REPORT
of the
OPEN SESSION
of the
STANDING TECHNICAL COMMITTEE
of the
EUROPEAN COMMISSION FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE
(EuFMD)

Held in
Vienna (Austria)
29 September-1 October 2010

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- Intervet International BV
- Merial
- Prionics

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OPEN SESSION OF THE EuFMD RESEARCH GROUP

Considerations and Recommendations of the Open Session

Considering that:

1. There is a need to improve information flow between field, laboratory, and disease management teams, national and internationally, to enable each level to better identify the significance of disease and virological patterns;
2. FMD free countries and those in endemic regions need to identify suitable vaccines/antigens for their emergency and preventive programs, but there is a lack of standardized framework or approach to deal with the uncertain incidence in endemic regions and risks of introduction, and to the level of cross-protection to be expected, and the duration of benefits of change in seed viruses;
3. To progress along the **Progressive Control Pathway** (PCP), countries are required to provide evidence of application of activities involving monitoring and surveillance of FMD and on the application and impact of control programs. To generate such information, countries are challenged with the issue of designing complex surveys;
4. Value/market chain analysis methods are means to identify critical FMD risk points in affected (and at risk) countries, building upon more traditional epidemiological and virological approaches;
5. In the last three years the PCP approach has been used in several parts of the world, and greater confidence in the results of stage assessment could be achieved with further definition of the requirements in each stage, and of the system for verification of control activities (particularly the requirement for release of results);
6. The dynamics of emerging FMD epidemics have usually been defined by molecular typing, but structured field investigation will provide a wider range of information and should not be overlooked;
7. Most countries in Subsaharan Africa are at PCP level 0 or 1;
8. Most countries have national reference laboratories (NRLs) for FMD but there are no Reference Centers (RCs) recognized by FAO or OIE within virus pools 4 and 5;
9. The economic and political importance of FMD appears to be increasing, but the economic assessment of control options has yet to be systematically undertaken in most countries;
10. Prerequisites for efficient control include early warning and early detection, rapid and efficient response measures, and national capacity to inform and assist decision makers based on a well tested combination of local and national expertise and validated models.

Recommends that:

Regarding recommended antigen strains maintained in vaccine banks

1. Greater effort is placed by the international organizations to integrate the virological typing data such as generated by the World Reference Laboratory (WRL), with information from official and unofficial sources as a result of regional projects and programs; this effort should include greater integration and support for FMD networks and laboratories connecting NRLs in each virus pool;
2. Development and evaluation of the model for ranking the risk posed by viral pools be continued, but it must be ensured that the limitations and assumptions are clearly identified and communicated with the results; both the ranking system and the results of virological assessments should be presented to the next EuFMD Commission Session;
3. More effort should be placed on integrating intra-regional animal movements information with other layers of risk information, to better assess risk associated with new virological findings;
4. Strain recommendations for inclusion in vaccines should be risk based, as well as taking account of international and regional vaccine availability and vaccine matching data;
5. To improve vaccine selection and stimulate the development of more cross-protective vaccines, research should be directed at understanding the repertoire and mechanism of action of the polyclonal antibody response as well as defining the protection associated with different paratopes;
6. International cooperation between vaccine producers and reference laboratories should be encouraged to help overcome problems in availability and access to vaccine matching reagents, i.e. vaccine strains, sequence data, field isolates and antisera; FAO and other vaccine purchasers should place conditions on suppliers to provide suitable reference reagents, or fund their production by independent laboratories, to ensure the vaccine matching system is sustainable;
7. Studies to better characterize and improve the reliability of vaccine matching methods should continue to be supported, not only for current vaccine selection but also for development of new in vitro and computer based (*in silico*) selection processes;
8. A fuller understanding is needed of the antigenic diversity within some serotypes and regions and of the significance that this has for vaccine induced protection. The EuFMD Research Group should assess the gaps in knowledge and their relative importance;
9. A more systematic approach should be developed and used to evaluate vaccine effectiveness in the field, and report made summarizing current studies and options, to the next Research Group Session.

Regarding vaccine control

10. In process control methods, for quantification of FMD-NSPs in vaccines should be further validated; if used by FMD vaccine producers, methods and results should be published when part of their claim for purity;
11. Indirect assessment of FMD vaccine potency and vaccine matching should be harmonized to obtain exchangeable/comparable results by close co-operation between vaccine producers and international FMD reference laboratories;
12. Antigen stability should be given greater priority in quality control, and standards and associated tests to measure stability should be further developed and validated by both vaccine producers and research laboratories.

Regarding antigenic diversity, vaccine selection and monitoring

13. Greater efficiency in the identification of significant variation in FMDV could be achieved through optimizing use of regional and world reference laboratories services; flowcharts describing sampling procedures could be agreed, covering sample selection, tests at national and regional level, and when to send samples to reference laboratories for advanced typing;
14. The EuFMD/FAO should support initiatives that connect NRLs and International Reference Centres (IRCs) to achieve greater efficiency in application of molecular typing, using protocols which meet the quality standards of the FAO RCs;
15. More should be done to investigate the performance of vaccines in the field as this may differ from what was found at the point of manufacture for reasons such as vaccine stability. Greater application of vaccine effectiveness measurements are needed, comparing the risk of disease in vaccinated animals to the risk of disease in non-vaccinated animals. Different vaccination regimes could also be compared to identify optimal strategies.

Regarding the Progressive Control Pathway for FMD in practice

16. That the EuFMD Working Group on the **Progressive Control Pathway** (PCP) guidelines be further expanded to involve those epidemiologists with expertise in design of surveillance programs, and who are working on FAO or other PCP support projects, to learn the lessons from application, to further develop practical guidelines, and standards for surveys and analysis of data;
17. FAO is encouraged to further develop the market chain analysis approach and promote its use and integration in the framework of PCP/Roadmaps;
18. FAO and OIE are encouraged to further define requirements for each stage, make them verifiable and consider the possibility of some form of PCP status acceptance;
19. Support necessity of cooperation of EU, OIE and FAO on the PCP.

Regarding diagnostics

Concerning pen-side tests

20. Information regarding performances of Lateral Flow Devices (LFD) for FMD antigen detection should be systematically collected, and guidelines developed for dealing with uncertainty (interpretation of weak results, false positive detected in the field, if any);
21. LFD for serotype-specific detection should be developed, and if the commercial market is uncertain, international organizations should consider taking the lead to commission LFD for field evaluation (EuFMD/FAO/EC);
22. Research on development of other pen-side tests for antigen detection based on alternative methodologies, such as dry ELISA, is encouraged;
23. Continued development of high sensitivity (better than LFD) and high speed (similar to LFD) penside tests using genome amplification methods are recommended;
24. Guidance on the use of pen-side tests should be updated, particularly regarding use in countries without a national reference laboratory capable of alternative confirmation methods, should be established.

Concerning genome detection

25. Given the wide variation in protocols applied for RNA extraction and RT-PCR, it is recommended that countries make greater use of the protocols validated at the WRL (for both Real Time and classic RT-PCR); the SOPs on the WRL Web site should be used;
26. Diagnostic development priorities should be:
    i) Development of ready-to-use kits for RT- PCR, similar to those already commercially available for other TADs;
    ii) Development and validation of serotype-specific RT-PCR;
    iii) Development and validation of multiplex RT-PCR appropriate for regional settings (as serotypes and strains differ).

Concerning Antigen detection and typing ELISA

27. Ready-to-use kits should be commercialized and the international organizations, representing buyers, should become early adopters for evaluation and to encourage sustainable supply.

Concerning immunoassays for antibody detection

28. Ready-to-use kits for SP antibodies should be further developed, validated and made available;
29. The WRL should make available on demand a cost recovery basis:
    i) International serological standards for Asia-1, SAT types, and for relevant antigenic variants (type A);

ii) Proficiency panels for calibration and evaluation of in-house assays, and for batch control;

30. The EuFMD Research Group should work on guidance / recommendations for use of Solid Phase Competition ELISA compared to the Liquid Phase Blocking ELISA.

Concerning QA/QC

31. FAO or the Research Group of the EuFMD should develop guidance on the minimum standards for QA/QC for laboratories performing services required for the different Stages of the PCP.
32. Diagnostic test producers should make available validation dossiers, for accreditation according to ISO17025.

Concerning new developments

33. Multiplex tests: priorities are a multiplex PCR with sufficient sensitivity, but also there should be attention to multiplex detection of FMD antigens and antibody, and greater use of recombinant antigens (VLP; universal ligands, etc).

Regarding FMD epidemiology – Eurasia and South America

34. Greater attention must be made to training in disciplines needed to rapidly assess "What is the impact on animal health? What are the possible routes of transmission and what are the socio-economical effects?". This information is necessary to identify and address the critical control points in the PCP, and is helpful to feedback to field veterinarians to support their activities in controlling FMD;
35. The time delay between disease event and results of molecular typing must be shortened to a few days, in order to support decision making in complex epidemic situations. Support should be given to use the power and throughput in high technology laboratories to test samples rapidly or transfer technologies to Regional RCs and NRLs in affected areas.

Regarding FMD epidemiology – Africa

36. International organizations should support countries in Sub-Saharan Africa to initiate PCP Stage 1 activities. Training of regional experts in PCP could assist to build confidence in design of activities, such as sero-surveillance and epidemiology and socioeconomic assessments needed to develop country strategies;
37. EuFMD or FAO should strengthen regional laboratories to support primary diagnosis in NRLs in West/Central and Eastern Africa.

Regarding Control of FMD in free countries

38. EuFMD reviews the use by countries of decision support systems, including models, in developing and testing their contingency plans for a variety of scenarios of disease introduction, and explore the impact of different control measures;
39. More research is conducted on the likely intra-community spread of FMD if introduced at different times and locations into the EU;
40. The continued development of expertise in FMD recognition and immediate response, but ensuring that laboratory veterinarians as well as field veterinarians are trained from each state, since they will need to work together to ensure rapid diagnosis and to establish surveillance;
41. Continued support for work to identify contact rates and critical points for transmission, with a view to develop practical methods for application in contingency planning or during the early epidemic response.

Regarding FMD training

42. Trainees from the *Real Time Training Courses* become, as far as possible, trainers in their own countries;
43. Better preparation for the training by the trainees would improve the team work and contribution to final output;
44. Refresher courses and workshops should be organized and update the trainees on developments; and that modules for additional FMD experience should be considered (outbreak management, use of decision support models, etc).

REPORT

Open Session of the Research Group, 29 September-1 October 2010

The Open Session of the Research Group of the Standing Technical Committee of the EuFMD was held in Vienna, Austria, from 29 September to1 October 2010, with the theme "**New tools and challenges for progressive control**". The Session was attended by over 240 participants from across the world, predominantly from Europe and Africa, but also with good participation from East Asia and South America. The program was organized into fourteen technical sessions, covering recent advances and ongoing technical constraints affecting *progressive control of FMD.* The Session considered six keynote papers and 82 presentations, relating to the 14 items. One evening debate was held, and forty-two posters presented. Two panel discussions were included in the program, one concerning the control and monitoring of vaccination and another with private industry representatives.

The Agenda of the Session is **Appendix 1** and the list of participants in **Appendix 2**.

Opening

The Session was opened by Dr. Sonja Hammerschmid, Rector of the University of Veterinary Medicine, Vienna, who reminded the participants of the long history of teaching and research in Vienna, from 1765. Control of infectious diseases had always been a major importance in Austria, being situated in the European landmass and at risk from sweeping epidemics arriving from far and near. Veterinary schools have an important role to ensure that the next generations of veterinarians have an understanding of their role, which can be crucial for early recognition and response.

Dr. Ulrich Herzog, CVO Austria and Chairman of the EuFMD Executive Committee, welcomed participants to Vienna; he was glad to see that several networks and projects had used the opportunity to organize side meetings, and trusted that the Open Session would not only a great scientific success but assist experts to work together in new ways and with new ideas. He considered the Open Session of major international Importance and looked forward to receiving ideas and views on how the role as a forum and meeting point could assist in further international collaboration on FMD science. He declared the meeting open.

Two plenary presentations were then given, one being the Frenkel Lecture, and the other on the management of the recent type O incursion into Japan in 2010.

Day 1

Frenkel Lecture

The main Plenary Lecture at the Open Session is named after the renowned Dutch scientist whose "Frenkel method" introduced the first possibility of mass vaccine production and application in the world.

The Lecture "*Integrated procedures to assess FMD vaccine quality and herd immunity in Argentina*" was presented by Dr. Jose La Torre (Argentina). This paper described the development of the scientific basis for current control of vaccine quality and potency, and of the vaccination programme monitoring, which have been a major part of the improvement in success of vaccination for the control of FMD in Argentina. The lessons learnt, he proposed, should be understood and applied in other regions where vaccination is to play the principal control measure in preventing virus circulation (**Appendix 3**).

Control of FMD in Japan

Dr. Toshiyuki Tsutsui presented a paper on "*The FMD disease epidemic in Japan, 2010*", which described the evolution and progression of this incursion which proved extremely challenging for control, and resulted in massive costs for the sector and Government, with stamping policy changed to vaccination during the crisis, which was followed by culling of vaccinated animals. Japan is a country that is usually FMD-free, and the area most affected was densely populated with pig, dairy and beef farms. He described some of the challenges encountered when implementing control in this area. (**Appendix 4**).

Progressive control of FMD - in practice

The Session introduced the **Progressive Control Pathway** (PCP) for FMD, which was first developed by EuFMD together with FAO projects at a workshop in November 2008 for development of Long Term Regional approach to FMD control in West Eurasia. The issues considered included: What have we learnt from applying the PCP approach in West Eurasia in 2008-10?. After national sero-surveys for FMD, is there a smart alternative to national vaccination campaigns in all species? Are the guidelines for monitoring and surveillance, and for lab capacity in each country, appropriate? How do we identify

critical control points, and bring in socio-economic assessments to identify how and what can be done better?

This first session began with a keynote addressed by Dr. Keith Sumption, Secretary of the EuFMD Commission (**Appendix 5** and **Appendix 5b** is the paper presented to the Paraguay conference on "*PCP and regional roadmaps towards a common framework for long-term action against FMD at national and regional levels*"). Dr. Kris de Clercq, representing the OIE, then described the work in progress on the relationship between the PCP Stages and the OIE Standards (**Appendix 6**). Further presentations illustrated the experience of applying the PCP to country situations, including design of surveys to achieve Stage 1 (Dr. Ferrari, **Appendix 7**), the monitoring of vaccination and control measures (Dr. Yadin, **Appendix 8**), the progress from zonal Stage 3 to official recognition of freedom under vaccination in Thrace (Dr. Potzsch, **Appendix 9**), and the activities needed to maintain freedom (Dr. Füssel, **Appendix 10**).

FMD risk assessment, threat detection

Issues in this section were: How do we quantify/prioritize the threats from each virus pool to Europe? How can we better use regional networks to give us "viral intelligence" on emerging threats? Can we predict epidemics within West Eurasia, based on long term monitoring and viral characteristics? What viral predictors could be used? How do we get closer to real-time information on FMD events? Does risk information change lead to any difference by national risk managers (e.g. to the Far-East type O epidemics in March/April 2010)?

This session featured a keynote presentation from Dr. Jef Hammond, Head of the FAO WRL for FMD, Pirbright (**Appendix 11**). This was followed by presentations considering if FMD epidemics can be predicted (Dr. Bulut, **Appendix 12**), on risk of FMD incursions from different regions to Europe (Dr. McLaws, **Appendix 13**), on the genetic basis of the type A epidemic development in Turkey (Dr. Ozyuruk), and the further development of the FMD BioPortal (Dr. Pérez, **Appendix 14**).

Session on Vaccine Development and Vaccine control

Developments in vaccine development and immune response to FMDV were presented p (**Appendices 15-19**) and four of these papers involved work with different approaches (reverse genetics and expression systems) to genetically engineer FMDV.

Seven papers on control of vaccines and vaccination programmes were presented (**Appendices 20-26**), covering important developments in emergency vaccine formulation procedures, work to

improve thermostability, indirect assessment of potency, serology for herd immunity, in process controls for NSP in vaccines, and effect of thiomersal on FMDV virions.

Antigenic diversity, vaccine selection and monitoring

This session was organized by the antigenic diversity and vaccine selection working group, under the EuFMD Research Group, led by Dr. David Paton. The Issues: How can we improve the guidance (cross-protection, antigenic relevance) to vaccine bank managers on which antigens to hold for the current risks?. What do new approaches (such as Antigenic Cartography) offer? How will these methods change our working practises in global viral threat identification and in countries using vaccination? Why do we seem to have a problem with type O vaccination, when type O is relatively antigenically stable?

