi[1], K. Fukai[1], K. Morioka[1], M. Yamakawa[1]
[1] Exotic Disease Research Station, National Institute of Animal Health, National Agriculture and Food Research Organization, 6-20-1 Josui-honcho, Kodaira, Tokyo 187-0022, Japan

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

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

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

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

Presentation available HERE.

Appendix 15

Complete Genome Sequence Analysis of over 140 FMD Viruses Iso- Lated from Free-Living African Buffalo (Syncerus Caffer) in Zimbabwe

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

J. Wadsworth1, B. Bolt1, L. Ferretti1, E.C. Anderson2, A. Gray1, P. Ribeca1, N.J. Knowles,1*

1 The Pirbright Institute, Pirbright, Woking, Surrey, GU24 0NF, United Kingdom; 2 Department of Veterinary Services, Veterinary Research Laboratory, Causeway, Harare, Zimbabwe (Present address: Rose Cottage, Lower Bearwood, Pembridge, Herefordshire, HR6 9ED, UK).

Introduction

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

Materials and methods

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

Results

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

Conclusions

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

Presentation available HERE.

Appendix 16

Evolution and Competition of Sat Strains During Buffalo Transmission in a Controlled Challenge Experiment

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

K. Scott[*,1], *E. Perez-Martin*[2], *F. Zhang*[2], *L. de Klerk-Lorist*[3], *L. van Schalkwyk*[3], *B. Beechler*[4,5], *A. Jolles*[4,5], *B. Charleston*[2], *F. Maree*[1]

[1] *Transboundary Animal Disease Programme, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 0110, South Africa;* [2]*The Pirbright Institute, Ash Road, Woking, Surrey, GU24 0NF, United Kingdom;* [3]*State Veterinary Services, P.O. Box 12, Skukuza, 1350, South Afri- ca;* [4]*College of Veterinary Medicine, Oregon State University, Corvallis, Oregan, USA;* [5]*Depart- ment of Integrative Biology, Oregon State University, Corvallis, Oregan, USA.*

Introduction

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

Material and methods

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

Results

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

Discussion

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

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

Presentation available HERE.

Appendix 17

FMDV Evolutionary Dynamics within Infected Buffaloes and Its Large-Scale Consequences

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

L. Ferretti, E. Pérez-Martín, A. Di Nardo, L. Lasecka-Dykes, B. Charleston, P. Ribeca The Pirbright Institute, Ash Road, Woking, United Kingdom

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 18

Antibody Responses to the Major Antigenic Sites of FMD Virus Serotype O After Primo-Vaccination, Re-Vaccination and After Natural Exposure

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

J.K. Biswal, R. Ranjan, S. Saravanan, B. Pattnaik*
ICAR-Directorate of Foot-and-mouth Disease, Mukteswar-263138, Nainital, Uttarakhand

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Appendix 19

Exploring Private and Public Sector Rights and Responsibilities in Prevention and Control of FMD: The Case of Right to Access Vaccines by Livestock Keepers

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

B. Vosough Ahmadi[1]
[1]*Land Economy, Environment and Society Research Group, Scotland's Rural College (SRUC), West Mains Road, Edinburgh EH9 3GH, United Kingdom.*

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

Presentation available HERE.

Appendix 20

FMD Research Gap Analysis Workshop 2018

APPENDIX 20: FMD RESEARCH GAP ANALYSIS WORKSHOP 2018

M. Pérez-Filgueira[1,2*]
[1]*Instituto de Virología, CICVyA, INTA, Argentina;* [2]*CONICET, Argentina*

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

Presentation available HERE.

Appendix 21

Gene Signatures Associated with FMDV Infection and Persistence Part I: Persistent FMDV Infection in An Air-Liquid Interface Model Of Bovine Soft Palate

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

S. Hägglund1*, E. Laloy2*, K. Näslund1*, F. Pfaff3, M. Eschbaumer3, A. Romey2, A. Relmy2, H. Huet2, K. Gorna2, M. Beer3, S. Zientara2, L. Bakkali-Kassimi2, S. Blaise-Boisseau2* and J.F. Valarcher1

[1] Host Pathogen Interaction Group, Section of Ruminant Medicine, Dept. of Clinical Science, Swe- dish University of Agricultural Sciences (SLU), Ulls väg 26, 75007 Uppsala, Sweden; [2] Université Paris-Est, Anses, Laboratoire de Santé Animale de Maisons-Alfort, Laboratoire national et OIE de référence pour la Fièvre Aphteuse, UMR Virologie 1161, 14 rue Pierre et Marie Curie, 94700 Maisons-Alfort; [3] Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493, Greifswald-Insel Riems, Germany; [*]Contributed equally to this work.

Introduction

Persistent infection with foot-and-mouth disease virus (FMDV) delays the process to recover a FMDV-free status. In carrier cattle, which harbour FMDV >28 days post infection (DPI), re- plicating virus has been detected in epithelial cells in the nasopharynx and soft palate (SP). To induce viral persistence, FMDV likely suppresses immune responses or changes to escape the- se responses. The aim of this study was the development of a model to be able to characterise gene signatures within FMDV and its target cells. The overall goal was to identify factors that can be used to prevent persistent infections or to improve diagnostics.

An air-liquid interface multilayer model of bovine SP cells was developed and characterised by immunostaining and electron microscopy. After five weeks of culture without further passage, the cells were infected with FMDV O/FRA/1/2001 at an MOI of 0.01 or 1, respectively. The infection was monitored until 28 DPI by virus isolation in cell culture, RT-qPCR, immunofluo- rescence, and immunohistochemistry.

Results

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

Discussion

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

Presentation available HERE.

Appendix 22

Gene Signatures Associated with FMDV Infection and Persistence Part II

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

F. Pfaff[1], S. Hägglund[2], S. Blaise-Boisseau[3], E. Laloy[3], L. Bakkali-Kassimi[3], A. Romey[3], A. Relmy[3], K. Gorna[3], K. Näslund[2], S. Köthe[1], D. Zühlke[4], K. Riedel[4], S. Zientara[3], J.F. Valarcher[2], D. Höper[1], M. Beer[1], M. Eschbaumer[1]

[1]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany; [2]Host Pathogen Interaction Group, Section of Ruminant Medicine, Dept. of Clinical Science, Swedish University of Agricultural Sciences (SLU), Ulls väg 26, 75007 Uppsala, Sweden; [3]Université Paris-Est, Anses, Laboratoire de Santé Animale de Maisons-Alfort, Laboratoire national et OIE de référence pour la Fièvre Aphteuse, UMR Virologie 1161, 14 rue Pierre et Marie Curie, 94700 Maisons-Alfort; [4]Department for Microbial Physiology and Molecular Biology, University of Greifswald, Felix-Haus- dorff-Straße 8, 17489 Greifswald, Germany

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Appendix 23

Transmision of Fmd from Persistently Infected Carrier Cattle to NaïVe Cattle via Transfer of Oropharyngeal Fluid

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

J. Arzt[1], G.J. Belsham[2], L. Lohse[2], A. Bøtner[2], C. Stenfeldt[1,3]

[1]Foreign Animal Disease Research Unit, USDA-ARS, Plum Island Animal Disease Center, Greenport, NY, USA; [2]The National Veterinary Institute, Technical University of Denmark, Lindholm, Kalveha- ve, Denmark; [3]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA

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

Materials and Methods

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

Results

All cattle challenged with OPF (challenge dose determined to 102 TCID50 on LFBK$_{\alpha v\beta 6}$ cells) developed clinical FMD of similar severity as animals that had been infected using a high-ti- ter inoculum. In contrast, pigs exposed via intra-oropharyngeal inoculation of OPF, or by ingestion of nasopharyngeal tissues, did not develop FMD.

Discussion

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

Presentation available HERE.