This session opened with a keynote presentation by Dr. David Paton (**Appendix 27**), followed by seven further presentations on antigenic diversity and methods to estimate antigenic relatedness and cross-protection, including progress to predict from sequence data, the use of serological data, including in antigenic cartography, and cross-protection studies between type O (Manisa and Campos). (**Appendices 28-34**).

Day 2

The Progressive Control Pathway for FMD; Papers on lessons learnt from practice

Six papers were presented in this session that described approaches to surveillance, economic considerations and FMD control within the PCP framework. The first two (Dr. Ferrari, FAO, and Dr. Bartels, EuFMD) considered design of surveys needed for risk assessment (PCP Stage1), followed by papers on methods for improving the identification of control measures in the market chain with socio-economic assessments, and on implementing the PCP approach in Ethiopia and the TransCaucasus (**Appendices 35 to 40**).

Monitoring Control and Vaccination Programmes Session and Panel

Two papers were presented in this session that described different approaches to evaluate vaccination at the herd level (**Appendices 41-42**). This was followed by a panel discussion.

FMD molecular characterization

This session comprised six presentations relating to advances in the molecular characterisation, starting with an overview on definitions and nomenclature given by Dr. Nick Knowles (**Appendix 43**), followed by presentations on applications and advantages of full FMD genomic sequencing when applied to understanding big epidemics and localised spread between and within herds (**Appendices 44-45**). The potential of next generation sequencing was presented by Dr. Caroline Wright (**Appendix 46**).

Diagnostics

This session was organized by the *Diagnostics Working Group* of the EuFMD Research Group, led by Dr. Emiliana Brocchi.

Issues: FMDV nomenclature: proposal for new system. Test validation – what's new, what are the gaps? What are the gaps in our diagnostic repertoire? Which diagnostic tests are needed at each PCP Stage, from endemic to near-freedom? What are we gaining from Full-Genome sequencing to guide diagnostics?. How do we rapidly type FMD to strain level, in affected West Eurasian countries?. Can we

profile herd infection using diagnostics, to identify how long infection has been in a herd? Carrier status of domestic buffalo.

This long and important session considered a keynote presentation (Dr. Vilna Wosloo, **Appendix 47**) and 11 papers concerning the detection of FMD virus, antigen and antibodies (**Appendices 48-58**). These papers covered important developments that could lead to preserved RNA rather than live virus being transported for reference centre typing, with use of transfection to recover live virus (project supported by EuFMD); the development of simple ELISA for antigen detection, the evaluation of penside tests, and comparison of rapid one-step RT-PCR assays. Global and regional harmonization and proficiency testing operated by WRL (for FAO and the EC) and regional examples (for SADC) were presented.

FMD epidemiology

Issues: What have we learnt on why, when, how epidemics or outbreaks occur? Risk based surveillance: experience and guidance. Surveillance in vaccinated populations: Still a problem with impure vaccines? What have we learnt from epidemics in free countries in 2010?.

– *Eurasia and South America:*

This Session consisted of 11 presentations on the epidemiology of FMD in Eurasia and South America (**Appendices 59-69**). These papers highlighted the resurgence of interest in FMD epidemiology, and in the move beyond molecular studies into understanding contact networks and a better description of the space and time dynamics of FMD. These studies fit well with requirements of PCP Stage 1 to define the risk populations and transmission risks.

– *Africa:*

This session included 10 papers related to issues important to FMD in Africa, including results from sero-surveys, molecular and virological studies (**Appendices 70-79**).

Control of FMD in free countries

This session included six scientific presentations concerning FMD preparedness and control in countries that are usually FMD-free (**Appendices 80-85**), highlighting the importance of a using different rigorous testing procedures for evaluation of contingency plans for FMD incursions.

Antivirals and other developments

Five papers were presented in this session (**Appendices 86-90**) concerning FMDV pathogenesis and recent advances in the development of antiviral drugs.

Surveillance technologies

Issues: Mobile phones and FMD: How do we communicate in a quicker and smarter way with those that need to know? Farmer-led reporting: Can we rapidly scale up information flows during a crisis? I-Phones and FMD. New diagnostics; Theoretical or practical? What quality standard must novel FMD vaccines meet for international acceptance?

Two papers were presented in this session, concerning technological and diagnostic technologies relevant to FMD surveillance (**Appendices 91-92**).

FMD Real Time Training and Private Sector Platform

Issues: This session reviewed lessons learnt from training of over 70 persons in FMD recognition and outbreak investigation practices, under the EuFMD/EC real-time FMD Training Program in 2009-10. How do we retain the experience and keep trainees up to date? Do we need refresher events/courses/online exercises, and to extend the experience to include FMD in pigs, etc? The program ended with an open session which included a description of FMD real-time training courses offered by EuFMD in 2010 (**Appendix 93)** and the platform was given to the private sector (representatives from Intervet ,Merial, Prionics and a talk on the Public/Private Sector arrangements in India).

Closure

The EuFMD Commission gratefully acknowledges the support of Dr. Ulrich Herzog, CVO Austria and President of the EuFMD Executive Committee, the local organizing agency AGES, as well as the companies who generously sponsored the meeting.

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Pag.169... **42. Can evaluation methods used for human vaccination programmes be used to improve the control of foot and mouth disease (FMD)?.** *T. Knight-Jones*

Pag.172... **43. FMDV Genotype definitions and nomenclature.** *N. Knowles*

Pag.175... **44. Full genome sequence analysis of FMD viruses in Western Eurasia.** *D. King*

Pag.177... **45. Inter- and intra-herd sequence variability of foot-and-mouth disease viruses recovered during the 2007 UK outbreak.** *B. Valdazo Glez.*

Pag.180... **46. Can next generation sequencing be used to unravel fine scale FMDV population dynamics?.** *C. Wright. (Full papers available in pag.360)*

Pag.183... **47. Keynote: Diagnostics.** *W. Vosloo*

Pag.189... **48. Rescue of highly pathogenic FMD viruses from preserved viral RNA samples collected in Pakistan and Afghanistan.** *G. Belsham*

Pag.191... **49. Development of a FMD diagnostic multiple bead immunoassay.** *B. Boisseau. (Full papers available in pag.370)*

Pag.194… **50. A simple antigen detection Elisa Kit for FMDV serotypes O,A,C, and ASIA1.** *E. Brocchi. (Full papers available in pag.375)*

Pag.196... **51. FMD and SVD combined proficiency scheme studies 2009 and 2010 - Virology and overview.** *Y. Li. (Full papers available in pag.384)*

Pag.200... **52. Use of Priochek® FMDV-NS Elisa and Priocheck® FMDV type O Elisa in Uganda.** *K. Tjørnehøj*

Pag.203... **53. Evaluation of lateral flow device 'SVANOVA' for detection of FMDV during the course of infection.** *S. Metwally*

Not available **54. Serology resuslts from the 2009 annual Proficiency Testing Scheme (PTS).** *P. Keel. Not available*

Pag.206... **55. Diagnostic performance of two commercially available cell viability assays in FMD research .** *T. Willems*

Not available **56. Harmonizing diagnostics standards and procedures for Sero-Surveillance in FMD risk countries in the SADC Region.** *G. Maltho. Not available*

Pag.209... **57. Comparison of two ONE-STEP REAL-TIME RT-PCR assays for FMDV diagnosis.** *V. Mioulet*

Pag.212... **58. Comparison of tests that detect Persistent FMDV Infection in cattle.** *S. Parida*

Not available **59. Genetic diversity and geographical distributions of FMDV in Pakistan and Afghanistan, 1997-2009.** *J. Syed. Not available*

Pag.215... **60. Monitoring of Foot-and Mouth disease in the Russian Federation.** *V. Borisov. Not available*

Not available **61. FMD evolution and prediction: Statistical analysis in Kerman provice of I.Iran.** *S. Kazemia*

Pag.217... **62. Modelling of FMD epidemiology evolution in I. R. Iran since 2006.** *V. Otarod*

Pag.220... **63. FMDV-A/IRN/2005 endemics in WestEurasia: estimation of coascelent events in the population history.** *F. Ozyorut*

Pag.223... **64. Molecular investigation of recent outbreak of the type O FMD Iran.** *N. Shirazi*

Pag.225... **65. Detection of FMDV in carrier buffalo in South East Asia.** *S. Parida*

Pag.227... **66. FMD NSP-seroprevalence in large ruminants in 5 Egyptian governorates under vaccination control – analytical results.** *C. Bartels*

Pag.230... **67. FMD NSP-seroprevalence in large ruminants in 5 Egyptian governorates under vaccination control – descriptive results.** *K. Van Maanen*

Pag.235... **68. Factors associated with within-herd transmission of serotype A FMD virus in Argentina, 2001.** *B. Brito*

Pag.239... **69. Temporal, spatial and phylogenetic assessment of full-sequenced serotype a FMD viruses from Argentina, 2001.** *G. Cabanne*

Pag.241... **70. Commodity-based trade in ruminant products: potentials and impacts for Sub-Saharan Africa.** *M. Carron*

Pag.244... **71. Foot and mouth disease in Mali and West Africa.** *A. Traore*

Pag.247... **72. FMD Virus situation in Nigeria.** *D. Lazarus*

Pag.252... **73. Indication of undetected SAT-1 infection after serological surveillance for foot-and-mouth disease in Eritrea.** *T. Tesfaalem*

Pag.256... **74. Antibodies against FMD virus in African buffalo herds in different national parks in Uganda (2001-2008).** *A Chrisostom*

Pag.259... **75. Bayesian evolutionary analysis of FMD type A virus in Africa.** *N. Knowles*

Pag.261... **76. A molecular biological study on recent Sudanese FMD virus isolates.** *Md. Habiela. (Full papers available in pag.382)*

Pag.264... **77. A review on FMD viruses collected in Tanzania from 1967 to 2009.** *C. Kasanaga*

Pag.267... **78. Descriptive and spatiotemporal analyses of FMD in Tanzania from 2001 to 2006.** *K. Fredrick*

Pag.271... **79. Developing a partnership for FMD research in Southern Africa.** *R. Mark*

Pag.273... **80. Quantitative analysis of FMDV secretion and excretion.** *C. Bravo de Rueda*

Pag.276... **81. FMD control - learning from the recent events in FMD free countries in Asia - the problems of high density swine/ruminant areas.** *S. Wainwright*

Pag.281... **82. Some elements of disease preparedness for highly contagious emerging and re-emerging diseases in Germany.** *M. Krammer*

Pag.284... **83. Developing and validating simulation models of FMD for informing policy decision-makers .** *K. Owen*

Pag.287... **84. Classic vs. contact network-based control strategies in FMD and other viral epidemics** *A. Rivas*

Pag.290... **85. Protective vaccination to combat FMD in Finland - does it pay?.** *N. Jarkko*

Pag.292... **86. Development of a FMD infection model in severe combined immunodeficient mice for the preliminary evaluation of antiviral drugs.** *D. Lefebvre. (Full papers available in pag.400)*

Pag.295... **87. Induction of type-1 interferon by bovine plamacytoid dentritic cells (pDCs) in response to FMD virus.** *E. Reid*

Pag.299... **88. Getting to the Heart of non-epithelial foot-and-mouth disease virus (FMDV) replication.** *R. Waters*

Not available **89. Mapping persistent determinants in foot-and-mouth disease virus genome.** *Z. Zhang. Not available*

Pag.303... **90. Induction of early specific local immune responses in the respiratory tract of FMD-infected cattle.** *M. Pérez Filgueira*

Pag.306... **91. Epicollect.** *D. Aanensen*

Pag.314... **92. Applications of REPLIKINS® in FMDV surveillance and vaccine production .** W. Preben

Pag.316... **93. Real-Time Training for improved FMD diagnosis and response in the field : Lessons learnt.** *A.B. Youssef*

Appendix 1
AGENDA

New tools and challenges for progressive control

eufmd FMD Week2010 programme

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

Wednesday 29 September

09:00	09:10	Welcome	
09:10	09:40	Frenkel lecture	
09:40	09:50	*Discussion*	
09:50	10:05	**FAO**	
10:05	10:30	Control of FMD in Japan	
10:30	11:00	*Coffee/Tea break*	

		PARALLEL SESSION 1		PARALLEL SESSION 2	
		Progressive control in practice		**Vaccine development**	
11:00	11:15	**Keynote** Keith Sumption	*One hour session with 5 minute opening pots (20 minutes) then 30 minutes Panel Discussion*	*Blignaut Belinda*	Custom-Engineered Chimeric FMD vaccine elicits protective immune responses in pigs
11:15	11:30	**OIE - on the PCP** in Relation to OIE Norms on FMD		*Zamorano Patricia*	Early protection induced by the use of a recombinant baculovirus as adjuvants for a FMD virus vaccine
11:30	11:45	Achieving **PCP Stage 1**: Lessons from West Eurasia		*Caracci Margot*	Production of a safe EMCV-FMDV recombinant
11:45	12:00	**PCP Stage 2**: Monitoring control versus eradication strategies		*Windsor Miriam*	The interaction of bovine MODC with FMDV
12:00	12:15	**PCP Stage 3 to 4**: Achieving freedom, example of Thrace region		*Grant Clare*	Characterisation of the T-dependend and T-independent immune responses in cattle
12:15	12:30	**PCP Stage 4 & 5**: Disease free regions: maintaining freedom - what can be improved?		*Su Mi Kin*	Replication of foot-and mouth disease virus RNAs lacking 3AB, 3B or 3D region
12:30	13:30	*Lunch*		*Lunch*	
		FMD risk assessment, threat detection		**Vaccine control**	
13:30	13:45	**Keynote** Jeff Hammond		*Goovaerts Danny*	Freezing of formulated FMDV vaccines and vaccines banks: Potential for a faster response in emergency situations
13:45	14:00	**Keynote** Jeff Hammond		*Fowler Veronica*	Thermostability and infectious properties of a FMD type A virus with a partially deleted VP1 G-H loop
14:00	14:15	*Bulut C, Ozyoruk F, Potzsch C, Sumption K*	Inter-epidemic periods in West Eurasia: are major FMD epidemic predictable?	*Mattion Nora*	Confidence in indirect assessment of FMD vaccine potency and vaccine matching by percentage of expected protection
14:15	14:30	*McLaws Melissa*	Priority setting for antigen banks -risk to Europe	*Willems Tom*	In vitro alternatives for FMD virus challenge in the PD50 vaccine potency test are serotype-dependent
14:30	14:45	*Ozyoruk Fuat*	FMDV-A/IRN/2005 endemics in WestEurasia: estimation of coascelent events in the population history	*Mattion Nora*	Quantitative single serum dilution liquid phase Elisa for the Assesment of herd immunity and expectancy of protection against FMD virus in vaccinated cattle
14:45	15:00	*Pérez Andres*	Update on the disease biopportal project at UC Davis	*Capozo Alejandra*	Development of an In Process Control Filtration-Assisted Chemiluminometric Immunoassay to Quantify Foot and Mouth Disease virus (FMDV) Non Capsid Proteins in Vaccine Antigen Batches
15:00	15:30	*Coffee/Tea break*		*Harmsen Michiel*	Effect of Thiomersal on dissociation of intact (146S) FMDV virions into 12S particles
		Antigenic diversity, vaccine selection and monitoring		*Coffee/Tea break*	
15:30	15:45	**Keynote** David Paton			
15:45	16:00				
16:00	16:15	*Reeve Richard*	Identifying epitopes and quantifying their role in loss of cross-neutralization		
16:15	16:30	*Blignaut Belinda (presenter F. Maree)*	Using antigenic data on the FMDV capsid for the selection of new vaccine candidates for the SAT types		
16:30	16:45	*Srinivasan V. A.*	Evaluation of cross-protection between O1 Manisa and O1 Campos in cattle vaccinated with O1 Manisa vaccines		
16:45	17:00	*Daryl Borley*	Predicting sites of antigenic importance of serotype O FMD viruses using serological and capsid sequence data		
17:00	17:15	*Ludi Anna*	Serotype A cartography (missing abs)		
17:15	17:30	*Srinivasan V. A.*	Clinical protection, sub-clinical infection and persistence following vaccination with different payloads of O1 Manisa vaccine and challenge in		



POSTER SESSION

Thursday 30 September 2010

		PARALLEL SESSION 1		PARALLEL SESSION 2	
		PCP in practise		**FMD Molecular characterisation**	
09:00	09:15	*Ferrari Giancarlo*	Progressive Control Pathway and serological surveys: Methodological approach to sampling	*Knowles Nick*	FMDV Genotype definitions and nomenclature
09:15	09:30	*Bartels Chris*	Seromonitoring for FMD in PCP stage 1: the choice between a random or targeted sampling design?	*Ayelet Gelagay*	The study on sequence variability in the structural protein-encoding region of foot-and-mouth disease virus serotype A and O of Ethiopian field isolates
09:30	09:45	*McLaws Melissa*	Integreting market chain analysis into the PCP process to identify critical risk control points	*King Donald*	Full genome sequence analysis of FMD viruses in Western Eurasia
09:45	10:00	*Guitian Javier*	Conceptual framework for the evaluation of the economic impact of FMD in small holders livestock systems of the Andean region	*Valdazo Gonzalez Begoña*	Inter- and intra-herd sequence variability of foot-and-mouth disease viruses recovered during the 2007 UK outbreak
10:00	10:15	*Rufael Tesfaye*	FMDV surveillance in Ethiopia during 2007-2010 towards national control pathway in risk reduction approach	*Wright Caroline*	Can next generation sequencing be used to unravel fine scale FMDV population dynamics?
10:15	10:30	*Potzsch Carsten*	Advancement in FMD control in the Trans-Caucasus countries - A regional field simulation exercise	*Jamal Syed*	Genetic diversity and geographical distributions of FMDV in Pakistan and Afghanistan, 1997-2009
10:30	11:00	Coffee/Tea break		*Sedighe Kazemi Nia*	FMD evolution & prediction: Statistical analysis in Kerman province of I.R. Iran
				Coffee/Tea break	
		Diagnostics		**FMD Epidemiology - Eurasia and South America**	
11:00	11:15	**Keynote** Wilna Vosloo		*Waheed Usman*	Molecular epidemiology of FMDV isolated from field outbreaks of FMD during 2005-2008 in Pakistan
11:15	11:30			*Otarad Vahid*	Modeling of FMD epidemiology evolution in I. R. Iran since 2006
11:30	11:45	*Belsham Graham*	Rescue of highly pathogenic FMD viruses from preserved viral RNA samples collected in Pakistan and Afghanistan	*Knowles Nick*	Recent events in the evolution of FMD in the Middle East
11:45	12:00	*Blaise Boisseau, Sandra*	Development of a FMD diagnostic multiple bead immunoassay	*Cabanne Gustavo*	Temporal, spatial and phylogenetic assesment of full-sequenced serotype a FMD viruses from Argentina, 2001
12:00	12:15	*Brocchi Emiliana*	A simple antigen detection Elisa Kit for FMDV serotypes O,A,C, and ASIA1	*Satya Parida*	Detection of FMDV in carrier buffalo in South East Asia
12:15	12:30	*Li Yanmin*	FMD and SVD combined proficiency scheme studies 2009 and 2010 - Virology and overview	*Rivas Ariel Luis*	Classic vs. contact network-based control strategies in FMD and other viral epidemics
12:30	12:45	*Tjørnehøj Kirsten*	Use of Priocheck® FMDV-NS Elisa and Priocheck® FMDV type O Elisa in Uganda	*Van Maanen Kees*	FMD nsp-seroprevalence in large and small ruminants in 5 Egyptian governorates under vaccination control
12:45	13:30	Lunch		Lunch	
				FMD Epidemiology -Africa	
13:30	13:45	*Metwally Samia*	Evaluation of lateral flow device 'SVANOVA' for detection of FMDV during the course of infection	*Traore A*	Foot and mouth disease in Mali and West Africa
13:45	14:00	*Keel P*	Serology results from the 2009 annual proficiency testing scheme (PTS)	*Ayebazibwe Chrisostom*	Antibodies against FMD virus in African buffalo herds in different national parks in Uganda (2001-2008)
14:00	14:15	*Van Maanen Kees*	Diagnostic laboratory capacity requirements in relation to each stage of the PCP	*Lazarus David*	FMD Virus situation in Nigeria
14:15	14:30	*Willems Tom*	Diagnostic performance of 2 commercially available cell viability assays in FMD research	*Ayelet Gelagay*	Epidemiological study on FMD in cattle: sero-prevalence and risk factors in South Omo zone, South-Western Ethiopia
14:30	14:45	*George Matlho*	Harmonizing diagnostics standards and procedures for Sero-Surveillance in FMD risk countries in the SADC Region	*Tekleghiorghis Tesfaalem*	Indication of undetected SAT-1 infection after serological surveillance for foot-and-mouth disease in Eritrea
14:45	15:00	*Miaulet Valerie*	Comparison of two ONE-STEP REAL-TIME RT-PCR assays for FMDV diagnosis	*Fasina Folorunso Odulayo*	Recent isolates of Nigerian FMD virus confirm widespread Sudan-Sahel epidemic cluster
15:00	15:30	Coffee/Tea break		Coffee/Tea break	
15:30	15:45	*Satya Parida*	Comparison of tests that detect Persistent FMDV Infection in Cattle	*Knowles Nick*	Bayesian evolutionary analysis of FMD type A virus in Africa
15:45	16:00	*To be decided*	Managing FMD infected materials in non-containment	*Habiela Mohammed*	A molecular biological study on recent Sudanese FMD virus isolates
16:00	16:15	*To be decided*		*Kasanga Christopher*	A review on FMD viruses collected in Tanzania from 1967 to 2009
16:15	16:30	*To be decided*		*Kivaria Frederik*	Descriptive and spatiotemporal analyses of FMD in Tanzania from 2001 to 2006
16:30	16:45	**Monitoring control and vaccination programmes panel (30 minutes)**		*Raweyemamu*	Southern African FMD research
16:45	17:00	*CONTINUED*			

Friday 1st October

		PARALLEL SESSION 1		PARALLEL SESSION 2	
		Control of FMD in free countries		**Anti-virals and other developments**	
09:00	09:15	*Bravo de Rueda, Carlo*	Quantative analysis of FMDV secretion and excretion	*Lefebre David*	Development of a FMD infection model in severe combined immunodeficient mice for the preliminary evaluation of antiviral drugs
09:15	09:30	*Wainwright Sherrylin*	FMD control - learning from the recent events in FMD feree countries in Asia - the problems of high density swine/ruminant areas	*Mattion Nora*	HSV-1 Amplicon vectors direct the in situ production of FMD virus like particles in mammalian cells
09:30	09:45	*Kramer Matthias*	Some elements of disease preparedness for highly contagious emerging and re-emerging diseases in Germany	*Zhidong Zhang*	Up-regulation of interleukin-10 gene expression associated with foot-and-mouth disease virus induced myocardial inflammation in ovine fetus
09:45	10:00	*Owen Katie*	Developing and validating simulation models of FMD for informing policy decision-makers	*Waters Ryan*	Getting to the Heart of non-epithelial foot-and-mouth disease virus (FMDV) replication
10:00	10:15	FMD Real Time Training		*Zhidong Zhang*	Mapping persistent determinants in foot-and-mouth disease virus genome
10:15	10:30	FMD Real Time Training		Mariano Pérez Filgueira	Induction of early specific local immune responses in the respiratory tract of FMD-infected cattle
10:30	*11:00*	*Coffee/Tea break*		*Coffee/Tea break*	
		Surveillance Technologies			
11:00	11:15	Aanensen David	Epicollect		
11:15	11:30	Willeberg Preben	Applications of REPLIKINS® in FMDV surveillance and vaccine production		
		OPEN			
11:30	11:45	*Niemi Jarkko*		Protective vaccination to combat FMD in Finland - does it pay?	
11:45	12:00	**PRIVATE SECTOR** PLATFORM:		Panel from private sector - 5 minutes each on their developments	
12:00	12:15	**PUBLIC PRIVATE SECTOR** FEEDBACK on Session panel from both		Panel from both public and private sectors, inc GFRA	
12:15	12:30	*CONTINUED*			
12:30	*13:30*	*Lunch*			
		Wrap Up and closure			
13:30	13:45	**The Way Ahead** - conclusions given by leaders of the Working Groups			
13:45	14:15	Recommendations			
14:15	14:30	Appreciations and prizes			
14:30	15:30	**Closure**			
		Coffee / Tea break			
		To place			
		Singh	FMD vaccine production under Private Public Partner mode in India (include under "Public/Private" sector)		
Total number		**80**			

new abstracts : under revision (to be included in the programme)	
Capozzo Alejandra	Recombinant aphtovirus chimera of the glycoprotein of vesicular stomatitis virus evaluated as DNA and protein-based vaccines in mice and cattle

Appendix 2.

LIST OF PARTECIPANTS

Research Group members are listed together.
Remaining participants are listed under **"Others"** in alphabetical order (by surname) and by **"Country"** at the end.

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Dan Haydon
Lynda Hendry
Donald King
Theodore Knight-Jones
Nicholas Knowles
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FMD Week 2010

INTEGRATED PROCEDURES TO ASSESS FMD VACCINE QUALITY AND HERD IMMUNITY IN ARGENTINA

José La Torre

ICT MILSTEIN - ANIMAL VIROLOGY CENTER - NATIONAL RESEARCH COUNCIL

VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER 1

RIIDFA
Argentine Inter-Institutional Network for R&D in FMD

National Research Council
Ministry of Science and Technology
CEVAN – ICT MILSTEIN

National Institute of Agriculture Tecnology
Ministry of Agriculture
VIROLOGY and BIOTECHNOLOGY CENTERS

National Service for Agrifood Health Quality
OIE –FMD- REFERENCE LABORATORY

Vaccine Company
FMD Vaccine and Antigen Bank



COOPERATION
1+1>2

FMD Week 2010 VIENNA, AUSTRIA – 27th SEPTEMBER – 1st OCTOBER RIIDFA - JLT 2

ARGENTINA FOOT-AND-MOUTH DISEASE STATUS (OIE 2010)

Vaccination of cattle, sheep, swine and goat populations

Vaccination of cattle population



FREE ZONE WITH VACCINATION

FREE ZONE WITHOUT VACCINATION

AREA UNDER INTENSIVE SURVEILLANCE (15 km)

Data: Modified from SENASA

FMD Week 2010 VIENNA, AUSTRIA – 27th SEPTEMBER – 1st OCTOBER RIIDFA - JLT 3

PROCEDURES ADOPTED TO IMPROVE VACCINE AND VACCINATION EFFICACY IN ARGENTINA

- STRINGENT VACCINE CONTROL (all batches, PPG)
- REPLACEMENT OF FORMOL BY AZIRIDINES
- INCORPORATION OF OIL ADJUVANTED VACCINES (long lasting immunity)
- ✓ UPDATING OF VACCINE STRAINS
- ✓ ADJUSTMENT OF ANTIGENIC PAYLOAD AND INTEGRITY OF 140 PARTICLES
- ✓ MAbs AND SEQUENCING FOR VACCINE CONTROL
- ✓ INDIRECT METHODS, HERD IMMUNITY AND VIRAL CIRCULATION

FMD Week 2010 VIENNA, AUSTRIA – 27th SEPTEMBER – 1st OCTOBER RIIDFA - JLT 4

VACCINE STRAIN UPDATE
"VACCINE MATCHING" 1983-1987

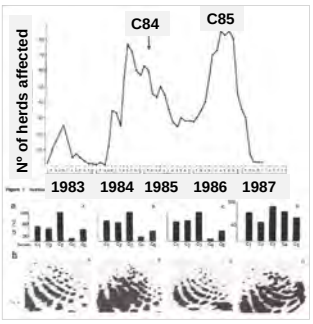
VACCINE STRAIN
C3 Resende

IDENTIFIED FIELD STRAINS
C Arg 84
C Arg 85

VN AND CROSS PROTECTION STUDIES

⇩

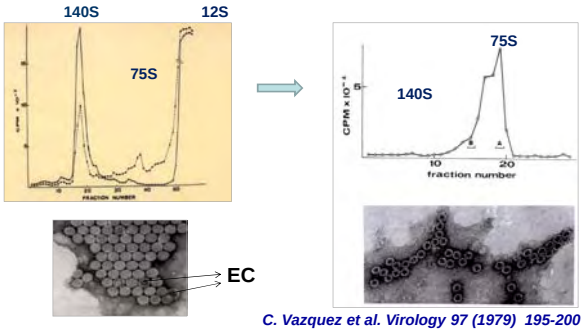
SELECTED VACCINE STRAIN
C Arg 85



I. E. Bergmann et al. Vaccine, Vol 6 (1988) 245-252

FMD Week 2010 VIENNA, AUSTRIA – 27th SEPTEMBER – 1st OCTOBER RIIDFA - JLT 5

ANTIGENIC PAYLOAD
IDENTIFICATION OF FMDV POTENTIAL ANTIGENIC PARTICLES



C. Vazquez et al. Virology 97 (1979) 195-200

FMD Week 2010 VIENNA, AUSTRIA – 27th SEPTEMBER – 1st OCTOBER RIIDFA - JLT 6

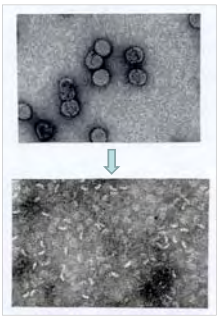
LABILITY OF 140 S

BREAKDOWN OF 140S

VPs: 1 - 4

12S SUBUNITS ANTIGENIC ???

VPs: 1- 3



TEMP. (>40°C)

pH

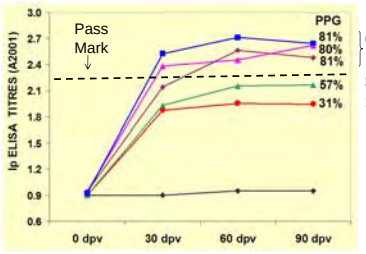
I. STRENGTH

PROTEASES

C. Vazquez et al. Virology 97 (1979) 195-200

EFFECT OF INCREASED Ag PAYLOAD ON THE INDUCTION OF ANTIBODY LEVEL AND PROTECTION

EXPERIMENTAL VACCINES CHALLENGE VIRUS: A/Arg/2001



NON-VACCINATED CONTROL

N. Mattion et al. Vaccine 22 (2004) 4149-4162

ANALYSIS OF VIRAL POLYPEPTIDES BY SDS-PAGE: CONTROL OF VP1 INTEGRITY

VIRUS SAMPLES + TRYPSIN

ISOLATION OF 140S PARTICLES

SDS-PAGE COOMASIE BLUE STAINING

C Tr Tr C

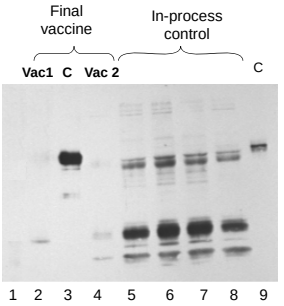


VP1-3

Tr. FRAGMENT

VP 4

ANALYSIS OF NSP BY WESTERN BLOT / ECL



C+: Recomb. 3ABC

Mab: 3H7 against 3A

3 ABC

3 AB

3 A

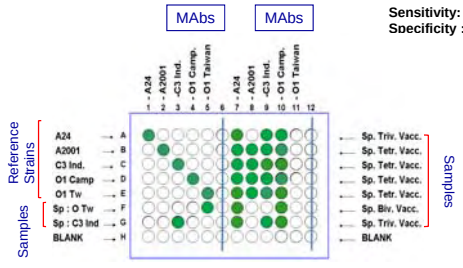
Capozzo et al. This meeting

APPLICATION OF MAbs TO VACCINE CONTROL

STRAIN IDENTIFICATION AND PURITY WITH SPECIFIC MAbs.