Appendix 24

Rapid, On Site, Diagnosis of FMD and Safe and Cost-Effective Shipment of Samples Using Lateral Flow Devices for Laboratory Diagnostics

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

L Bakkali Kassimi[1], G.J. Belsham[2], A.N. Bulut[3], K. Gorna[1], C. Hamers[4], P. Hudelet[4], S. Jamal[5], E. Laloy[1], A. Relmy[1], A. Romey[1], H.G. Ularamu[6], S. Zientara[1] and S. Blaise-Boisseau[1]

[1] Laboratoire de Santé Animale de Maisons- Alfort, Laboratoire de référence Nationale et OIE pour la Fièvre Aphteuse, UMR Virologie 1161, Université Paris-Est, Anses, Maisons-Alfort, France; [2] DTU National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark (DTU- Vet); [3] SAP/FMD Institute, Dumlupinar Bulvard,35, 06510 , Ankara, Turkey; [4] The Veterinary Public Health Center, Boehringer Ingelheim Animal Health, 29 Avenue Tony Garnier, 69007 Lyon France; [5] Department of Biotechnology, University of Malakand (UM), Chakdara, Pakistan; [6] FMD Research Centre, Nat. Vet. Res. Inst. (NVRI), PMB 01 Vom, Nigeria

Introduction

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

Materials-methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 25

The Utility of Pooled Milk for Fmd Surveillance in Nakuru County, Kenya

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

B. Armson[1,2], V.L. Fowler[1], K. Bachanek-Bankowska[1], V. Mioulet[1], P. Kitala[3], D. Machira[3], B. Sanz-Bernado[1], A. Di Nardo[1], E. Chepkwony[4], D.P. King[1], N.A. Lyons[1,5]*

[1] The Pirbright Institute, Pirbright, UK; [2] Institute of Biodiversity, Animal Health and Compara- tive Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, UK; [3] The University of Nairobi, Faculty of Veterinary Medicine, Nairobi, Kenya; [4] Foot-and-Mouth Disease Laboratory, Ministry of Agriculture and Irrigation, Embakasi, Nairobi, Kenya; [5] European Commis- sion for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture Organisation of the United Nations, Rome, Italy

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 26

Evaluation of Environmental Sampling As a Low Technology Method for Surveillance of FMDV in an Endemic Area

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

C. Colenutt[1], E. Brown[1], N. Nelson[2], J. Wadsworth[1], J. Maud[3], B. Adhikari[3,4], S.c. Kafle[5], M. Upadhyaya[6], S.K. Pandey[7], D.J. Paton[1], K. Sumption[3], S. Gubbins[1]

[1] The Pirbright Institute, Ash Road, Pirbright, Surrey, GU24 0NF, UK; [2]The Met Office, FitzRoy Road, Exeter, Devon, EX1 3PB, UK; [3] European Commission for the Control of Foot-and-Mouth disease (EuFMD), Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy; [4] Food and Agriculture Organisation of the United Nations, Nepal Country Office; 5 National FMD and TADs Laboratory, Department of Livestock Services, Ministry of Livestock Development, Nepal; [6]Veterinary Epidemiology Centre, Department of Livestock Services, Ministry of Livestock Development, Nepal; 7Directorate of Animal Health, Department of Livestock Services, Ministry of Livestock Development, Nepal.

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

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

Presentation available HERE.

Appendix 27
Environmental Sampling: A Surveillance Tool for FMDV in Marketplaces

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

E. Brown[1], C. Colenutt[1], J. Maud[2], D. Paton[1], B. Adhikari[3], N. Nelson[4], M. Mahapatra[1], S. Pari- da[1], S. Chapagain Kafle[5], M. Upadhyaya[6], S. Kafle Pandey[7], K. Sumption[2], S. Gubbins[1]
The Pirbright Institute, Ash Road, Pirbright, Surrey, GU24 0NF. United Kingdom European Commission for the Control of Foot-and-Mouth disease (EuFMD), Food and Agricul- ture Organisation of the United Nations (FAO), Rome, Italy; Food and Agriculture Organization of the United Nations Representation in Nepal, United Nations Building, Pulchowk, Lalitpur, Kathmandu, Nepal; Met Office, FitzRoy Road, Exeter, Devon, EX1 3PB. United Kingdom; FMD and TADs Laboratory, Department of Livestock Services, Ministry of Livestock Development, Nepal; Veterinary Epidemiology Centre, Department of Livestock Services, Ministry of Lives- tock Development, Nepal; Directorate of Animal Health, Department of Livestock Services, Ministry of Livestock Development, Nepal

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

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

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

Discussion
Movement of goats through markets has been implicated in the spread of FMDV in Nepal. Detection of FMDV RNA in environmental swabs demonstrates FMDV infected goats have been present at the Khasibazar goat market, although the source of virus cannot be linked to specific animals.
Environmental sampling provides a simple, non-invasive method of detecting FMDV at mar- kets, where clinical signs in animals may not be apparent and where individual sampling is not practical. Environmental sampling data could be used to supplement epidemiological data to identify and predict patterns of spread and highlight potential transmission risks in endemic and FMD- free countries.

Presentation available HERE.

Appendix 28
Effective in Silico Sequence-Based Prediction of FMDV Vaccine Matching

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

M. Mahapatra[1], S. Mahendran[2], L. Ferretti[1], S. Parida[1], P. Ribeca[1]
[1] The Pirbright Institute, Ash Road, Woking, United Kingdom; [2] School of Veterinary Sciences, University of Surrey, Guildford, United Kingdom

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

Materials and methods
A large dataset of titres obtained from neutralisation assays on different FMDV types was used to train machine-learning algorithms. Quantities derived from whole-capsid sequen- cing information (including biochemical and structural indicators) were used to inform the prediction.
The predictors are able to reproduce observed titres with great accuracy (much greater than methods previously published in the literature). Accuracy appears to be largely independent of the FMDV type and vaccine used.

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

Presentation available HERE.

Appendix 29
In-Vitro Correlates of Heterologous Protection Using Avidity and Igg-Subtyping Elisas

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

A. Capozzo[1], R. Reeve[2], D. Paton[3], A. Ludi[3]
[1]Instituto Nacional de Tecnologia Agropecuaria (INTA). Institute of Virology. Buenos Aires., Ar- gentina; [2]Boyd Orr Centre for Population and Ecosystem Health, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK; [3]The Pirbright Institute, Ash Road, Woking, Surrey, GU24 0NF, UK

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

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

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

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

Presentation available HERE.

Appendix 30
Assessment of Existing and Future Vaccine Selection Techniques –Moving Forward

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

R. Reeve[1,], D. Paton[2], A. Ludi[2], A. Capozzo[3]*
[1] Boyd Orr Centre for Population and Ecosystem Health, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK; [2] World Reference Laboratory for foot-and-mouth disease, The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 0NF, UK; [3] Instituto Nacional de Tecnologia Agropecuaria (INTA) Institute of Virology. Buenos Aires, Argentina.

Introduction

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

Materials and Methods

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

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

Presentation available HERE.

Appendix 31
Reinforcement Learning for Context-Dependent Control of Emergency Outbreaks of FMD

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

W.M. Probert[1], C.J. Fonnesbeck[2], M.J Keeling[3], M.C. Runge[4], M.J. Tildesley[3], K.Shea5,6, M.J. Ferrari[5,6]
[1] Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, Nuffield Department of Medicine, University of Oxford, Oxford, UK; [2] Department of Biostatistics, Vanderbilt University, Nashville, Tennessee, USA; [3] Department of Biological Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK; [4] US Geological Survey, Patuxent Wildlife Research Center, 12100 Beech Forest Rd, Laurel, Maryland, USA; [5] Center for Infectious Disease Dynamics, Department of Biology, Eberly College of Science, The Pennsylvania State University, University Park, Pennsyl- vania, USA; [6] Department of Biology and Intercollege Graduate Degree Program in Ecology, 208 Mueller Laboratory, The Pennsylvania State University, University Park, Pennsylvania, USA

Introduction

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

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 32

Investigating The Benefits of an Adaptive Management Approach Involving Emergency Vaccination Using Simulated FMD Outbreaks in New Zealand

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

R. Sanson[*,1], *Z. Yu*[2], *T. Rawdon*[2], *M. van Andel*[2]
[1] *AsureQuality Limited, Palmerston North, New Zealand;* [2] *Ministry for Primary Industries, Welling- ton, New Zealand*

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

Materials and methods
InterSpread Plus was used to simulate 5000 FMD outbreaks in New Zealand. Four response strategies were evaluated: Stamping-out only (SO) and SO plus vaccination initiated by three mechanisms: randomly started between days 11-35 of the response (VAC); started on day 21 (VACf); or deployed if the number of infected premises (IPs) or the estimated dissemination rates exceeded certain threshold values between days 11-35 (TRV).
Parameters that were varied randomly included the number of personnel, whether airborne spread occurred, farm classes vaccinated and surveillance visits per person per day. Outputs, including time to first detection, total IPs, outbreak duration, total man-days, vaccine doses used and if and when the EDI trigger was fired, were collected for each iteration. Data were analysed using contingency table analyses, univariable tests and multivariable analyses.
Simulations were stopped if 90 days had elapsed without IPs or when the outbreak reached 365 days.