MAbs MAbs

Sensitivity: 5 - 15 ng/ ml
Specificity : 100%



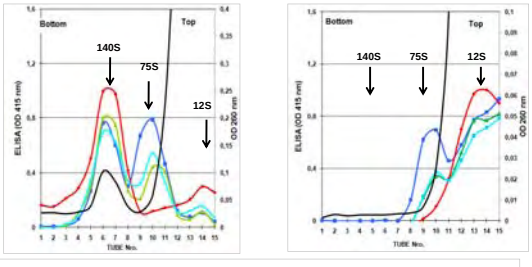
C. Seki et al. Veterinary Microbiology 133 (2009) 239-251

INTEGRITY OF 140S IN MULTIVALENT FMD VACCINES

STRAIN SPECIFIC MAbs ACROSS SUCROSE GRADIENTS

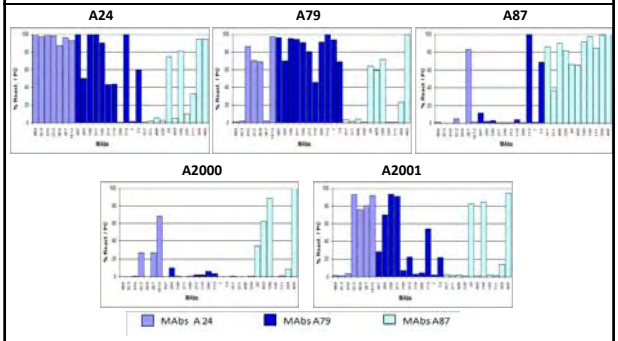
CONTROL

12' at 60ºC



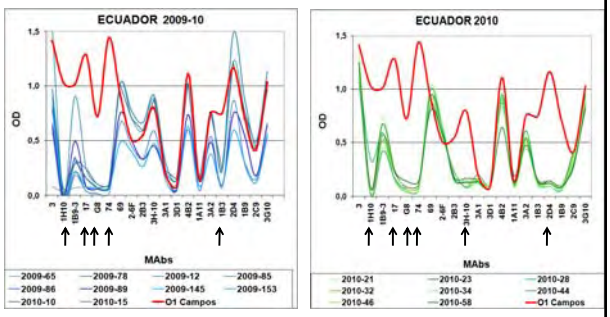
C. Seki et al. Veterinary Microbiology 133 (2009) 239-251

RAPID AND SENSITIVE MAb ANTIGENIC PROFILING OF VACCINE AND FIELD STRAINS



N. Mattion et al. Vaccine 22 (2004) 4149-4162

TYPE O FMDV FIELD ISOLATES IN ECUADOR (2009-2010)



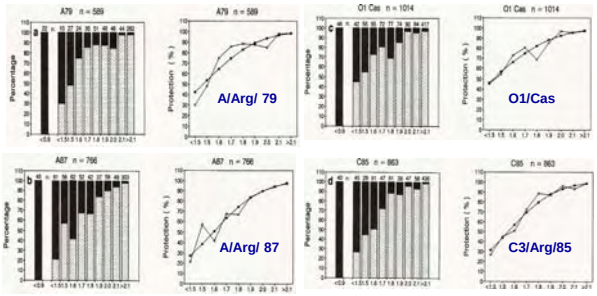
INDIRECT POTENCY CONTROL, MONITORING OF VACCINATION EFFICACY AND VIRAL CIRCULATION

- ✓ **IpELISA: CORRELATION BETWEEN PPG AND Ab LEVELS FOR ARGENTINE VACCINES (1995)**
  Adapted from Hambling et al. J.Immunol. Methods 93 (1986) 115-121
- ✓ **INCORPORATION OF IpELISA FOR INDIRECT VACCINE POTENCY CONTROL**
  (control of every batch and every vaccine strain included in the vaccines)
- ✓ **MONITORING OF HERD IMMUNITY (sIpELISA)**
- ✓ **MONITORING OF VIRAL CIRCULATION (NSP TESTS)**

VACCINE POTENCY (DIRECT vs. INDIRECT METHODS)

CORRELATION OF PROTECTION BY PPG WITH IpELISA TITERS

3252 BOVINES VACCINATED, BLED AND CHALLENGED

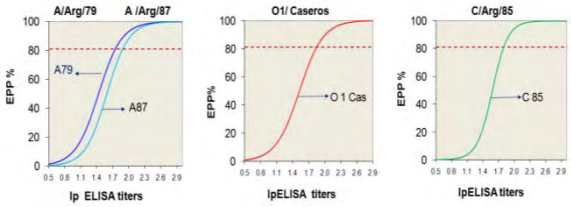


O. Periolo et al. Vaccine 11 (1993) 754-760 *B. Robiolo et al. Vaccine 13 (1995) 1346-1352*

CORRELATION OF PPG WITH IpELISA TITERS

(LARGE SCALE TESTING)

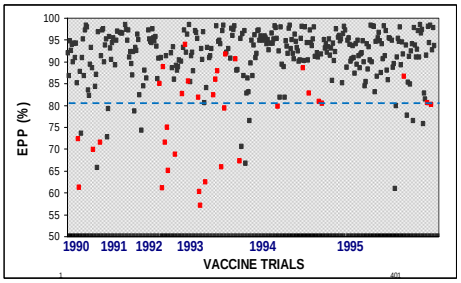
7000 BOVINES VACCINATED, BLED AND CHALLENGED
409 VACCINE BATCHES OF TETRAVALENT VACCINES



EPP (IpELISA) vs. PPG

401 COMMERCIAL VACCINES

PPG TRIAL: APPROVED REJECTED

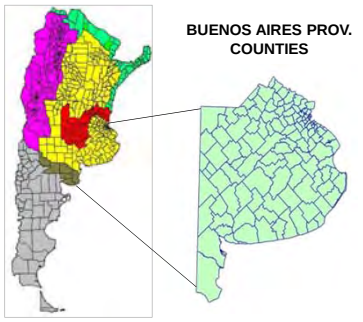


HERD IMMUNITY

374 VACCINATION UNITS (FARMERS ASSOCIATION) TRAINED AND CONTROLLED BY SENASA

Circa 55.000.000 BOVINES

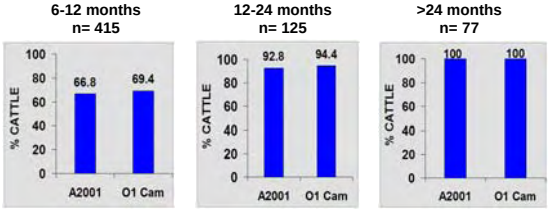
Circa 1/3 CALVES <1 YEAR



HERD IMMUNITY

SINGLE SERUM DILUTION slpELISA IN NSP NEGATIVE SERA

% OF ANIMALS WITH slpELISA TITERS COMPATIBLE WITH EPP ≥75%



B. Robiolo et al. J. Virological Methods 166 (2010) 21-27

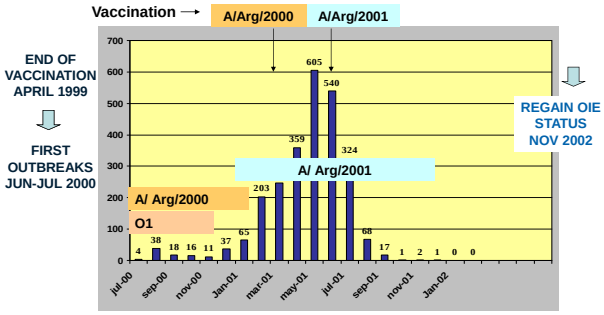
N. Mattion, this meeting

IMPACT

- 1995: ARGENTINA WAS DECLARED FREE OF FMD WITH VACCINATION (OIE)
- 1999: (April) LAST DOSIS OF FMD VACCINE WAS APPLIED
- 1999: (Nov) ARGENTINA WAS DECLARED FREE OF FMD WITHOUT VACCINATION (OIE)

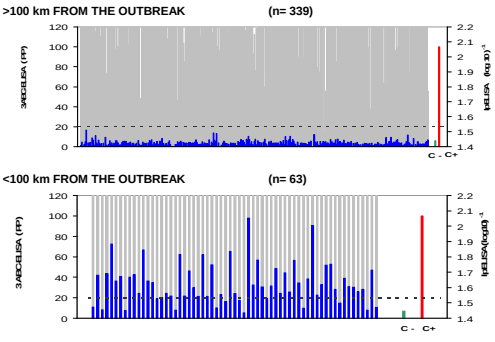
HOWEVER, HAPPINESS IS NOT FOREVER….

FOOT AND MOUTH DISEASE EPIDEMIC IN ARGENTINA 2000 - 2001



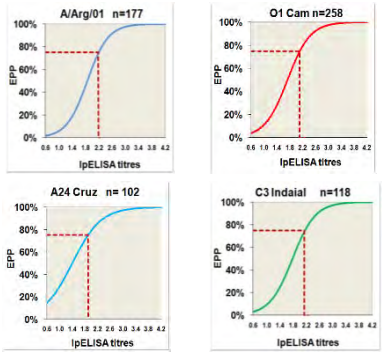
N. Mattion et al. Vaccine 22(2004) 4149-4162

VIRAL CIRCULATION IN THE FIELD IN 2001 (3ABC-ELISA)



B. Robiolo et al. Vaccine 24 (2006) 997-1008

2001: VACCINE STRAIN UPDATING



E. Maradei et al. Vaccine 26 (2008) 6577-6586

CURRENT SITUATION

- 2003: RIIDFA- INTENSIVE VACCINE MATCHING ACTIVITIES. COOPERATION WITH VAR INST OF BELGIUM. STRENGTHENING OF REGIONAL COOPERATION
- 2007: PUBLIC LABORATORIES OF RIIDFA ENTER UNDER ISO QA CERTIFICATION
- 2009: SENASA WAS DESIGNATED AS OIE FMD REFERENCE LABORATORY



FINAL REMARKS

1. VACCINATION IS A VALID AND IMPORTANT TOOL TO CONTROL FMD WITHOUT THE RISK OF DISEASE SPREADING
2. HERD IMMUNITY SURVEYS ARE RELEVANT FOR MONITORING THE EFFECTIVITY OF THE VACCINATION PROCESS
3. MAbs ARE VALUABLE TOOLS FOR FIELD STRAIN IDENTIFICATION AND VACCINE CONTROL
4. INDIRECT METHODS ARE A RELIABLE ALTERNATIVE TO IN VIVO POTENCY TESTING
5. IT IS IMPORTANT TO BUILD AND MAINTAIN INTERNATIONAL REFERENCE BANKS OF SERA, INCLUDING MAbs
6. CONSOLIDATE AND EXPAND VACCINE AND Ag. BANKS AND PROMOTE NETWORKING ACTIVITIES

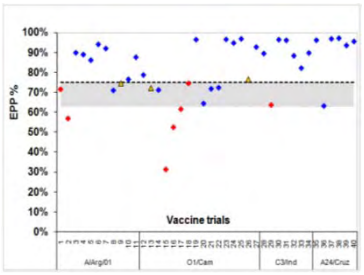
THANK YOU….



INTEGRATED PROCEDURES FOR VACCINE CONTROL SUMMARY

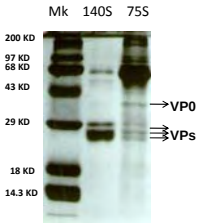
I	EPIDEMIOLOGICAL STUDIES	• MATCHING OF VACCINE AND FIELD STRAINS • MAbs/SEQUENCING • VN/CROSS PROTECTION
II	IN PROCESS CONTROL	• CELL AND VIRUS BANKS. IDENTITY AND PURITY OF VACCINE STRAINS (MAbs) • QUANTIFICATION OF 140S (SUCROSE GRADIENTS + MAbs) IN MONOVALENT AND POLYVALENT SUSP. • FREE OF NSP (rAg + MAbs)
III	FINAL PRODUCT	• INOCUITY • ALL SEROTYPES AS 140S (SUCROSE + MAbs) • POTENCY: INDIRECT (lp ELISA) • POTENCY PPG (ALEATORY) • FREE OF NSP
IV	EFFECTIVITY OF VACCINATION	• HERD IMMUNITY (slp ELISA) • VIRAL CIRCULATION (3ABC ELISA)

2001: VACCINE STRAIN UPDATING

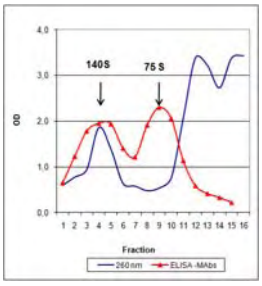


E. Maradei et al. Vaccine 26 (2008) 6577-6586

PAGE-SDS



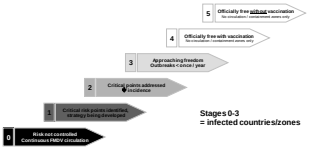
SUCROSE GRADIENT



Regional Roadmaps and the Progressive Control Pathway (PCP): - lessons learnt in promoting monitoring and risk based FMD management in endemic regions of Eurasia

[1] Prepared by:
Sumption K (1) , BenYoussef, A (1), Potzch, C (1), McLaws, M (1) , Ferrari G (2) and Lubroth J (2) ,

(1) European Commission for the Control of Foot-and-Mouth Disease (EuFMD), FAO, Rome, Italy, (2) EMPRES Animal Health, AGAH, FAO, Rome, Italy



Stages 0-3
= infected countries/zones

The challenge

- To initiate monitoring of FMD in all countries considered endemic
- To support countries interested to develop (or improve) their FMD risk management
- To enable countries to compare their FMD risk management progress
- To create a system that fairly rewards progress in reducing risk

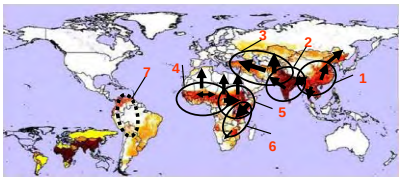
- →→PCP-FMD
- Progressive Control Pathway for FMD

Global Control through Regional Roadmaps for each of the seven virus pools

- a recommendation of the 2008 Open Session of the EuFMD research group
- 7 virus pools recognised by the OIE/FAO FMD lab network
  differ in FMDV antigenic types/required vaccines, risk factors and control capacities, requires tailored approach
- FAO follow-up has been to develop the PCP approach – first applied at the Shiraz Regional Workshop in November 08

Virus pools and Regional Roadmaps

- SEVEN FMDV virus pools - common strains/ risk
- Regional Roadmaps exist - for South-East Asia (SEAFMD campaign) and South America
- NEW - West Eurasia Roadmap -since 2008
- African Roadmaps (3) developed 2009
- Gaps – South Asia (Pool 2), implementation (Africa)



The Progressive Control Pathway PCP) for FMD control

- developed by FAO in 2008
- pathway leading from “”endemic”” towards “free status””
- applied in West Eurasia, and for developing Roadmap for Africa ;
- enables assessment of country progress
  - within a Region
  - between Regions
  - self-assessment at National level
  - provide progress indicators for donors/investment
- under study by OIE as a major tool in a Global Approach (FAO/OIE)

2010 Session – brings PCP practitioners and methods developers together - considers uptake of refinements into PCP practice

- Sessions to come –
  - PCP in Practice 1: Lessons from application, relevance to free regions (5 speakers)
  - PCP in practice 2: Methodologies – monitoring, identification of intervention points (6 papers)
- Lab services needed for each stage:
  - Diagnostics session (E. Brocchi, K van Maanen)
- Epidemiology Sessions: relevant to control strategy development (PCP Stage 1-2)
- Vaccine development, control, vaccination campaign monitoring:
  - Relevant to improving PCP Stage 2 (monitoring programmes for control and eradication)
- FAO Consultative Group (expert) meeting on the PCP: next week (4-6th October)
- OIE Scientific Commission - considering the PCP criteria and may set up recognition process for national control strategies that aim at eradication (PCP Stage 3)

Conclusions

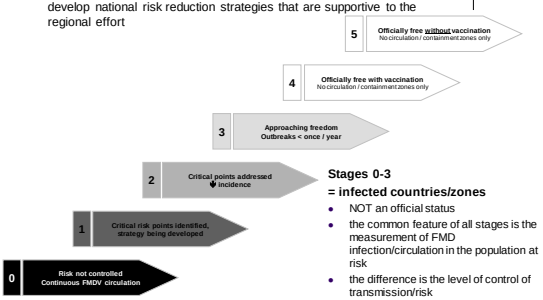
- The Progressive Control Pathway (PCP) for FMD is a FMD risk based management approach which has wide applicability at national to regional scale;
- More technical work is needed :
  - **On Stage 1**, to improve identification of critical risk control points, and socio-economic aspects of strategy optimisation;
  - **On Stage 2** , to include both ""disease control"" and ""pro-eradication"" as valid management options
  - Monitoring vaccination campaigns – what indicators to use?
  - define criteria and assessment processes
  - identify assessment criteria for quality of national FMD risk management (Stage 2)
  - validate the approach in Stage 2 countries
  - resolve issues on sharing (sero-) monitoring data (which has regional-global value)

Conclusions/2

- Need for more "political work" –
  - With OIE, on recognition of National FMD *Pro-Freedom* Control Strategy (= recognition of PCP Stage 3)
  - International application in Roadmaps,
  - Processes for evaluation of country progression

FAO Progressive control pathway - risk reduction approach

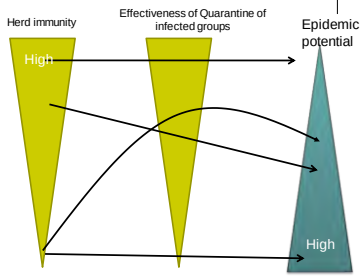
•not a top down prescribed approach: but each MS encouraged to develop national risk reduction strategies that are supportive to the regional effort



Stages 0-3
= infected countries/zones

- NOT an official status
- the common feature of all stages is the measurement of FMD infection/circulation in the population at risk
- the difference is the level of control of transmission/risk

Wide variation in effectiveness of national FMD management



The four PCP Principles

The PCP approach is based on the following principles:

1. active monitoring for FMDV circulation is the basic requirement of a control program, and therefore common action in all stages
   - the monitoring of outcomes (indicators of control), within a national FMD management system, is required at the higher stages;
2. activities in each PCP stage should be appropriate to the required reduction in virus circulation and risk of disease to be achieved;
3. activities and their impacts in each stage are measurable, comparable between countries, and generate information and potential benefits at national as well as to international stakeholders;
4. the optimisation of use of scarce resources for FMD control through the targeting of measures to the husbandry systems and critical risk points where the impact on disease control and/or virus circulation will be greatest;