Results
IPs were highly right skewed (median = 10, interquartile range (IQR) = 3 – 34, range = 0 – 50,582); as was duration (median = 20 days, IQR = 7 – 39, range = 0 – 360). Logistic regres- sion showed that the number of veterinarians available, the response strategy and the EDI trigger firing were associated with large or long epidemics.
Vaccination reduced the number of IPs and duration compared to SO, and the TRV strategy performed the best in terms of the fewest IPs and the shortest duration.

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

Presentation available HERE.

Appendix 33

Evaluating Optimal Control Strategies for FMD with the Us Disease Outbreak Simulation

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

S. Sellman[1], L.M. Beck-Johnson[2], C. Hallman[2], T. Lindström[1], R.S. Miller[3], D. Murrieta[2], K. Portac- ci[3], M.J. Tildesley[4], K. Tsao[2], C.T. Webb[2], U. Wennergren[1].
[1]Department of Physics, Chemistry and Biology, Division of Theoretical Biology, Linköping Uni- versity, Sweden; [2]Department of Biology, Colorado State University, Fort Collins, CO, USA; [3]USDA APHIS Veterinary Services, Center for Epidemiology and Animal Health, Fort Collins, CO, United States; [4]Mathematics Institute, University of Warwick, Coventry, UK.

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

Materials and Methods
The US Disease Outbreak Simulation (USDOS) is a data-driven, spatially explicit disease spread model simulating continental scale outbreaks. It was developed for the U.S. livestock demo- graphic in a collaborative effort between Colorado State, Warwick, and Linköping Universities with support from the U.S. Department of Homeland Security Science and Technology Direc- torate.
Using USDOS, the effect of spatial clustering of premises on FMD outbreaks was analyzed analytically and through stochastic simulations in two sets of different spatial distributions of the U.S. cattle population—one random and one where the premises' locations were simula- ted to provide more realistic distributions.
Control strategies based on combinations of movement bans, culling and vaccination strate- gies were evaluated for simulated FMD outbreaks. The outbreaks were initiated in all parts of the U.S. and included realistic constraints on amount of time and resources that could be allocated regionally.

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

Presentation available HERE.

Appendix 34

Between-Herd Transmission Dynamics of FMD in Kenya Rangelands

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

K. VanderWaal[*], *V. Obanda*[2], *M. Alkhamis*[1], *A. Sangula*[3], *F. Gakuya*[2], *E. Hartwig*[4], *S. Pauszek*[4], *G. Smoliga*[4], *B. Brito*[4], *A. Perez*[1], *J. Arzt*[4], *G. Omondi*[1]
[1]*University of Minnesota College of Veterinary Medicine, 1365 Gortner Ave, 55108, St. Paul, MN USA;* [2]*Kenya Wildlife Service, 40241-00100, Off Langata Road, Nairobi, Kenya;* [3]*Foot-and-Mouth Disease Laboratory, P.O. Box 18021-00500 Enterprise Road, Embakasi, Kenya;* [4]*Plum Island Animal Disease Center, 40550 Route 25, 11957, Orient Point, NY USA*

Introduction

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

Materials and Methods

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

Results and Discussion

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

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

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

Presentation available HERE.

Appendix 35

Using Networks of Livestock Mobility to Improve Control of Endemic FMD in Northern Tanzania

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

D. Ekwem[1,2], J. Enright[3], T. Morrison1, J. Buza[2], G. Shirima[2], G. Hopcraft[1], R. Reeve[1], T. Lembo[1]
[1]Boyd Orr Centre for Population and Ecosystem Health, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Science, University of Glas- gow, UK; [2]Nelson Mandela African Institution of Science and Technology, School of Life Sciences and Bioengineering, Arusha, Tanzania; [3]Global Academy of Agriculture and Food Security, Univer- sity of Edinburgh, UK.

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

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

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

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

Presentation available HERE.

Appendix 36
Livestock Mobility in West Africa: Network Analysis and Applications

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

A. Apolloni[1], R. Lancelot[1], C. Coste[1]

[1]CIRAD, UMR ASTRE, Campus International de Baillarguet , Montpellier, France

Introduction

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

Materials and Methods

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

Results

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

Discussion

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

Appendix 37

Qualitative Aspects of the Immune Responses Related to Protection Against FMDV Challenge in Cattle

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

S. Di Giacomo[1], F. Barrionuevo[1,2], D. Bucafusco[1,2], M.C. Miraglia[1,2], J.M. Schammas[1], A. Ayude[1], A.V Capozzo[1,2], M. Pérez-Filgueira[1,2]*
[1]Instituto de Virología, CICVyA, INTA, Argentina. 2CONICET, Argentina

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

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

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

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

Presentation available HERE.

Appendix 38

Secure Beef Supply in The U.S. Planning for Control and Continuity of Business in an FMD Outbreak

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

M.W. Sanderson[1], C.J. Hanthorn[1], D.A. Bickett-Weddle[2], R.D. Dewell[2], M.J. Lee[2], J.A. Roth[2]
[1] Center for Outcomes Research and Epidemiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; [2] Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University, Ames, IA

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

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

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

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

Presentation available HERE.

Appendix 39

Modelling the Impact of Regional Movement Control Policies for FMD Outbreaks in Disease Free Countries

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

M.J. Tildesley[1], S. Brand[1], N. Bradbury[1], E. Brooks Pollock[2], M. Werkman[3] & M.J. Keeling[1]
[1] Zeeman Institute for Systems Biology and Infectious Disease Epidemiology, School of Life Scien- ces and Mathematics Institute, University of Warwick, Gibbet Hill, Coventry, CV4 9YG, United Kingdom; [2] Bristol Veterinary School, University of Bristol, Langford House, Langford, Bristol, BS40 5DU, United Kingdom; [3] Department of Infectious Disease Epidemiology, St Mary's Campus, Imperial College London, United Kingdom

Introduction

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

Methods and Results

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

Discussion

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

Presentation available HERE.

Appendix 40

Modelling Management Strategies for Vaccinated Animals After an Outbeak of FMD and the Impact on Return to Trade

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

R.A. Bradhurst[1], M.G. Garner[2,3], I.E. East[2], C.E. Death[2], A.J. Dodd[1] and T. Kompas[1]*
Centre of Excellence for Biosecurity Risk Analysis, University of Melbourne, Parkville, VIC, Austra- lia; Epidemiology and One Health Program, Animal Health Policy Branch, Department of Agri- culture and Water Resources, Canberra, ACT, Australia; European Commission for the Control of Foot-and-Mouth Disease, FAO, Rome, Italy

Introduction
An incursion of foot-and-mouth disease (FMD) in a previously FMD-free country can cause significant economic damage from the immediate and prolonged closure of FMD-sensitive markets. Whilst emergency vaccination may help with disease containment, the presence of vaccinated animals complicates post-outbreak surveillance and the recovery of FMD-free status.
We present enhancements to the Australian Animal Disease Spread Model (AADIS) that allow comparisons of post-outbreak management strategies for securing proof-of-freedom from FMDand return to trade. These include: surveillance of previously infected areas (according to configurable sampling regimes), in order to obtain statistical confidence of freedom from FMD, and economic assessment of retaining vaccinated animals in the population compared to remo- ving them to waste or for salvage.

Materials and methods
A case study is presented that compares vaccinate-and-retain, vaccinate-and-remove-to-was- te and vaccinate-and-remove-for-salvage strategies, and their impact on the recovery of FMDsensitive markets (per OIE guidelines).

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

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

Presentation available HERE.

Appendix 41

Vaccine Efficacy of FMD Virus-Like Particles Produced by the Baculovirus Expression System

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

E. van den Born[1], S. Loureiro[2], C. Porta[3], E. Perez[3], E. Fry[4], H. Hoenemann[1], A.J. Melsió[1], R. Segers[1], D. Stuart[4], I. Jones[2], B. Charleston[3]
[1] R&D Swine Biologicals, MSD Animal Health, Boxmeer, The Netherlands; [2] Animal and Microbial Sciences, University of Reading, Whiteknights, Reading, United Kingdom; [3] The Pirbright Insti- tute, Pirbright, Woking, United Kingdom; [4] Division of Structural Biology, The Henry Wellcome Building for Genomic Medicine, Headington, University of Oxford, Oxford, United Kingdom.