Stage 0: risk not controlled

- Criteria: no systematic FMDV monitoring system in past 12 months
- Stage 0: characteristics
  - *when:*
  - level of virus circulation (prevalence in serological studies) has not been studied in past 12 months;
  - and/or: outbreaks occur every year
  - and: the impact of control measures (vaccination, quarantines) on virus circulation is not studied or measured

Risk not controlled
Continuous FMDV circulation

Stage 0:

- West Eurasia in 2009
- many countries!
- whether vaccinating or not
- countries that [deliberately?] do not report FMD ....and do not report results of serological surveys are automatically in Stage 0



Stage 0 in red

Lessons learnt

- Stage 0 Criteria - clear
- Countries do not wish to remain in Stage O
- political incentive to undertake Stage 1

Stage 1: critical FMD risk points assessed, national strategy under development

- Main criterion: systematic information gathering on FMD circulation to define possible high risk groups and critical control points
- Stage 1: characteristics
  - when:
  - level of virus circulation (prevalence - NSP positives) has been studied in past 12 months, and indicates virus circulation has occurred
  - the critical risk points associated with the major husbandry/marketing chains are being identified ;
  - and:
    - a strategy is under development to address the CRP

Critical risk points identified, strategy being developed

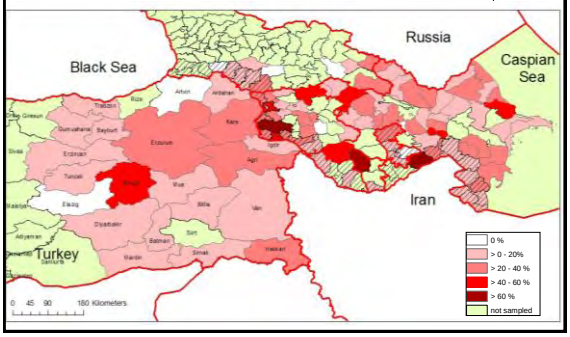
Stage 1 – low cost

- serological survey to identify incidence and risk groups
- identify FMDV strains
- identify Critical Control Points (CCP)
- identify capacity to control and identify willingness to pay
- develop strategy

- *provides valuable surveillance data for risk assessment therefore Stage 1 activities of regional value*

Regional NSP situation – four country sero-survey

Sampling Mid-2008 in 6-24 month animals (true prev.)



Critical control points.....



Lessons learnt

- Keep principles simple, easy to communicate
- Ensure sufficient depth
- Analyse production systems at risk/Market chains first?
- Before designing sero-surveys?

- Application Practiced
- (FAO Projects):
  - 5 Central Asian Countries
  - Bhutan (2010)
  - Egypt (2010)
- Successes, great improvement in information base - but strategy development?
- What about countries not wishing to progress ? (*to implement control)*
- Can they remain in Stage 1? - a case for requiring minimum monitoring?

Stage 2: FMD under control, circulation is progressively reduced

- Main criterion: FMD control strategy has been implemented and monitored (repeated sero-surveys)
- Stage 2: characteristics
  - when:
  - each new outbreak(s) is investigated and potential sources identified
  - level of virus circulation (prevalence in serological studies) has been studied repeatedly for at least 24 months, and evidence of FMDV exposure found in each survey
  - the risk associated with the major husbandry/marketing chains identified, and strategies implemented for each ;
  - and: the impact of control measures (vaccination, quarantines, measures at borders) on virus circulation is being measured

Critical points addressed
↓ incidence

Stage 2: can be high cost

- usually involves vaccination
- but does not prescribe national mass vaccination
- expected that some countries will choose not to effectively implement Stage 2
  - lack of economic incentives and finance
  - importance of regional political pressure and support
  - potential incentives: FMD controlled compartments/commodity based trade

Vaccination is often not enough.....

- very high Ro of virus
- high vaccination cover rarely enough
- gaps remain
- critical control points need to be addressed - stop virus finding gaps



Kevenlik, Turkey: June 4th, 2009

Stage 2: issues

- Should we differentiate between “disease” and “”pro-eradication”” management options?
  - Or is this only real difference between what is acceptable (incidence /trend in critical groups)
- Measuring impact of measures - use of indicators and tolerances (e.g acceptable levels of vaccination performance)
- Can we –should we –compare key indicators across countries (harmonisation?: post-vaccination, incidence surveys?)
- Stage 2 Criteria –include expectations of national decision making processes ?
  - Issues include decentralisation of FMD management
- *Application :* targetting control measures : new, difficult for decision makers
- Need to validate approach -examples include I.R of Iran, Turkey

Stage 3: Approaching freedom; effective prevention and containment measures

- main criterion: FMD outbreaks are *exogenous (no continual circulation)*
- Stage 3: characteristics
  - *when:*
  - each new outbreak(s) is shown to originate outside of the country or zone, not originate within;
  - level of virus circulation (prevalence in serological studies) has been studied repeatedly for at least 24 months, and evidence of FMDV exposure found but being restricted to limited foci or limited time periods;
  - each cluster of infection or outbreaks have a plausible explanation, through outbreak tracing;
  - each outbreak or evidence of infection is followed up by **immediate measures and post-outbreak surveillance**, and review of the impact of control measures (vaccination, quarantines, measures at borders)

Approaching freedom
“FMD Events” < once / year

Stage 3:

- This is where early warning and response are critical
- Surveillance, rather than monitoring – new events require response
- Therefore : - contingency planning is critical
- access to vaccine reserves/banks for emergency
- Control of incursions requires prevention (border..)
- rapid response to risk
- ability to regulate animal movement;
- example:
  - several North African countries in response to the type A Iran 05 incursion into Libya in 2009

Stage 3: lessons

- Some countries claim to be in Stage 3 (but cannot supply sero-monitoring evidence - therefore annual assessment resulted in downgrade)
- Possible recognition of this Stage by OIE as a "recognised control strategy" (aimed at eradication/freedom)
- Proving every outbreak/cluster is an incursion – emphasis on invetigation and molecular typing
- Proving short lived circulation; focus on serosurveillance capacity
- Some countries with continual, short lived incursions (cannot control borders) – really should be Stage 2?

PCP and Roadmaps –application

- West Eurasia (Virus Pool 3)
  - Roadmap -14 countries -developed 2008
  - 1st assessment of progress – October 2009
  - progress on track to achieve 2020 Vision
- Subsaharan Africa (Virus Pools 4-6)
  - Continental Roadmap - developed January 2009 (Nairobi1 meeting)
  - composed of three subregional Roadmaps
  - progress in year 1 mainly to establish FMD Lab networks (part of information base)
  - formidable obstacles !
  - Progress meeting late 2010

West EurAsia Roadmap for FMD Control: Vision : *freedom from clinical disease by 2020*

Regional cooperation among Eurasian countries .....

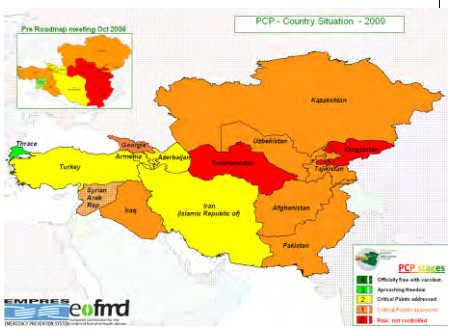
for the progressive control of FMD through public and private partnerships

leading towards freedom of clinical disease by 2020 for regional economic development, food security, and poverty alleviation.



Progress in 2009

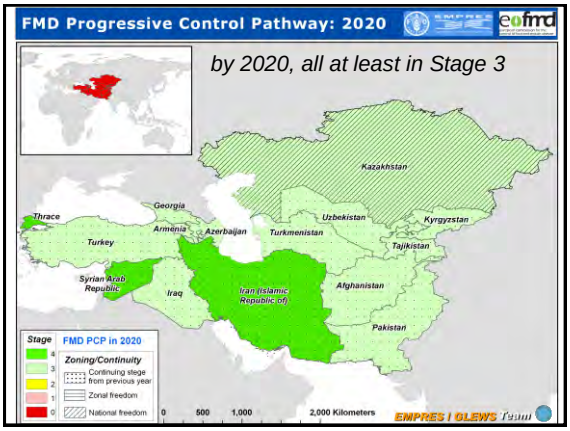
- 10 of the 14 countries undertook sero-monitoring program in 2009
  - TUR, GEO,AZB, ARM, IRAN (pilot areas), PAK, AFG, UZB, TAJIK, IRAQ
  - results pending: Syria, Turkenistan
  - breakthrough - first FMD *reported* serosurveys in several countries
  - high incidence in all (5-10% yearly exposure) except UZB
- incentives largely project driven – but no country wants to be seen as lagging in the Roadmap Annual Progress meeting
- *Negative progress: two countries "downgraded"" - on lack of monitoring evidence submitted*



West EurAsia Roadmap- country Stage position following the Progress Review of 2009, and expected progression to 2020

FINAL assessment of country Stage position for 2009, together with the expected progression to 2020. (Chart2)

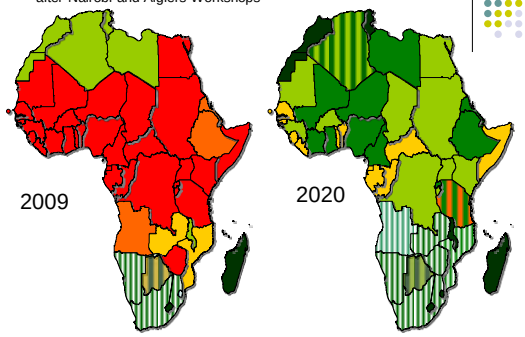
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Comment
West Eurasia	Kazakh													
	Kyrgyz		new											FMD seromonitoring system therefo assumed 2, 3 and 5 years to move
	Tajik		new											progress to stage 1
	Turkmen													
	Uzbek		new											progress to stage 1
	AFG		new											progress to stage 1
	IRN													
	PAK		new											2009: progress to stage 1. Progress
	TURK		new											progress to stage 2
	Finace (TR)			new										easier to OIE in 2010
	Withd zones (TR)													
	Syria		new											Syria considered to be in stage 1 re reporting of seromonitoring expecte
	Iraq													
	Armenia													
	Azerbaijan													
	Georgia	pending	new											2009 : re-assessed as Stage 1, exp Stage 2 in 2011



PCP criteria and processes

- Subject of the FAO Consultative Group meeting in Pirbright, October 4-6th
- criteria – tested in 4 workshops/surveys
- surveillance principles developed
  - require refinement, validation
- technical developments
  - Through application in FAO and other projects in 3 continents
  - Ideas emerging in this Session
- linkage to OIE
  - Under study; recognition of Stages, link to PVS
  - criteria for progress could include PVS evaluation and follow-up

Africa Roadmap progression to 2020
– after Nairobi and Algiers Workshops

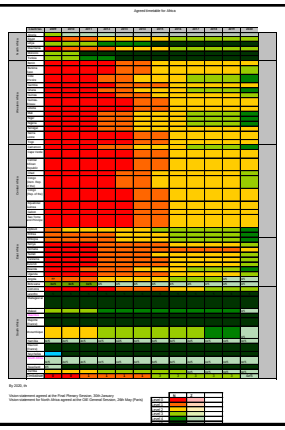


Africa Roadmaps to 2020

Expected PCP progression,

North, West/Central, East and Southern Africa

(Nairobi Workshop, Jan 09 and Algiers, Feb 09)



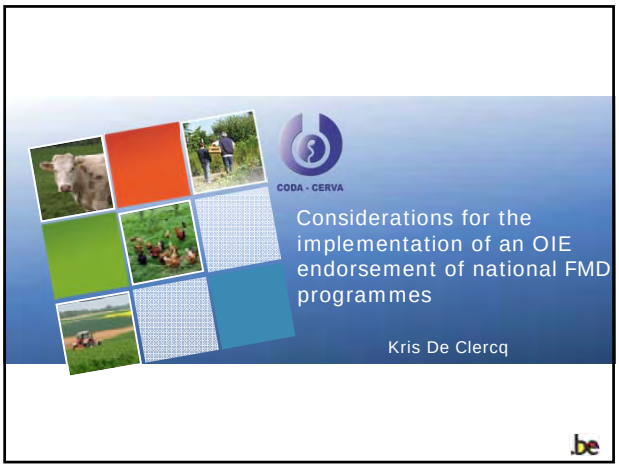
PCP and global FMD intelligence

- If enough countries implement PCP Stage 1 or above,
  - countries in a region can act on the risk
  - Global level: improved information for risk assessment based on incidence and virological threat
- What level of PCP application needed - for sufficient regional and global ""viral intelligence"" monitoring?
  - How many countries per Pool would be enough?
  - How often to repeat sero-monitoring? (if the country does not intend to progress to control?)
- High potential for PCP take-up to address regional –and global information gaps

Acknowledgements

- EUFMD Commission member states
- EC (DG-SANCO –Regional workshops)
- FAO (J. Lubroth, G Ferrari, J Pinto)
- J. Domenech
- OIE (G. Bruckner)
- African Union-IBAR (Pan African Workshop)
- FAO World Reference Laboratory (WRL) Pirbright (D Paton, Jef Hammond)

- Supporting centres:
  - EUFMD Secretariat staff (Nadia Rumich)
  - RAHCs in Nairobi, Bamako, Beirut, Tunis, Gaborone, Nepal



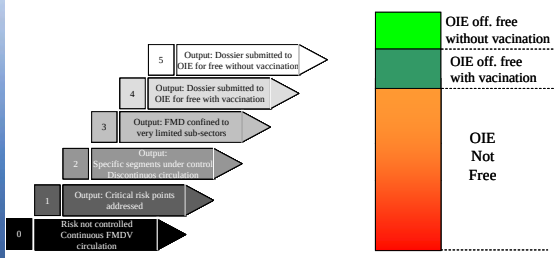
Progressive control pathway for FMD (PCP) / OIE standards

- A possible strategy for global FMD control by linking the FAO PCP approach to the OIE's international standards and the existing procedures of official recognition of FMD free status
  - The OIE ad hoc Group on the evaluation of FMD STATUS of MEMBERS
  - The OIE Scientific Commission for Animal Diseases (SCAD)
  - The OIE Scientific Department
  - FAO representatives

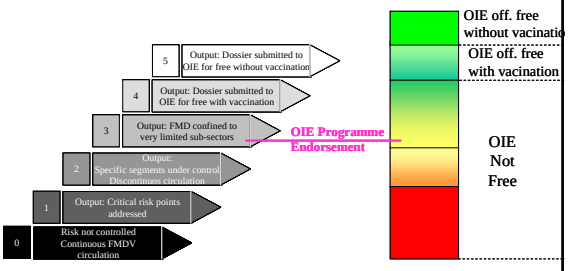
THE OIE AD HOC GROUP ON THE EVALUATION OF FOOT AND MOUTH DISEASE STATUS OF MEMBERS

- Identification of the key components of regional or national FMD control plans and how those plans are implemented
- Review of the FAO Progressive Control Pathway for FMD
- Considerations for the implementation of an OIE endorsement of national FMD programmes
- Amendments to the existing FMD Chapter of the Terrestrial Code in support of recognition by the OIE of 'endorsed national FMD control programmes'
- Consideration of the need for a questionnaire to support Members wishing to apply for endorsement of a national FMD control programme

Relationship between FAO's Progressive Control Pathway and OIE's official FMD statuses.



Proposal to modify the categories recognized by the OIE in regard to FMD



Proposal for amendments to the existing FMD Chapter of the Terrestrial Code

Article 8.5.6.
FMD infected country or zone

An FMD infected country is a country that does not fulfil the requirements to qualify as either an FMD free country where vaccination is not practised or an FMD free country where vaccination is practised.
An FMD infected zone is a *zone* that does not fulfil the requirements to qualify as either a FMD free zone where vaccination is not practised or an FMD free zone where vaccination is practised.