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

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

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

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

B. Charleston - Presentation available HERE

E. van den Born – Presentation available HERE.

Appendix 42
A Current Perspective on Adenovirus 5-Vectored FMD Vaccines

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

T. de los Santos, PhD[1]
[1] *Plum Island Animal Disease Center, Agricultural Research Service, North East Area, U.S. Depart- ment of Agriculture, Orient, NY, USA.*

Introduction
Over the years, viral vectors have been used to deliver FMDV structural proteins, with the aim of synthesizing virus-like particles (VLPs) in infected cells, potentially inducing both, humoral and cell-mediated immunity. Numerous groups have focused on using different viral vectors, including vaccinia virus, fowlpox virus, attenuated pseudorabies virus (PRV) or "single cycle" Semliki forest virus (SFV). Most of these FMD vaccines were tested in a limited number of livestock species, usually requiring vaccination boosts and offering partial protection in swine and/or cattle.
To date, the most successful strategy to induce protection against FMD in livestock, as an alternative to the inactivated whole FMDV vaccine, is the use of a recombinant-replication- defective human adenovirus type 5 coding for FMDV capsid proteins and also the 3Cpro that allows for the in vivo assembly of VLPs (Ad5-FMD). These vaccines protect swine and cattle from clinical disease as early as 5 days post vaccination. In fact, an Ad5-FMD serotype A24 vaccine has been recently granted licensure for emergency use in cattle in USA. The main advantages of this vaccine platform are: i) it does not require a high containment facility for production; ii) it has intrinsic DIVA capabilities; iii) it does not require the adaptation of field strains to vaccine production in cell culture; and iv) it is genetically stable. However, the Ad5-FMD technology requires further industrial development for large scale production and broad use internationally, especially in developing countries. In this section we will discuss recent developments aimed at improving vaccine potency, vector stability, route of delivery, all strategies focused on achieving fast protection with one vaccine dose, at the lowest cost possible and with not significant side effects. Furthermore, we will discuss current challenges and possible solutions aimed at improving Ad5-FMD vaccine performance.

Presentation available HERE.

Appendix 43

FMD-LL3B3D Vaccine Platform: Safe, Highly Potent, Fully Diva Compatible, Inactivated FMDV Vaccines

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

J. Hardham[1], A. Urniza[2], C. Murray[3], S. Dixon[1], M. Huether[4], N. Martinon[1], I. Correas[1], N. Oien[1], K. Sellam[5], J. Stegner[1], J. Thompson[1], P. Dominowski[1], C. Gay[6], S. Uddowla[7], J. Pacheco[7], A. Kloc[7], P. Krug[7], L.L. Rodriguez[7], and E. Rieder[7]

[1] Zoetis Inc, Kalamazoo, MI, US; [2]Olot, Spain, [3]Walton Oaks, UK; [4]Lincoln, NE, US; [5]Parsippany, NJ, US; [6]Office of National Programs, USDA-ARS, Beltsville, MD, US; [7]Plum Island Animal Disease Center, ARS, USDA, Greenport NY, US

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 44

An Overview of Reverse Genetic Approaches to Enhanced FMD Vaccines in Africa

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

F. Maree[*1,2], *K. Scott*[1], *P. Opperman*[1], *M. Chitray*[1,2]

[1]*Agricultural Research Council, Onderstepoort Veterinary Research, Vaccines and Diagnostic Development, Private Bag X05, Onderstepoort 0110, South Africa.;* [2]*University of Pretoria, Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, Pretoria 0002, South Africa.*

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 45

Rational Design of Attenuated FMDV Vaccines by Elevation of –Cpg- And –Upa- Dinucleotide Frequencies

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

M.D. Ryan
Biomedical Sciences Research Complex, School of Biology, University of St. Andrews, North Haugh, St. Andrews KY16 9ST, Fife, Scotland, UK.

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

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

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

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

Presentation available HERE.

Appendix 46

Field Trial to Estimate the Effectiveness of the Vaccination Program Implemented in the Maghreb Region

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

E. Brocchi[1], N. Abouchoaib[2], F. El Mellouli[2], M. Bugnetti[1], F. Rosso[3], A. Ripani[4], G. Pezzoni[1], S. Grazioli[1]
[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy; [2] Office National de Sécurité Sanitaire des Produits Alimentaires (ONSSA), Laboratoire Régio- nal d'Analyses et de Recherches, Casablanca, Morocco; [3] European Commission for the control of Foot-and-Mouth disease (EuFMD), FAO Rome; [4] OIE Sub-Regional Representation for North Africa, Programme officer

Introduction
Routine or emergency vaccination are strategic tools to control FMD. Preliminary estimates of vaccine effectiveness can be obtained by confined field studies, contributing to optimizing control programs.
Field trials to evaluate effectiveness of FMD vaccines currently used in the Maghreb region have been designed; here we report the results obtained from the trial conducted in Morocco.

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

Results
In previously vaccinated cattle, vaccination elicited a strong and fast increase (up to 10X, 5 DPV) of neutralizing antibodies, suggestive of protective immunity, with overlapping titres against the two type O vaccine strains and the field virus O-ALG/1/2014 (lineage O-Ind2001); antibodies had further increased at 30 DPV, reaching average titres of ≥ 3 Log10. Analogous trend was observed for type A, though titres to the vaccine strain were 3-fold higher than those against the field virus A/Algeria/1/2017 (despite both belong to the same lineage A/ Africa/G-IV).
All naïve calves seroconverted from negative to positive after vaccination, but the level of VN antibodies remained lower than in the boost-vaccinated group. The best immune response was observed against the vaccine strain A/Eritrea-98, with 95% animals overcoming the pre- sumed protective threshold, whilst only about 50% achieved sufficient immunity against the other FMDV strains tested.
ELISA provided results consistent with VNT for boost-vaccinated cattle, whilst it was less sen- sitive to detect antibodies in prime-vaccinated calves.

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

Presentation available HERE.

Appendix 47

Modelling the Impact of Farming Practices upon Vaccine Effectiveness in Endemic Settings – A Case Study in Kenya

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

S. Cant[1], A. Holmes[1], B. Miller[1], E. Southall[1], X. Xi[1], E.C. Chepkwony[2], A.K. Sangula[2], N.A. Lyons[3,4] & M.J. Tildesley[5]

[1] EPSRC and MRC Centre for Doctoral Training for Mathematics in Real World Systems, University of Warwick, Gibbet Hill, Coventry, UK ; [2] Foot-and-Mouth Disease Laboratory, Embakasi, Nairobi, Kenya; [3] The Pirbright Institute, Ash Road, Pirbright, Woking, UK; [4] European Commission for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture Organization of the United Nations, Rome, Italy; [5] Zeeman Institute for Systems Biology and Infectious Disease Epidemiology, School of Life Sciences and Mathema- tics Institute, University of Warwick, Gibbet Hill, Coventry, UK.

Introduction

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

Methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 48

Genetic Characterization of FMDV Responsible for Outbreaks in Nigeria During 2016: Resurgence of the Novel Fmd- Sat1 Topotype

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

D.O. Ehizibolo[1], I. Fish[2], B. Brito[3], S. Pauszek[2], C. Stenfeldt[2], M. Bertram[2], G.H. Ularamu[1], Y.S. Wungak[1], D.D. Lazarus[1], A.G. Ardo[4], C.I. Nwosuh[1], and J. Arzt[2]

FMD Laboratory, Viral Research Division, National Veterinary Research Institute, Vom, Nigeria; Foreign Animal Disease Research Unit, ARS/USDA-Plum Island Animal Disease Center, 40550 Route 25, 11957, Orient Point, NY USA; The ithree institute, University of Technology Sydney, 15 Broadway, Ultimo NSW 2007, Australia; Extension Unit, National Veterinary Research Institute, Vom, Nigeria.

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Appendix 49

Serological and Molecular Epidemiology of FMD Viruses in Agro-Pastoralist Livestock Herds in the Kachia Grazing Reserve, Nigeria

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

D.O. Ehizibolo[1], A.R. De Vleeschauwer[2], A. Haegeman[2], D. Lefebvre[2], C.I. Nwosuh[1], J.U. Umoh[3], E.C. Okolocha[3], H.M. Kazeem[4], S. Van Borm[5], K. De Clercq[2]

[1]FMD Laboratory, Viral Research Division, National Veterinary Research Institute (NVRI), Vom, Nigeria; [2]Unit Exotic Viruses and Particular Diseases, Sciensano, Brussels, Belgium; [3]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; [4]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; [5] Platform Biotechnology and Bioinformatics, Sciensano, Brussels, Belgium

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Appendix 50
Complex Concomitance of FMDV Strains in Nigeria

APPENDIX 50: COMPLEX CONCOMITANCE OF FMDV STRAINS IN NIGERIA

H.G. Ularamu[1], D. Lefebvre[2,], A. Haegeman[2], Y.S. Wungak[1], D.O. Ehizibolo[1], D.D. Lazarus[1], A. De Vleeschauwer[2], K. De Clercq[2]*

[1] FMD Laboratory, Viral Research Division, National Veterinary Research Institute (NVRI), Vom, Nigeria; [2] Service for Exotic Viruses and Particular Diseases, Department of Infectious Diseases in Animals, Sciensano, Groeselenberg 99, 1180 Brussels, Belgium

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Appendix 51
Foot-And-Mouth Disease in Small Ruminants and in Wildlife In Northern Nigeria

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

Y.J. Atuman[1], D. Lefebvre[2,], H.G. Ularamu[1], Y.S. Wungak[1], A. De Vleeschauwer[2], K. De Clercq[2]*
[1] FMD Laboratory, Viral Research Division, National Veterinary Research Institute (NVRI), Vom, Nigeria; [2] Service for Exotic Viruses and Particular Diseases, Department of Infectious Diseases in Animals, Sciensano, Groeselenberg 99, 1180 Brussels, Belgium

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

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

Results
In sheep and goats 21.7% of the samples tested positive whereas 78.3% tested negative. Of the positive samples, 50.8% reacted with SP and NSP, 21.5% reacted with SP only and 27.7% with NSP only. Of the samples reacting with SP, 17.0% was mono-specific for serotype O, 23.4% mono-specific for A, 31.9% mono-specific for SAT2 and 27.7% reacted with 2 or 3 of these serotypes.
In wildlife, 44.7% of the samples tested positive whereas 55.3% tested negative. Of the po- sitive samples, 76.5% reacted with SP and NSP, 11.8% reacted with SP only and 11.8% with NSP only. Of the samples reacting with SP, 6.7% was mono-specific for serotype O, 40.0% mono-specific for A and 53.3% reacted with 2 or 3 serotypes (O, A and SAT2).

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

Presentation available HERE.

Appendix 52

Serotyping Seroprevalence of FMD in Cattle from Uganda Surveillance 2014-2018

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

K. Scott[*,1], *F. Mwiine* [3,4], *O. Sylvester*[4], *J. Lutwaama*[5], *M. Chitray*[1], *K. van der Waal*[6], *E. Rieder*[7], *F. Maree*[1,2]
[1]*Vaccine and Diagnostic Development Programme, Transboundary Animal Diseases, Onderste- poort Veterinary Institute, Agricultural Research Council, Private Bag X05, Onderstepoort, 0110, South Africa;* [2]*Department of Microbiology and Plant Pathology, Faculty of Agricultural and Natural Sciences, University of Pretoria, Pretoria 0002, South Africa;* [3]*Ministry of Agriculture, Animal Industry and Fisheries, Livestock Health and Entomology, Uganda;* [4]*College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, Kampala, Uganda;* [5]*Department of Emerging and Re-emerging diseases, Uganda Virus Research Institute, Entebbe, Uganda.* [6]*University of Minnesota Twin Cities, Veterinary Population Medicine, Minnesota, USA;* [7]*ARS-USDA, Plum Island Animal Disease Center, Agricultural Research Service, New York, USA.*

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

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

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

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

Presentation available HERE.

Appendix 53
Foot-And-Mouth Disease in Burundi

APPENDIX 53: FOOT-AND-MOUTH DISEASE IN BURUNDI

A.I. Estevez Garcia[1], D. Lefebvre[1], L. Nyabongo[2], A. Haegeman[1], C. Nkundwanayo[2], A. De Vleeschauwer[1], D. Ntakirutimana[2], I. De Leeuw[1], D. Nsanganiyumwami[2], T. van den Berg[1], A. Niyokwishimira[2], K. De Clercq[1,]*
[1] Service for Exotic Viruses and Particular Diseases, Department of Infectious Diseases in Animals, Sciensano, Groeselenberg 99, 1180 Brussels, Belgium; [2] Laboratoire National Vétérinaire (LNV), Bujumbura, Burundi.

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

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

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

Discussion
Serological data suggests the presence of FMDV st O, A, SAT2 and perhaps SAT1 in Burundi. Seroprevalence for C and SAT3 was around 20%, presumably due to cross-reactivity. This % of cross-reactivity is comparable to previous data in Nigeria (Ehizibolo et al., 2017).
Data demonstrates that SAT2/IV, previously reported in Tanzania (EuFMD Global Monthly Reports), is widespread in Burundi, supporting the hypothesis of introduction by cattle impor- ted from Tanzania. A/Africa/G-I, previously reported in Tanzania and D.R.C. (EuFMD Global Monthly Reports), was only isolated in the most north-western province, bordering D.R.C. and Rwanda, suggesting another source of FMD.

Presentation avaliable HERE.

Appendix 54

Does the African Buffalo Really Spread FMD? A Sero-Survey of FMD in Cattle Around Mana Pools Conservation Park of Northern Zimbabwe

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

W. Chikurunhe[1], G. Matope[2], D. Pfukenyi[2], P. Tshabalala[3], M. de Garine Wichatitsky[2, 4, 5]
[1]Presenting author: Department of Veterinary Services, Zimbabwe, 1327 Artherstone Road, Bin- dura, Zimbabwe; [2] University of Zimbabwe, Harare, Zimbabwe; [3] Veterinary Research Laboratories, Harare; [4] CIRAD, UMR-ASTRE, Montpellier, France; [5] Kasetsart University, Bangkok, Thailand

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

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

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

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

Presentation available HERE.

Appendix 55
Control Methods of FMD in Benin by Trial Vaccination and Medi-Cinal Plants

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

E. Houndje[1], Y. Akpo[2], C. Ogni[1], N. Noudèkè, A. Youssao[1], G. Aplogan[2], S. Farougou[1], M. Kpodekon[1]
[1]University of Abomey-Calavi, EPAC, 01 BP 2009 Cotonou, Benin; [2]Animal Husbandry, Benin.

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

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

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

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

Appendix 56

Molecular Characterisation of FMDV Detected During 2015 - 2018 in Tanzania: Insights for Virus Diversity and Evolution In Africa

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

C.J. Kasanga[1], S. Kandusi[1], A. Msomi[1], R. Juma[1], H. Mpete[1], R. Sallu[2], F. Mramba[2], N.J. Knowles3, J. Wadsworth[3], V. Mioulet[3], D. Paton[3], P.N. Wambura[1], M.M. Rweyemamu1 and D.P. King[2]

[1] Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P. O. Box 3019, Morogoro, Tanzania; [2]Tanzania Veterinary Laboratory Agency, Ministry of Lives- tock and Fisheries, Dar-es-Salaam, Tanzania; [3] The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK.

Introduction

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

Methods

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

Results

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

Discussion

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

Acknowledgement

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

Appendix 57
FMD Surveillance and Control in Mali

APPENDIX 57: FMD SURVEILLANCE AND CONTROL IN MALI

Dr. Abdoulaye Diaouré VSF-international

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

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

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

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

Key words

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

Presentation available HERE.