Article 8.5.6bis.
Country or zone with an OIE endorsed FMD control programme

Countries or zones may apply for endorsement of their FMD control programme when they have implemented a control programme that has lead to a reduced occurrence of FMD, identification of the circulating viruses, vaccine matching and a better understanding of the epidemiology.
To qualify as a country or zone having an OIE endorsed FMD control programme, a Member should:
1. have submitted documented evidence on the efficiency of the veterinary services to control FMD. This evidence can be provided through an *Evaluation of Performance of Veterinary Services* (PVS assessment) followed by a gap analysis to address the potential weaknesses and capitalise on the current strengths of the system to sustainably control FMD;
2. submit documentation indicating that the control programme is sustainable;

Consideration of the need for a questionnaire to support Members wishing to apply for endorsement of a national FMD control programme

COUNTRY WITH AN OIE ENDORSED FMD CONTROL PROGRAMME

Report of a Member which applies for recognition of status, under Chapter 8.5. of the *Terrestrial Code* (2010), as a Member with an endorsed FMD control programme applicable to the entire territory or to a zone within the entire territory

Please address concisely the following topics. National regulations laws and Veterinary Administration directives may be referred to and annexed as appropriate in one of the OIE official languages:

1. Introduction

a) Geographical factors. Provide a general description of the country and the zone, including physical, geographical and other factors that are relevant to FMD dissemination, countries or zones sharing common borders and other countries or zones that although may not be adjacent share a link for the potential introduction of disease.
b) If the endorsed plan is gradually implemented
c)

Future Procedure

a) OIE Code Commission

b) OIE Bureau

c) Consultation Member States

d) Present for adoption by the General Session May 2011

Thank you !

Animal Health Service

Achieving stage 1
lessons from West Eurasia Roadmap

Giancarlo Ferrari

AGAH/EMPRES
FAO

Agriculture Department
Animal Production and Health Division

Animal Health Service

Progressive Control pathway (to summarize .....)

Stage	Monitoring	Preventive measures	Surveillance
0	no	no	no
1	yes	no	no
2	yes	yes	no
3	yes	yes	yes

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Progressive Control pathway (to summarize .....)

Stage	Monitoring	Preventive measures	Surveillance
0	no	no	no
1	yes	no	no
2	yes	yes	no
3	yes	yes	yes

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Animal Health Service

Progressive Control pathway (to summarize .....)

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Animal Production and Health Division

Animal Health Service

Progressive Control pathway (to summarize .....)

Stage	Monitoring	Preventive measures	Surveillance
0	no	no	no
1	yes	no	no
2	yes	yes	no
3	yes	yes	yes

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PCP Framework – activities supported for moving from 0 to 1

- Nation-wide serologic survey for the detection of antibodies against non-structural-proteins (NSP) of the FMD virus;
- Collection of tissue samples in reported outbreaks from clinically affected animals and support to ship to World Reference Laboratory;
- Periodical collection of oral swabs from non-clinically affected animals (targeting live animal markets) to be tested in an International reference laboratory (LoA with DTU in Lindholm – Denmark);
- Target studies in specific farming systems (dairy colonies in Pakistan);
- Training for field veterinarians and provision to peripheral veterinary services of FMD rapid diagnostic test;
- Training for laboratory specialist in NSP ELISA (LoA with IZSLER in Brescia) and provide support for laboratory reagents;

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West Eurasia Regional Roadmap



Status of the member countries as self-assessed in Shiraz 2008

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West Eurasia Regional Roadmap



Status of the member countries as determined during the Regional Meeting held in Istanbul, October 2009

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PCP Framework (moving further)

- Moving from stage 0 to stage 1 does not have major implications and implementation of monitoring activities is usually well accepted;
- Moving from stage 1 to stage 2 is a critical step;
- The monitoring system implemented in the previous stage may provide evidence of virus circulation (in absence of official reports);
- Veterinary Services must accept the challenges of designing and implement control activities based on measurable indicators and evaluation of the performances of the control program adopted;

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What has been essential to measure to obtain (or manage) progress? What can we further improve?

Model:

FMD Control in Israel

Hagai Yadin



What has been essential to measure to obtain (or manage) progress? What can we further improve?

Model:

FMD Control in Israel

Hagai Yadin

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FMD Control in Endemic Areas **Consist on**

Control policy by Prevention

- **Veterinary Services**
- **Registration**
- **Surveillance**
- **Vaccination**
  - Vaccine availability



FMD Control in Endemic Areas

Consist on

Control policy
By Prevention

- **Veterinary Services** - V
- Registration -
- all livestock tagged & central registered.
- Surveillance
- Vaccination



What has been essential to measure to obtain (or manage) progress? What can we further improve?

1

- Progress in FMD control = Progress in vaccination campaign .
- Progressive expanding the number of vaccinated herds:
- Dairy cattle.
- Native cattle / beef.
- Small ruminants.
- Aim – 150% vaccinations (young animals twice a year)

FMD Control in Israel an endemic area

FMD Susceptible live stock in Israel

- Dairy cattle- 120.000
- Feed lot - 100.000
- Beef cattle 200.000
- Sheep -1.200.000
- Goats - 50.000
- Pigs - 30.000



Composition of FMD Vaccine
used in Israel

- Cattle Vaccine – Tri valent

O O1 Manisa
O - Geshur Isr/2/85
Prefer O1 Iran 2005
O – 3039

A A22
A Iran 2005
A 4165

Asia1 Shamir

- Sheep vaccine – Bi valent

O O1 Manisa
O - Geshur Isr/2/85
Prefer O1 Iran 2005
O – 3039

A A Iran 2005

What has been essential to measure to obtain (or manage) progress? What can we further improve?
2

- Evaluate status presence of FMD.
- Virus isolation out suspected cases.
- Trained staff for samples collection from suspected cases.
- Laboratory facilities and staff for evaluating the suspected samples.

What has been essential to measure to obtain (or manage) progress? What can we further improve?
3

- Evaluate status presence of FMD.
- During FMD free period
- samples collection according to statistical and districts planed surveillance program for Virus isolation & NSP .

- **Trained staff for samples collection.**
- **Laboratory facilities and staff for evaluation of the collected samples.**

FMD Control in Endemic Areas

Control policy

- **Vaccine Control**
- **Upon arrival each batch**
- **Tested for serological response in 10-15 calves.**



FMD Control in Endemic Areas

Control policy - Surveilance

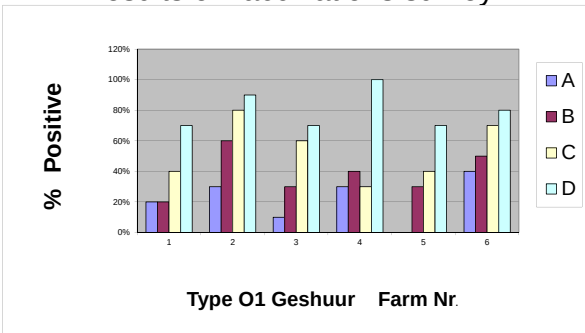
Dairy cattle
June / November
6 Dairy farms
10 animals of 4 age groups

Beef cattle
1Xyear, 4 herds

Sheep 1Xyear
6 herds
10 sheep
15 lambs

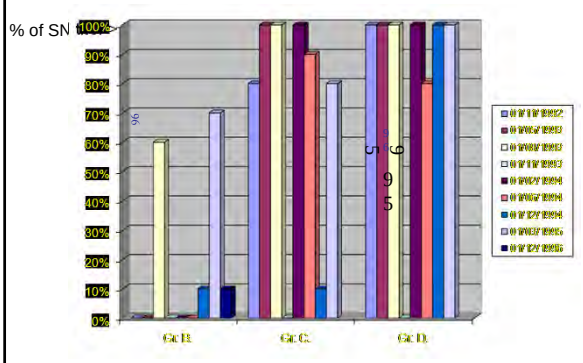


Results of vaccinations survey



Sero survey of annual FMD vaccinations

92 – 96



What has been essential to measure to obtain (or manage) progress? What can we further improve?

4

- Progress in FMD control = Progressive cooperation of FMD control between neighboring countries

- Adaptation the same control policy.
- Adaptation the same vaccine composition – tailored to the isolates in the region

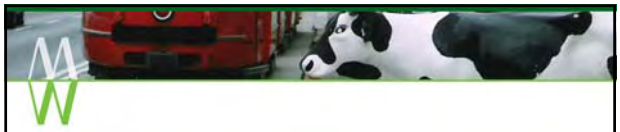
in Endemic Areas

Veterinary Services

8 Regional offices

- Akko
- Kiryat Shmona
- Tiberyas
- Afula
- Nazareth
- Hadera
- Rechovoth
- Beer-Sheba
- Head Quarter & KVI





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EUFMD

Thrace – from PCP stage 3 to stage 4

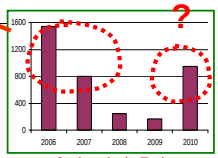
FMD exposure limited in space & time → FMD free zone with vaccination, since 2010



EUFMD

FMD outbreaks in Thrace

Year	No. of outbreaks	Serotypes
2000	-	-
2001	1	O
2002-2005	-	-
2006	16	A, O
2007	4	A, O
2008-2010	-	



Outbreaks in Turkey, until 9/2010

Problems:

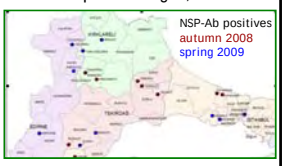
- ruminant movements (prices & meat preferences)
- situation around Kurban Bayram (Sacrifice Festival)
  → large scale live ruminant movements, informal markets, return animals from markets
  → Istanbul demand: 80,000 cattle & 200,000 sheep

EUFMD

Measures in Thrace



- Sero surveys in cattle since 2000 and small ruminants; follow-up investigations of NSP-Ab positives since 2003
- Outbreak investigations in Thrace since 2006
- Slaughter of animals infected, in-contact or suspected of being infected with FMD since 2006, and compensation
- Live ruminants moved to Thrace from Anatolia only for immediate slaughter in authorised abattoirs
- Checks for animal transportations at the two Bosporus bridges, infra red cameras since 2009/10
- Kurban Bayram:
  - 2008/09: only authorised markets and slaughter sites, slaughter of all animals
  - 2010: only animals from free countries



EUFMD

… and in Turkey



- Political support to control FMD & transparency about the FMD situation
- FMD outbreak control measures incl. movement control, vaccination & biosecurity
- Mass vaccination: 2x p.year LR, 1x p.year SR (free of charge since 2008)
- Increase of FMD awareness and notification
- transport of ruminants only from provinces with ≥ 85% vaccination coverage, since 2008
- Animal ID system (TurkVet); linked with FMD vaccination status, automated movement checks since 2009

EUFMD

International assistance

FAO/EuFMD/EC:

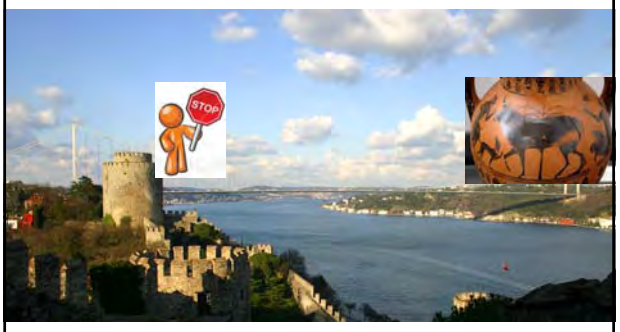
- sero-surveillance for Thrace (2003-08) and Anatolia (2007/08), incl. diagnostic support
- FMD outbreak investigations in Thrace & Anatolia (2004-08)

EC:

- 2007-10: mass vaccination and sero-surveillance (TR 060302)
- 2009-10: FMD awareness and support of OIE FMD freedom dossier (TR 0603.02-08/001)



EUFMD



European Commission | Directorate - General for Health and Consumers

Maintaining FMD Freedom

Dr. Alf-Eckbert Füssel

Unit for Animal Health and Standing Committees

This presentation does not necessarily represent the views of the Commission

Main Principles

1. Keep it out
2. Detect it fast
3. Control it vigorously
4. Re-establish free status quickly

2

Keep it out

- Import policy
  - Live susceptible animals
  - Products possibly carrying FMD virus
  - Personal luggage and mail
- Keep and move animals with disease in mind
  - biosecurity on holdings
  - responsible animal husbandry
  - prohibition on swill feeding
- Control disease at source
- Obtain FMDV intelligence

3

Detect it fast

- Passive surveillance
  - Notifiability
  - Compensation and Enforcement
  - Training
- Active surveillance
  - Targetted surveillance (similar to AI and BT)
  - Health programmes (other diseases)
  - Trade and export inspection and testing
  - Ante- and post mortem at slaughterhouses
- Identification & traceability
- Investigation and follow-up of suspicions
- Laboratory capacity for confirmation

4

Control it vigorously

- Legislation on FMD control measures
- Budget !!!!!!!!!!
- Contingency planning/ exercises
- Antigen / Vaccine stocks
- Limit impact
  - Zoning and compartmentalisation
  - Release save animal products as early as possible

5

Re-establish free status

- Trained personell for clinical inspection
- Laboratory capacity for mass screening
- Intelligent post-outbreak surveillance
- Cleansing and disinfection
  - sufficient equipment
  - approved disinfectants
- Equipment to restrain animals

6

FMD Week2010

FMD risk assessment and threat detection

Dr Jef M Hammond, Donald P. King, Nick J. Knowles, Jemma Wadsworth, Bob Statham, Yanmin Li, Phil Keel, Jo Stoner, Anna Ludi, Pip Hamblin, Ginette Wilsden, Geoff H. Hutchings, Nigel Ferris, Valerie Mioulet, Faizah Hamid, Miki Madi, Elizabeth Wilson.

Institute for Animal Health, Ash Road, Pirbright, Surrey, GU24 0NF, UNITED KINGDOM

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Background

- FMD most contagious disease of livestock
- 7 serotypes and multiple subtypes
- No single vaccine
- No single test
- Constant threat to free countries
- Major constraint to endemic countries
- Very high costs – preparedness : control

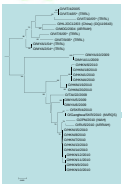


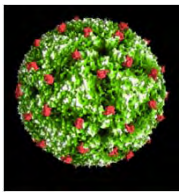
Talk Overview

- Middle and Near East
- Far-East
- Africa
- Asia
- South America
- Somewhere new?

•Where is it? risk assessment and threat detection

Talk Overview









•What is it?

•and What is it doing?

Talk Overview





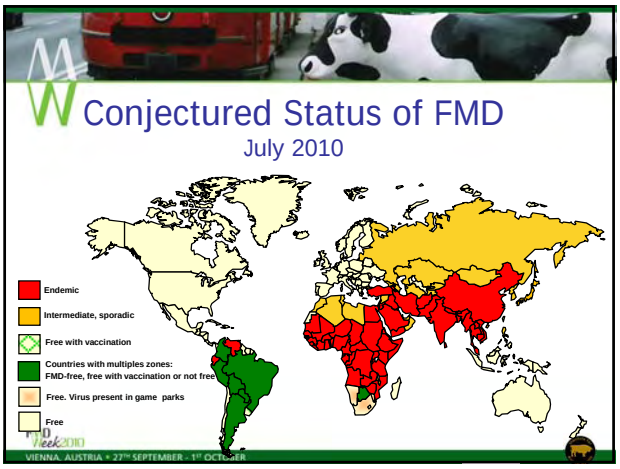
•Do we need to do something about it?

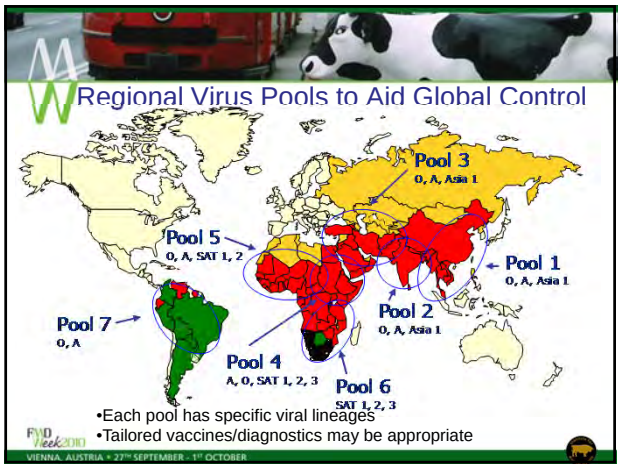
•What can we do?

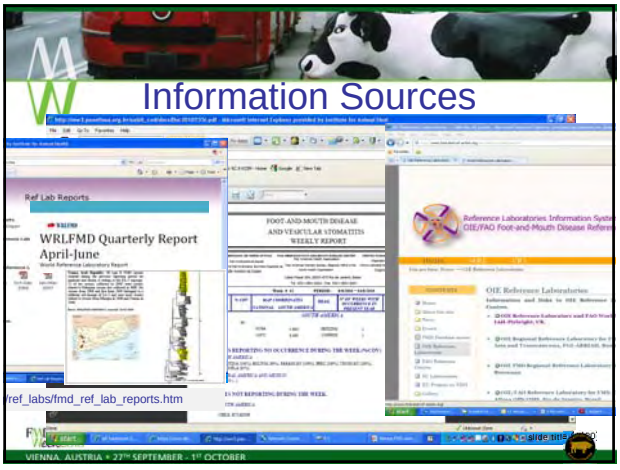
Where is it?