Appendix 58

A Gvii-2015, A New High Potency Vaccine With Broad Protection Against A/Asia/G-Vii Threat

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

B. van Schaijk[1], MF. Pollet-Ogier[2], L. Mouton[2], F. Fraisse[2], A. Dekker[3], P. Hudelet[2], J. Coco-Martin[1], B. Thenoz[2], H. Gaude[2]

[1] Boehringer Ingelheim Animal Health - Lelystad, The Netherlands; [2] Boehringer Ingelheim Animal Health - Lyon, France; [3] Wageningen BioVeterinary Research - Lelystad, The Netherlands

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 59
Intradermal Application of FMD Vaccines for Pigs

APPENDIX 59: INTRADERMAL APPLICATION OF FMD VACCINES FOR PIGS

J. Horsington[1], E. van den Born[2], G. Paul[3], N. Singanallur[1], W. Vosloo[1]

[1]CSIRO-Australian Animal Health Laboratory, Private Bag 24, Geelong, Victoria 3220, Australia; [2]R&D Swine Biologicals, MSD Animal Health, Boxmeer, The Netherlands;[3]R&D Swine Biologicals, MSD Animal Health, Cologne, Germany

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Appendix 60

Efficacy of A/MAY/97 FMDV Vaccine Against Heterologous Cha-Llenge With a Field Virus from the Emerging A/ASIA/G-VII Lineage in Cattle

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

P.L. Eblé[1], A.B. Ludi[2], B. Sanz-Bernardo[2], D.P. King[2], N.B Singanallur[3], W. Vosloo[3], A. Dekker[1]*
[1] Wageningen Bioveterinary Research, Wageningen University & Research, Lelystad, The Netherlands; [2] The Pirbright Institute, Ash Road, Pirbright, Surrey GU24 0NF, United Kingdom; [3] Australian Animal Health Laboratory, CSIRO-Health & Biosecurity, 5 Portarlington Road, Geelong, 3220, Australia

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

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

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

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

Presentation available HERE.

Appendix 61

A Simple Universal Test to Quantitate 146s Antigen During Pro- Duction of FMD Vaccines

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

A. Asfor[1], N. Howe[1], S. Grazioli[2], E. Brocchi[2] and T. Tuthill[1]
1The Pirbright Institute, Ash Road, Pirbright UK; 2Istituto Zooprofilattico Sperimentale della Lom- bardia e dell'Emilia Romagna, Brescia, Italy

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

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

Results
The monoclonal antibody reacted with virus of all serotypes. The sandwich ELISA has specifi- city for 146S of all serotypes tested.
Discussion
In summary, we have developed a pan serotypic detection system specific for 146S particles. This has potential to be further validated as a high throughput test for quantitation of FMDV 146S particles during vaccine manufacture and quality control.

Presentation available HERE.

Appendix 62
Improving the Duration of Immunity for FMD Vaccines

APPENDIX 62: IMPROVING THE DURATION OF IMMUNITY FOR FMD VACCINES

S Parida[*1], *K Lloyd- Jones*[1], *M Mahapatra*[1], *R Herbert*[1], *K Parekh*[1], *A Babu*[1], *D J Paton*[1], *G Taylor*[1], *R Lingala*[2], *M Madhanmohan*[2] , *S B Nagendrakumar*[2], *C Rajnathan*[2], *A Milicic*[3] *and V A Srinivasan2.*
1The Pirbright Institute, Ash Road, Woking, Surrey, UK, GU240NF; 2 Indian Immunologicals Limi- ted, Gachibowli, Hyderabad, India- 500 032; [3] *Jenner Institute, University of Oxford, Oxford, UK, OX3 7DQ.*

Introduction

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

Materials and methods

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

Results and Discussion

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

Presentation available HERE.

Appendix 63

The Use of Reverse Genetics to Facilitate the Growth of FMDV for the Production of Vaccines

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

S. Berryman[1], F. Feenstra[2], J. Coco-Martin[2], and T. Tuthill[1]
[1]The Pirbright Institute, Ash Road, Pirbright UK; [2]Boehringer-Ingelheim Animal Health, Houtri- bweg 39, Lelystad, The Netherlands.

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

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

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

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

Presentation available HERE.

Appendix 64

Multiplex Real-Time Rt-Pcr for Detection Of Fmdv, Rift Valley Fever Virus and Bovine Viral Diarrhea Virus in Bulk Tank Milk

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

M. Eschbaumer[*,1]*, K. Wernike2*
[1]*National Reference Laboratory for FMD;* [2]*National Reference Laboratory for BVD, Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Sue- dufer 10, 17493 Greifswald-Insel Riems, Germany.*

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

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

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

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

Presentation available HERE.

Appendix 65
Emergency Supply of Fmd Diagnostic Kits: Reagent Banks and Idvet Solutions

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

L. Comtet, M. Roche, F. Donnet, A. Carpentier, P. Pourquier IDvet, France

Introduction

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

Materials and Methods

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

Conclusion

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

Presentation available HERE.

Appendix 66

Results From an Inter-Laboratory Exercise to Evaluate Non-Structural Protein Elisa Kits

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

C. Browning1, L .Henry1, G. Wilsden1, L. Hendry2, T. Pollard2, S. Parida1, A. Ludi1, D. P. King1

1 The Pirbright Institute, Ash Road, Pirbright, Surrey, United Kingdom; 2Animal and Plant Health Agency, New Haw, Surrey, United Kindom.

Introduction

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

Method and Results

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

Discussion

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

Presentation available HERE.

Appendix 67

Results of the 2016 and 2017 Proficiency Testing Schemes for FMD Diagnostic Methods

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

A.B. Ludi, G.Wilsden, C. Browning, H. Baker, V. Mioulet, B. Wood, A. Gray, L. Henry, J. Wadsworth, J. Maryan, S. Belgrave, D.P. King
The Pirbright Institute, Ash Road, Pirbright, Woking, UK, GU24 0NF on behalf of partner labora- tories in the EURL, OIE/FAO Networks

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

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

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

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

Presentation available HERE.

Appendix 68

The Interdependence of FMDV Pathogenesis, Challenge System, and Outcome of Vaccine Studies

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

C. Stenfeldt[1,2], E. Rieder1, L. Rodriguez1, J. Arzt1
[1] Foreign Animal Disease Research Unit, USDA-ARS, Plum Island Animal Disease Center, Greenport, NY, USA; [2] Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, USA.

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

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

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

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

Presentation available HERE.

Appendix 69

Good Correlation Between Vaccine Match in Potency Tests and R1-Value

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

A. Dekker[*,1], *A.B. Ludi*[2]
[1] *Virology department, Wageningen Bioveterinary Research, Houtribweg 39, 8221 RA Lelystad, the Netherlands;* [2] *World Reference Laboratory for FMD, The Pirbright Institute, Ash Road, Pirbri- ght, Woking GU24 0NF, UK.*

Introduction

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

Heterologous potency = match x homologous potency.

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

Materials and Methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 70
Potency Assessment of FMD Vaccines Using Standardised Serological Assays

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

S.M. Jamal[1,], N. Goris[2], K. De Clercq[3], G. Wilsden[4], Y. Li[5], A. Dekker[6]*

[1] Department of Biotechnology, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakis- tan; [2] ViroVet, Ambachtenlaan 1, B-3001 Leuven, Belgium (formerly CODA-CERVA, Belgium); [3] Sciensano, Exotic and Particular Diseases, Ukkel, Belgium (formerly CODA-CERVA, Belgium); [4] Institute for Animal Health, (IAH), Pirbright, the United Kingdom; [5] The Chinese National/OIE Refe- rence Laboratory for FMD, Lanzhou Veterinary Research Institute, Lanzhou, Gansu, P.R. of China (formerly Institute for Animal Health (IAH), Pirbright, the United Kingdom); [6] Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

Introduction

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

Materials and methods

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

Results and discussions

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

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

Presentation available HERE.

Appendix 71
Detection of FMFV O/ME-SA/IND-2001E in Jordan

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

M. Ababneh[1], W. Hananeh2, Z. Ismail3, M. Hawawsheh1, M. Al-Zghoul[4], N. Knowles[5] and C. van Maanen[6]
[1] Department of Basic Medical Veterinary Sciences, Faculty of Veterinary Medicine, Jordan Univer- sity of Science and Technology, P. O. Box 3030, Irbid, 22110-Jordan; [2] Department of Veterinary Pathology and Public Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, P. O. Box 3030, Irbid, 22110, Jordan; [3] Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, P. O. Box 3030, Irbid 22110, Jordan; [4] Animal Health Division, Ministry of Agriculture in Jordan, Queen Rania Al Ab- dullah St 39, Amman, Jordan; [5] The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK; [6] European Commission for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

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

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

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

Discussion
During this period, viruses of the FMDV/O/ME-SA/Ind-2001e sublineage have spread to the Middle East, North Africa and Southeast Asia. The nucleotide sequence of the V1 gene of the O/JOR/1/2017 sublineage is very similar to that of viruses isolated from Saudi Arabia in 2016, indicating possible introduction of this strain to Jordan through animal movement or other transmission routes from Saudi Arabia.