- Middle and Near East
- Far-East
- Africa
- Asia
- South America
- Somewhere new?



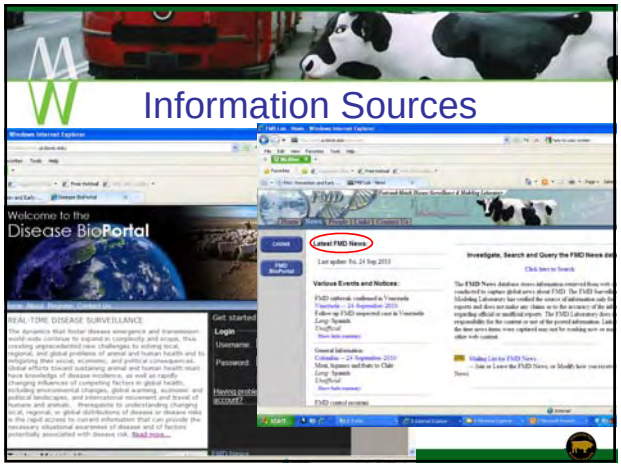

Conjectured Status of FMD
July 2010
Endemic
Intermediate, sporadic
Free with vaccination
Countries with multiples zones: FMD-free, free with vaccination or not free
Free. Virus present in game parks
Free
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Regional Virus Pools to Aid Global Control
Pool 3
O, A, Asia 1
Pool 5
O, A, SAT 1, 2
Pool 1
O, A, Asia 1
Pool 7
O, A
Pool 2
O, A, Asia 1
Pool 4
A, O, SAT 1, 2, 3
Pool 6
SAT 1, 2, 3
•Each pool has specific viral lineages
•Tailored vaccines/diagnostics may be appropriate
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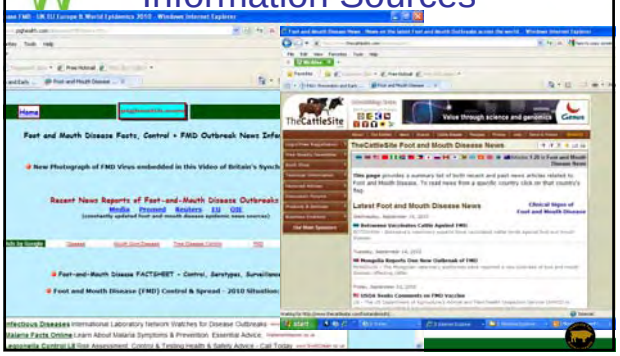

Information Sources
Ref Lab Reports
WRLFMD Quarterly Report
April-June
/ref_labs/fmd_ref_lab_reports.htm
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Information Sources
EMPRES
WAHID Interface
Weekly Disease Information
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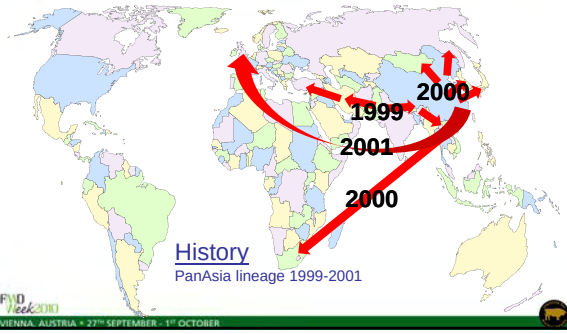

Information Sources
SEAFMD Campaign
SEAFMD Campaign - Home Page
News and Events
VIENNA. AUSTRIA


Information Sources
Welcome to the
Disease BioPortal
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Information Sources



Information Sources



Information Sources



Samples sent to Pirbright 2009

- ~1000 samples from 36 countries
-
- ~500 positive (523 in period 2006-2008)
- ~483 sequences (330 in 2008)
- Predominantly O and A serotype
  - A-Iran-05 lineage 30%
  - O-PanAsia 2 44%

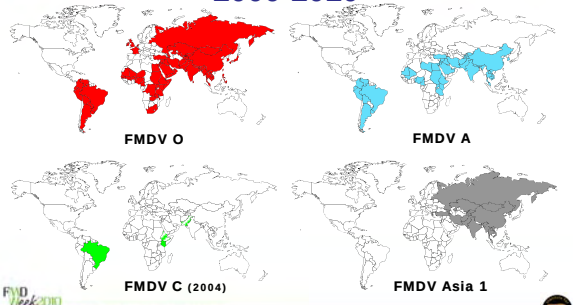
WRLFMD

Samples sent to Pirbright 2010

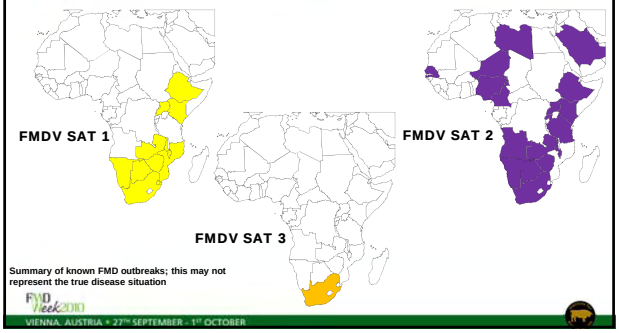
- ~1000 samples upto August from 20 Countries
- ~600 positive
- ~350 sequences
- Predominantly O and A serotype
  - A-Iran-05 lineage
  - O-PanAsia 2

WRLFMD

FMDV Eurasian Serotypes 2000-2010

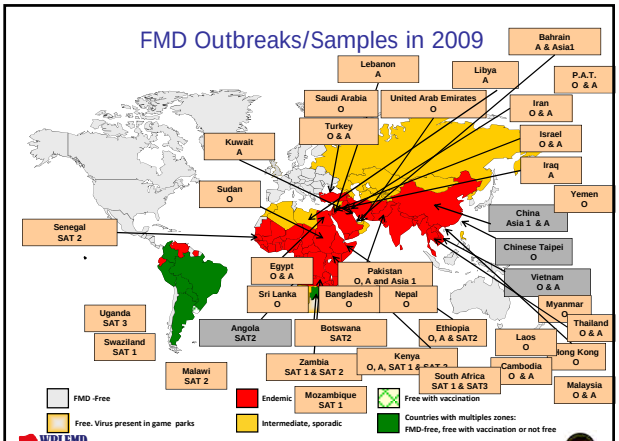


FMDV SAT Serotypes: 2000-2010

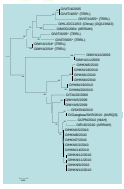


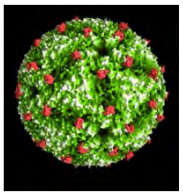
Summary of known FMD outbreaks; this may not represent the true disease situation

FMD Outbreaks/Samples in 2009



What is it?









- What is it?
- and What is it doing?

Diagnosis



Approaches:

- Detection of FMD virus, antigen or nucleic acid
  - Virus Isolation
  - Antigen Elisa
  - LFD
  - Automated TaqMan® RT-PCR



- Detection of FMDV-specific antibody (SP/NSP)
  - Virus Neutralization Test
  - Liquid Phase Blocking Elisa
  - Solid Phase Competition Elisa
  - 3ABC Elisa- (NSP)



Vaccine Matching by Serology

38 isolates tested by VNT

9/38 = match for A22 vaccine

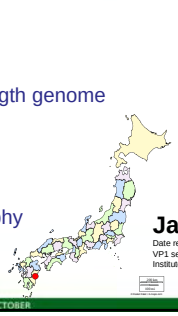
36/38 = match for new vaccine - A Tur 06

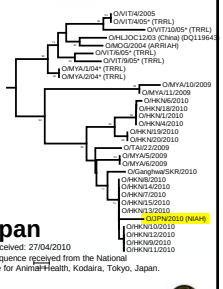
Field Isolate (WRL Ref No.)	Topotype	Strain	A22 Iraq	A TUR 06
			VNT	VNT
Bar 06/2008	Asia	Iran-05BAR-08	0.23	0.60
Bar 02/2009	Asia	Iran-05AFG-07	0.38	0.72
Irn 36/2007	Asia	Iran-05	0.21	0.39
Irn 39/2007	Asia	Iran-05	0.13	0.69
Irn 01/2008	Asia	Iran-05	0.15	0.74
Irn 02/2009	Asia	Iran-05BAR-08	0.35	0.93
Irn 06/2009	Asia	Iran-05BAR-08	0.1	0.38
Irn 23/2009	Asia	Iran-05BAR-08	0.07	0.35
Irn 25/2009	Asia	Iran-05BAR-08	0.08	0.35
Irq 10/2009	Asia	Iran-05BAR-08	0.27	0.74
Irq 17/2009	Asia	Iran-05BAR-08	0.32	>0.83
Irq 21/2009	Asia	Iran-05BAR-08	0.11	0.6
Irq 24/2009	Asia	Iran-05BAR-08	0.53	
Isr 02/2009	Asia	Iran-05BAR-08	0.28	

Characterisation by Sequencing

- Sequencing VP1
- Sequencing P1
- Sequencing full length genome

- Phylogeny
- Tracing
- Antigenic profiling
- Antigenic cartography





Japan

Date received: 27/04/2010

VP1 sequence received from the National Institute for Animal Health, Kodaira, Tokyo, Japan.

FMD Reports in 2010

Japan: In early April 2010, FMDV type **O** was reported

- almost 300 outbreaks were reported.
- The virus identified as Southeast Asia (SEA) topotype (Mya-98 lineage)
- and found to be closely related to viruses detected in the P.R. China, Hong Kong SAR, Republic of Korea, Myanmar and Thailand.
- First outbreak of FMD in Japan since 2000.

Mongolia: Outbreaks of FMD type **O** reported in cattle.

- A VP1 sequence submitted to the WRLFMD by ARRIAH showed was also of the SEA topotype, (Mya-98 lineage);
- Importantly, it was most likely a different introduction to outbreaks above, as it is most closely related to viruses from Thailand and Malaysia from 2009.

What can we do?



- Do we need to do something about it?
- What can we do?

Build Bonfires



Form Networks

- OIE/FAO FMD Reference laboratory network
- FMD Vaccine Bank holders network
- Global FMD Research Alliance

To make available accurate and timely global surveillance and research information

- Value of sharing information
- Developing trust and shared vision

Enhanced Surveillance: OIE/FAO Lab network

- WRLFMD: Pirbright, UK
- RRLSEA: Pakchong, Thailand
- LVRI: Lanzhou, China
- FGI ARRIAH: Vladimir, Russia
- PDFMD: Mukteswar, India
- RRLSSA: Gabarone, Botswana
- FMD-Laboratory: Embakasi, Kenya
- PANAFTOSA: Rio de Janeiro, Brazil
- LFADLCT: Argentina
- ARC-OVI: Onderstepoort, RSA
- PIADC: Plum Island, USA
- CODA-CERVA-VAR: Ukkel, Belgium



Approximately ~2400 samples tested during 2009

Vaccine Recommendations (Antigen Banks)



VACCINATE

HIGH PRIORITY	O Manisa O BFS or Campos A-Iran-05* A24 Cruzeiro A22 Iraq Asia 1 Shamir SAT 2 Saudi Arabia (or equivalent)
MEDIUM PRIORITY	A Argentina 01 A Iran 96 A Iran 99 A Eri A Ira 87 Saudi Arabia 23/86 (or equivalent) [illegible] 97 (or Thai equivalent such as A/NPT/TAI/86) O Taiwan 97 (pig-adapted strain or Philippine equivalent) SAT 1 South Africa SAT 2 Zimbabwe
LOW PRIORITY	A15 Bangkok related strain A Kenya A87 Argentina related strain SAT 1 Kenya SAT 2 Kenya SAT 3 Zimbabwe C Noville

Vaccine Recommendations (Antigen Banks)

Vaccine recommendations for free countries included in each WRLFMD quarterly report:

Based on current information

Can we?

- Improve process for vaccine recommendations
- Increase information made generally available
- Provide a clearer understanding of risk
- More integrated approach

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So how do we improve the situation?

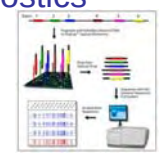
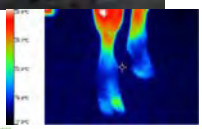
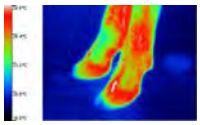
- Information
- Awareness and reporting
- Surveillance
- Strong veterinary service
- Strong policies
- Highly skilled laboratory staff
- Correct samples
- Rapid diagnosis
- Communication/Cooperation/Collaboration
- Networks
- Good vaccines
- A plan!

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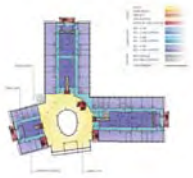
So how do we improve the situation?

Next Generation Diagnostics




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Build a new lab
The New Pirbright

But also
New FLI
New Plum Island!



What Can We Do? Global Activities

- Development of new vaccines
- Training
  - Interlaboratory proficiency tests
  - Field and Lab
  - Bio-security
- Improve information flow to laboratories
- Improve information flow to decision makers
- Next 4 talks address some of these issues

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Institute for Animal Health

Food and Agricultural Organisation of the United Nations www.wrlfmd.org

World Reference Laboratory for Foot-and-Mouth Disease

Improve Quality of Information for

- **Pools**
- **Regions**
- **Countries**
- **Governments**
- **Industry**
- **Animal Health Agencies**
- **Farmers**




And Farmers of Tomorrow

Thank you for your attention

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INTER-EPIDEMIC PERIOD IN WEST EURASIA:

ARE MAJOR FMD EPIDEMIC PREDICTABLE?

The CLOSED SESSION of the STANDING TECHNICAL COMMITTEE of the EUFMD, 29th September-1st October 2010, Vienna

BULUT, A.Naci[(1)]; OZYORUK, Fuat[(1)]; ALKAN, Musa[(1)]; and POTZSCH, Carsten[(2)] , SUMPTION,J- K.[(2)]

1. **Sap (FMD) Institute, Ankara, Turkey**
2. **FAO/EUFMD Rome, Italy**

Conclussion

- Contries in the West EurAsia Regions are endemic, some intermediate sporadic by FMD.
- The region has been experienced highly effective epidemic situation periodicly.
- It is essential that FMD epidemic could be predicted before the occurence in order to put into force control measures.
- It is possible by monitoring and analysing of following indicators:
  - Molecular epidemiological (nucleotid sequencing analysis) data,
  - Meat price and animal trading/movement patterns
  - R values as indicator of vaccine sufficiency
  - Serosurveillance results- NSP positivity rate and PI/PV antibody levels

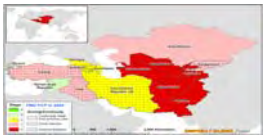
Conclussion(2)

- All the indicators are highly valuable tools to predict the epidemic situation.
- However, some of them is not practical and able to gain the information in time.
- Serosurveillance results is one of the most important data showing the risk; but not supply the warning early enough. However, analyzing cross-border differences of positive ratio can help to understand coming the risk.
- Tracing duration of PI antibody is a good signal for prediction. Because, the changing the generation and fading away PI antibody in a period are generally resulted high risk for a new epidemic.
- PV antibody level, particularly differences young population from the older can provide a meaningful information
- R value is another important signal, but not all time supply us prediction value (like 2007 Turkey O PanAsia II epidemic). And also indication is most likely take place lately the event.

Conclussion(3)

- Animal movement and trading has high risk on the a new virus incursion. Since meat price chainging cross-border countries is highly effective on the movement and trade pattern, it should be monitored together with movement and trade pattern in order to asses upcoming the epidemic risk. These tools are practical, easy and valuable to predict the FMD epidemic.
- Molecular epidemiological analysis data is the most important method for prediction. The method can supply us crucial information on the early warning. However, the method should covered systematically all countries into the region in order to supply us timely meaningful information. Current system is not enough for achievement this goal.
- It is concluded that it is possible to predict the major FMD epidemic before the spread using mainly molecular methods, epidemiological investigation and monitoring meat prices/animal movement helping the others indicators.