Presentation available HERE.

Appendix 72

The Association Between Border Provinces of Turkey and FMD Outbreaks

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

I. Keskin1, F. Rosso2, N. Bulut3, S. U. Uzun[4]
[1] Epidemiology Department, Etlik Veterinary Control Central Research Institute, Ministry of Agri- culture and Forestry, Ahmet Sefik Kolayli Street, 21-21A, 06020, Etlik, Ankara, Turkey; [2] European Commision for the Control of FMD (EuFMD), Food and Agriculture Organization, Viale delle Terme di Caracalla, 00153, Rome, Italy; [3] Diagnosis Department, Sap Institute, Ministry of Agriculture and Forestry, Dumlupinar Avenue, 35, 06044, Ankara, Turkey; [4] Epidemiology Section, Public Health Department, Ankara University Medicine Faculty, 06590, Cebeci, Ankara, Turkey.

Introduction
Foot-and-Mouth Disease (FMD) which is endemic in the Anatolian region of Turkey, affects susceptible livestock like cattle, sheep and pigs, with a socio-economic importance, is an im- portant viral contagious disease. Globalization-related factors such as movements of animal, animal products and human, and interaction between domestic and wildlife populations, in- crease the risk of FMD virus spread. Trading of animal and animal products varies depending on environmental and ecological factors such as drought and pasture availability, and changes in export and meat demand, including religious festivals and national celebrations. In this sen- se, illegal animal movements and animal trade between neighboring countries, as well as local animal movements and common pasture are important in spreading of FMD.
The objectives of this study are to (1) statistically identify the association between 23 border provinces of Turkey which are categorized as "North East", "East" and "South" and located next to at least one of the six eastern neighbour countries and FMD outbreaks, (2) determine the difference between the risk of FMD from the western part of the country and the east, excluding the Thrace region, and (3) determine the effect of the geographical region on FMD outbreaks.

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

Results
The results of this study were presented in the OS18.

Discussion
The discussion were presented in the OS18.

Presentation available HERE.

Appendix 73

Integrated Risk-Based Strategic Plans for Five Priority Diseases in the Palestinian Authority

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

C.J.M. Bartels, Melissa McLaws Animal Health Works, The Netherlands

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Key words

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

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

Presentation available HERE.

Appendix 74

Embedding Progressive Control for FMD in the Policy Agenda For Livestock Production in Three Countries in Southeast Asia

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

C.J.M. Bartels1, R. Abila2, I. Dacre2, Y. Qiu2, Melissa McLaws1
1Animal Health Works, Bakhuizen, The Netherlands; 2OIE Sub-regional Representation for Sou- th-East Asian, Bangkok, Thailand

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

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

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

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

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

Presentation available HERE.

Appendix 75

Trans-Pool Movement of Two FMD Virus Serotype A Lineages: A/ASIA/G-VII and A/AFRICA/G-IV

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

K. Bachanek-Bankowska, A. Di Nardo, J. Wadsworth, A. Gray, D.P. King, N.J. Knowles. The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, United Kingdom*

Introduction

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

Materials and methods

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

Results

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

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

Discussion

The globalisation in livestock trade and the increased access to the global export markets in- crease the risk of introduction of emerging FMDV lineages into previously unaffected regions. Here, two different lineages within the A serotype are shown to spread outside of their nor- mal distribution adversely affecting and/or becoming established in new geographical areas. This stresses the importance of close and careful surveillance as well as highlights the comple- xity of application of robust FMDV control measures.

Presentation available HERE.

Appendix 76

Socio-Economic Impact of FMD Outbreaks And Control Measures At Different Scales in Mongolia: From National Level Gross Los-Ses To Herders' Food Security

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

G. Limon1, G. Ilziibat2, B. Sandag2, S. Dorj2, D. Purevtseren2, B. Khishgee2, G. Basan2, T. Bandi3, M. Bruce[4], J. Rushton[5], P.M. Beard[1,6], N.A. Lyons[1,7]
1The Pirbright Institute, UK; 2State Central Veterinary Laboratory, Mongolia; 3Veterinary and Bre- eding agency, Mongolia; [4]Murdoch University, Australia; [5]Institute of Infection and Global Health, University of Liverpool, UK; [6]The Roslin Institute, UK; [7]European Commission for the Control of Foot-and-Mouth Disease (EuFMD), Food and Agriculture Organisation of the United Nations, Rome, Italy.

Introduction

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

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

Material and Methods

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

Results

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

Discussion

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

Presentation available HERE.

Appendix 77

Retrospective FMD Outbreak Reports From Uganda and Tanzania Border Districts (2011-2016): Implications for FMD Control by Vaccination

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

[1, 2] Kerfua Susan D, [1]Shirima Gabriel, [3]Kusiluka Lughano, [4]Ayebazibwe Chrisostome, [4]Mwebe Robert, [5]Cleaveland Sarah and [5]Haydon Daniel T*

[1] Nelson Mandela African Institution of Science and Technology, P.O. box 447, Arusha; [1,2]National Livestock Resources Research Institute, P.O.Box 96, Tororo, Uganda; 3Mzumbe University P.O.Box 1, Morogoro, Tanzania; [4]National Animal Diseases Diagnostics and Epidemiological Centre, P.O. Box 53, Entebbe, Uganda; [5]University of Glasgow, G128QQ, United Kingdom.

Introduction

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

Materials and methods

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

Results

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

Discussion

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

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

Presentation available HERE.

Appendix 78
Update of FMD in the Maghreb Region: Vaccination Issues

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

S.El Azhari[1,2], O. Fassi Fihri2 , B.klonjkowski3 , L. Bakkali-Kassimi3 , C. Loutfi1
1service de virologie, société de production biologique et pharmaceutique vétérinaire, Biopharma, Rabat, Maroc; 2Département de Pathologie et de Santé Publique Vétérinaires,Institut Agronomi- que et Vétérinaire Hassan II, Rabat, Moroc; 3UMR Virologie, INRA, ANSES, École Nationale Vétéri- naire d'Alfort, Laboratoire de Santé Animale, Maisons-Alfort, F-94700, France.

Introduction
Lately, North Africa suffered from several outbreaks of FMD caused by new viral lineages. This review aims to present the problematic of the choice of vaccine strains in North Africa.
Materials and methods
This study is based on the results of world, European and national FMD reports, as well as the synthesis of different related works. Also, a contribution to the evaluation of the prophylactic strategy adapted by the countries of the Maghreb based on the risk analysis is realized.

Results
The viral lineage of serotype O that exist in North Africa is O/ME-SA/Ind-2001, which is nor- mally present in the Indian Subcontinent, which proves the long distance movements of ge- notype
The most used method to choose the vaccine strain is in vitro vaccine matching. This test, gives different results of 2 isolates (Morocco, Algeria) belonging to the same topotype, and even more, a difference in the results for 2 viruses of the same topotype isolated in Algeria has been shown.
The virus prevailing in Morocco and Algeria, have a poor in vitro vaccine matching of O1Ma- nisa vaccine strain, while a study using the in vivo efficacy, support the use of the O1 Manisa vaccine to control the same topotype. The A/AFRICA G-IV was isolated in Algeria in 2017. The vaccine matching shows that the isolates correspond to two available vaccine strains, with r1 limit values.

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

Presentation available HERE.

Appendix 79
Assessment of FMD Vaccines in Mongolia and the Role of Bactrian Camels

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

G. Ilziibat1, B. Sandag2, T. Jargalsaikhan1, A. Tsolmon1, T. Otgon3, O. Galbadraa3, Z. Kadeyi[4], U. Sukhbaatar[5], O. Myagmarsuren1, B. Khanui1, T. Sainnohoi1, G. Basan1, T. Bandi2, G. Wilsden[6], C. Browning[6], A. Ludi[6], N.A. Lyons[6,7]

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

Introduction

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

Material and methods

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

Results

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

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

Discussion

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

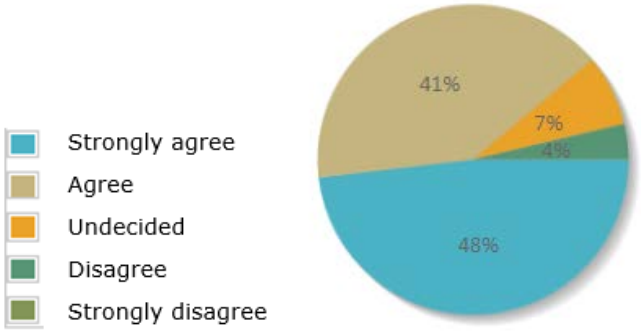
Presentation available HERE.

Appendix 80
Mobile Application Results

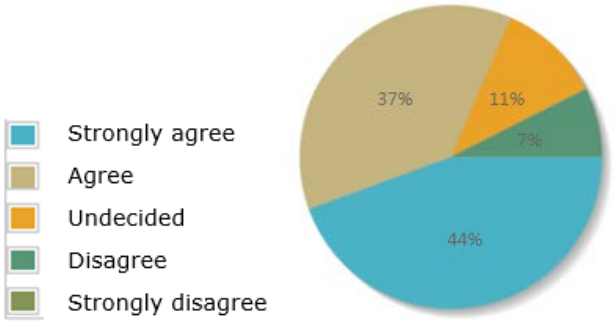
APPENDIX 80: MOBILE APPLICATION RESULTS

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

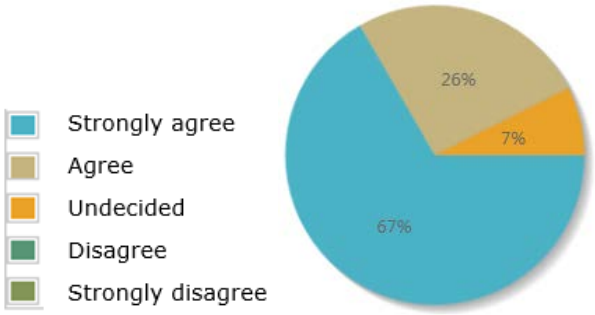
Idea 1: Improving vaccine availability needs urgent attention by both the public and private sectors and a new form of partnership is needed to achieve real change in vaccine supply.



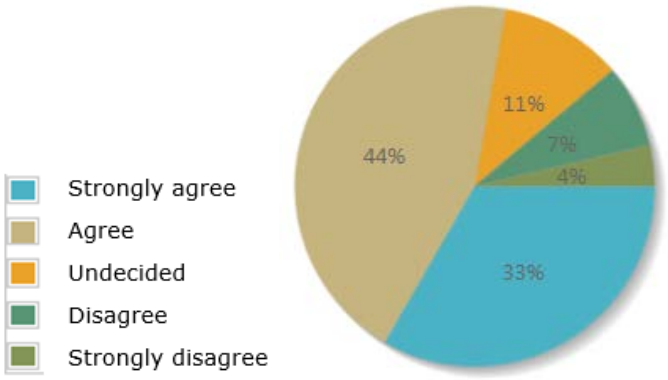
Idea 2: Development of a public-private professional network is needed to increase global security in the supply of effective vaccines.



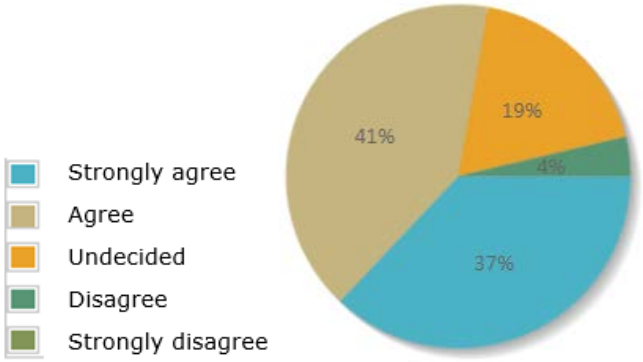
Idea 3: Quality vaccines are not enough, the barriers that prevent their availability must be addressed.



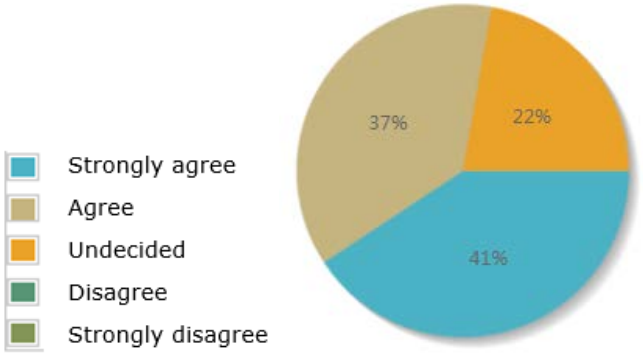
Idea 4: Inadequate access to effective vaccines in endemic regions is an animal welfare issue.



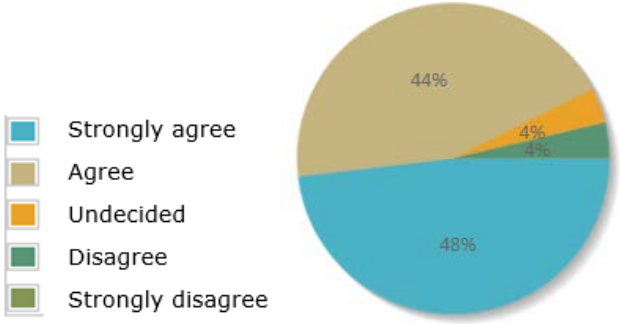
Idea 5: There exists a willingness to pay for vaccine for FMD, but further work is needed to understand the drivers for adoption and barriers to uptake.



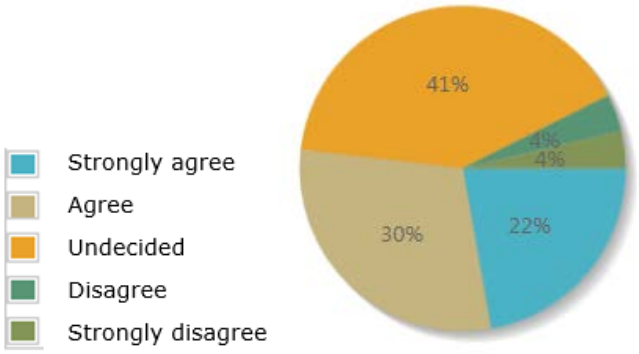
Idea 6: In many endemic settings, livestock keepers should have the right to access effective vaccines to protect their livestock and livelihoods.



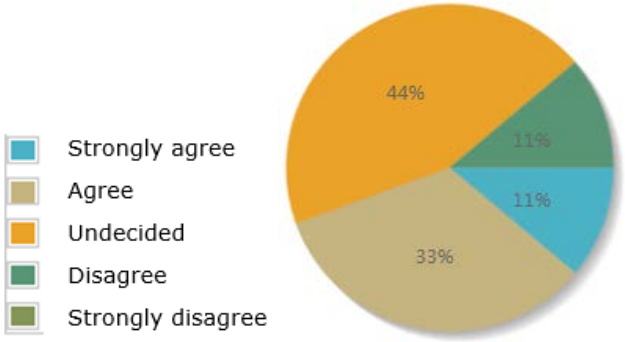
Idea 7: Further work to quantify the unmet demand for vaccines and predict future growth is needed.



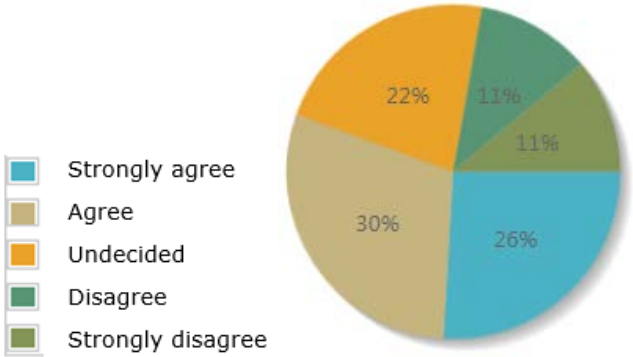
Idea 8: The traditional vaccine bank model is poorly equipped to respond to trans-pool movements of viruses, and innovative approaches to vaccien resources are required.



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



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



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