Introduction



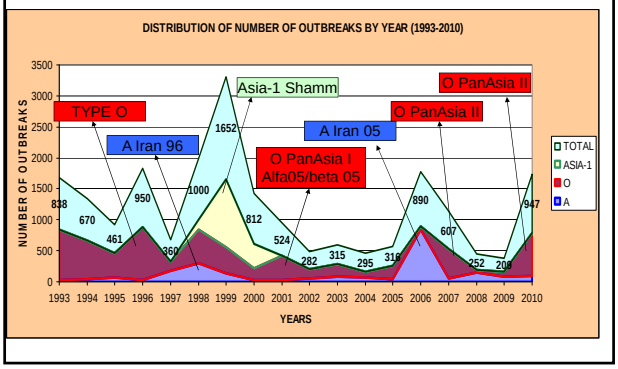
- The West EurAsia Region has almost the same unique condition regarding to disease spread dinamics and take place in the virus pool 3.
- Contries in the regions are endemic, some intermediate sporadic by FMD.
- The region has been experienced highly effective epidemic situation periodicly.
- The common border areas are extremly important for maintaining FMD.
- No exact data whether FMD persists mainly independantly in each country or continual transborder movement. However, observation and latest molecular analysis suport the second one is more important for maintaining of the disease
- Highly vaccination coverage achieved, but important gaps remain; biosecurity, quarantine…
- FMD risk related to young animal and lack of effective early and booster vaccination,

FMD EPIDEMICS PROGRESS INTERVALY?

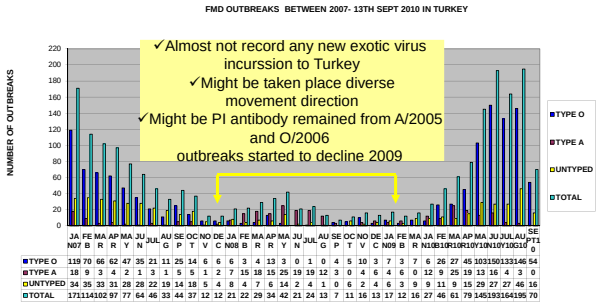
When it is analysed the progress of the FMD in Turkey:

- Altough the disease has persisted all time due to the different serotype of FMDV, some lineages cause the major epidemic periodically (mainly 5 years interval).
- Why the disease declines in this interval?
- It might be probable that;
  - PI antibody remained from the previous major aoutbreak can repress the circulated virus
  - No new virus incursion
  - PV antibody level is sufficient to immune the population
  - Means by a good control measures, virus sirculation can be stopped in the place in which it detected as earlier as possible and no further transmission to another place.
- Period of 2008-2009 in Turkey is a good example of this scenerio.
- This rewiev tries to explain possibility of prediction of the major FMD epidemic using mainly this Turkish progressive disease data and also sometimes the others in the region.

Progressive Major Epidemic In Turkey



Disease Situation



8

Factors affecting occurrence

Host population (built up of susceptible population)
- Immunity (vaccination, booster vaccination, field immunity)
- age, breed, constitution
- …

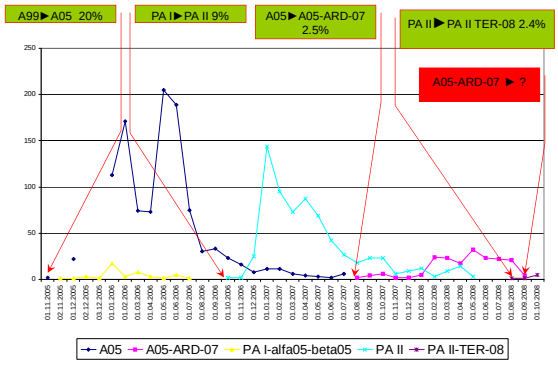
FMD virus
- Antigenic drift induced by mass vaccination
- Antigenic variation (molecular technices)
- New introductions
- Amount of introductions
- …

Environment
- Trade, movements
- control measures, biosecurity
- Climatic, weather
- …

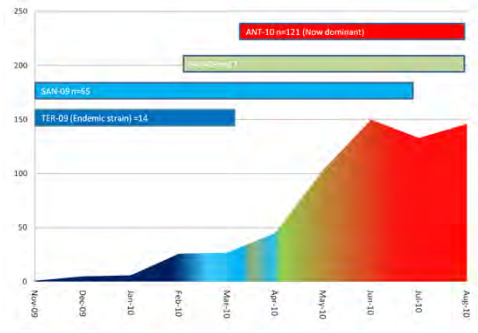
Molecular Epidemiological Analysis for Prediction of FMD Epidemic

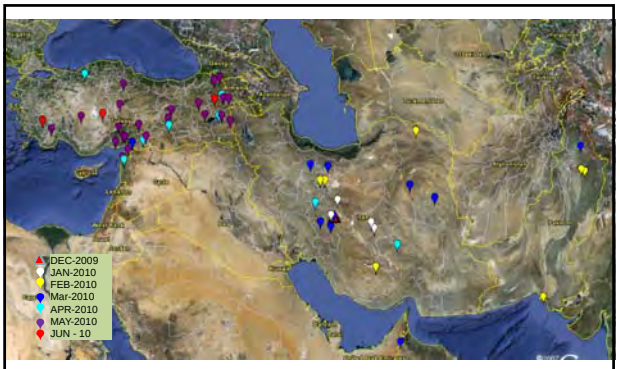
- Genetic change of FMDV can be easily developed inherintly.
- During the epidemic period, clade/genetyping transitions can be formed and resulted as genotyping diversity.
- Although some of lose the their virulence in a short period, the others can be persist dominantly in the population.
- The persistant clade/genotype of the virus causes a severe epidemic in population, when introduced.
- However, this virus also can lose its virulence, when accomplish its the evolution. But it can be stillstand the risk for another population.
- Major FMD epidemic can be predicted succesfully by performing genetic analysis used molecular technics in order to follow the cycle of the virus evolution.
- Although this analysis has been carried out partly in the region, it is not enough to fully prediction.
- It should be done as possible as in time and in place in the region

Clade&genotype transitions and VP1 diversity in the last 3 years



Recent events in FMD type O in Turkey





O PanAsia – II ANT-10 GLOBAL SITUATION

Importance of early detection of enterance new exotic viruses!

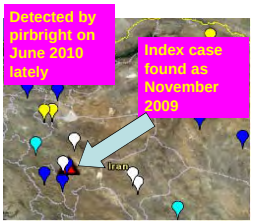
Detected first time by Şap Institute on 19th March 2010

ISLAHİYE, ANTEP



Detected by pirbright on June 2010 lately

Index case found as November 2009



- **Risk for entrance exotic virus is very high any time, depend on occurrence animal movement.**
- **It is not possible to estimate as possible earlier, unless sample sent to WRL in time or carried out the molecular analysis in place.**
- **To eliminate this high risk in current situation, it needs to implement proper and sufficient control measures in order to stop the virus circulation in first detected place.**

Monitoring the Meat Price

Early 2009

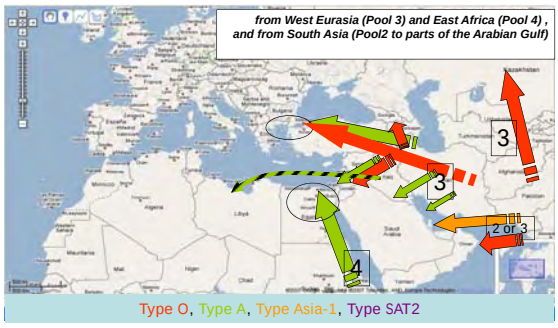
in USD	Cattle/beef				Sheep				Pigs/pork			
	districts		capitol		districts		capitol		districts		capitol	
Country	live	meat	live	meat	live	meat	live	meat	live	meat	live	meat
Turkey	3.7	6.2	5.9	9.5	3.7-4	8.1	4.0-4.4	8.7-10	n.a.	n.a.	n.a.	n.a.
Iran	2.3-3.3	7	3.3-4.3	9	3.2-4.2	7	3.9-4.9	9.5	n.a.	n.a.	n.a.	n.a.
Georgia	1.5-2	4.2-5	n.a.	5-6.5	3-3.7	5.5-6	3.7-4.3	6-8	3	6-7.3	n.a.	6.5-7.3
Armenia	1.6-2.5	3.9-4.4	n.a	4.1-4.7	2.4-3.7	4.9-6.8	n.a.	5.9-6.8	2.9-3.5	5.4-7.1	n.a.	6.8-8.2
Azer-baijan	4-4,5	6,2-7.2	5-5,5	8-9	5,5-6,5	8-9	6-7	10-11	n.a.	n.a.	n.a.	n.a.

Mid 2010

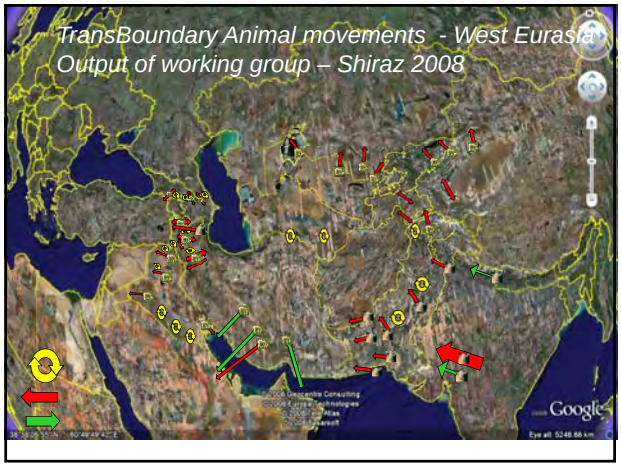
in USD	Cattle/beef				Sheep				Pigs/pork			
	districts		capitol		districts		capitol		districts		capitol	
Country	live	meat	live	meat	live	meat	live	meat	live	meat	live	meat
Turkey	6.7	10.5	7.5	11.5	6.9	12.5	7.5	13.7	n.a.	n.a.	n.a.	n.a.
Iran									n.a.	n.a.	n.a.	n.a.
Georgia	2.0-2.2	4.3-5.0	n.a	4.3-6.5	3.5-4.3	5.0-5.5	4.0-4.5	6.0-6.5	2.2-2.9	4.3-4.9	n.a	4.3-6.5
Armenia	2.7-3.5	5.4-5.9	n.a	5.3-5.9	3.3-4.3	6.8-8.1	n.a.	7.3-8.2	2.9-3.1	5.4-6.7	n.a	5.9-7.1
Azer-baijan	4,5-5	6,5-7,5	5,5-6	9-10	5,5-6,5	8-9	6,5-7	10-11,5	n.a.	n.a.	n.a.	n.a.

Monitoring the Animal Movement and Trade Pattern

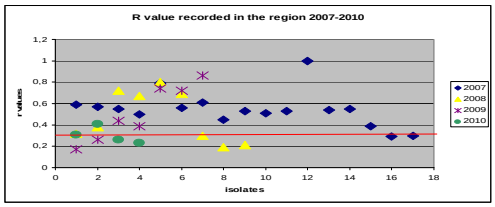
- Movement direction can be possible cross-border in the region which is highly depend on change of the meat price.
- A traditional movement direction is so common, from the east to the west.
- Epidemiological study(2007) about animal movement effect on the dynamics of the spread suggest that almost 70% of the disease spread was related to animal movement
- Monitoring of movement and trade pattern togetter with meat price is essential for early prediction.



Type O, Type A, Type Asia-1, Type SAT2



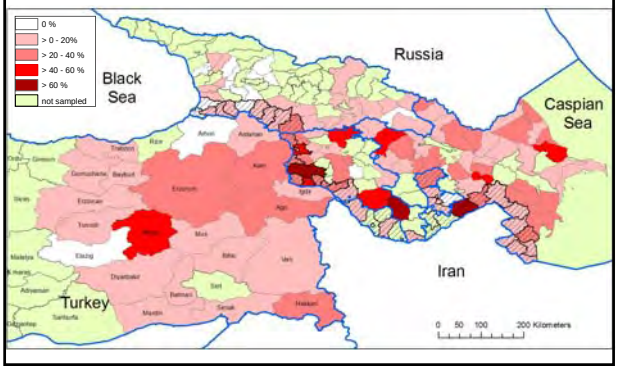
R Values as Indicator to predict the epidemic



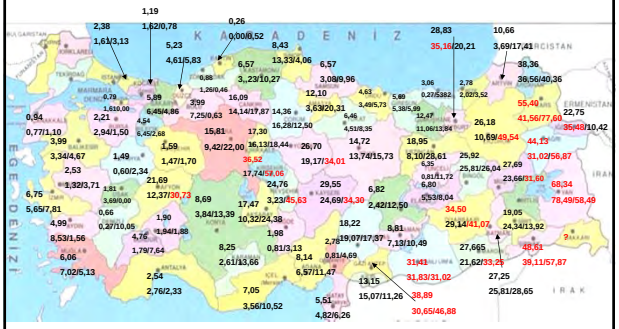
- R values is highly valuable data for assesment vaccine sufficiency.
- However, current avaliable data for the region is not enough to use evidence as proof for prediction of the major epidemic
- It also is not pratical always to say in time, mainly data is available lately.

Serosurveillance data

NSP antibody results – regional -



Map Distribution of Estimated Prevalance by Province



*= cumm prevalance

LR/SR

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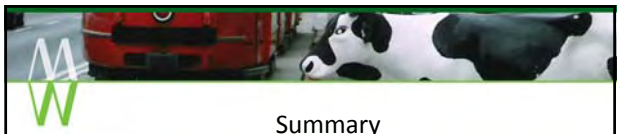


FMD Week2010

ASSESSING THE THREAT TO EUROPE FROM GLOBAL FMD VIRAL POOLS: A TOOL TO INFORM ANTIGEN PRIORITIES FOR VACCINE BANK STORES

Melissa McLaws & Keith Sumption

VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER



Summary

- Method **ranks viral pools** in terms of risk for being source of FMDV incursion to Europe
- Biggest risk from illegal imports – lack data and so use surrogates (Proximity, Legal trade, Passenger arrivals)
- Tool for vaccine bank decision makers, to be used in conjunction with other information
  - Circulating antigens
  - Vaccine matching
- Work in progress… & we are looking for input!



38th EUFMD General Session (2009) :

- development of a procedure for advising on antigens for European vaccine banks was set as **priority of research group**.

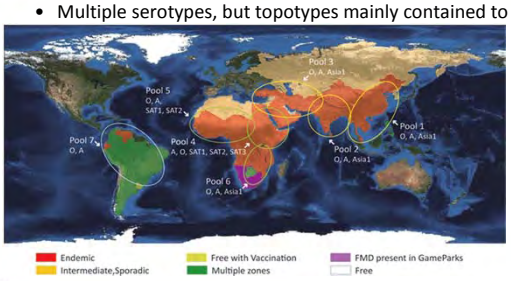
2009 EuFMD Research Group meeting:

- should include consideration of the **proximity** of the threat and whether it is heightened by **trade patterns**.
- 2 step methodology:
  1. an assessment of the comparative importance of different source regions for FMDV entry into Europe
  2. Identification of most appropriate vaccine seed viruses for strains circulating in these pools (WRL)



7 virus pools recognised by the OIE/FAO FMD lab network :

- Multiple serotypes, but topotypes mainly contained to pool



Source: FMD Reference Laboratories Information System - ReLaIS



Methodology: rationale

- EFSA risk assessment 2006 – *Is the EU likely to suffer new FMD outbreaks in the future*?
- 3 main routes of incursion:
  1. Infected live animals
  2. FMDV-contaminated animal product
  3. (Fomite)

  – FMDV will enter Europe through importations (commercial or personal use)



Methodology

Imports:
- ILLEGAL
- LEGAL (V LOW RISK; NORMALLY FMD-FREE COUNTRY)

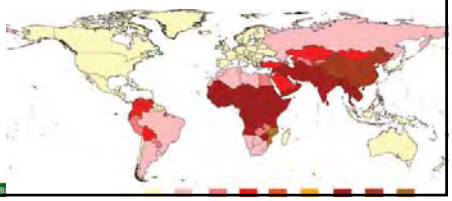
- Focus on illegal imports
  - Little data available
  - Data on seizures not available at European level (at least not publically)

Surrogates used to represent illegal imports:

- Live animal imports → 1) geographic proximity
- Animal products → 2) legal trade volume
  → 3) No. Passengers (air, ferry)
  → 4) Geographic proximity (?)

Risk of FMD introduction from each regional pool:
≈ ***quantity imports*** *from pool * pool* ***FMD prevalence***

- Prevalence: many gaps in knowledge worldwide
- Sumption et al (2008) grouped countries by their conjectured incidence



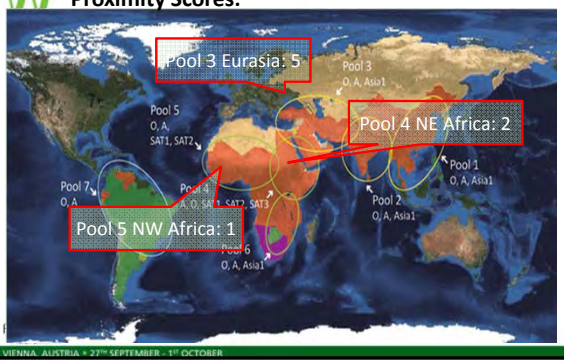
Method:

quantity *imports from pool * pool FMD* ***prevalence***

Quantity:

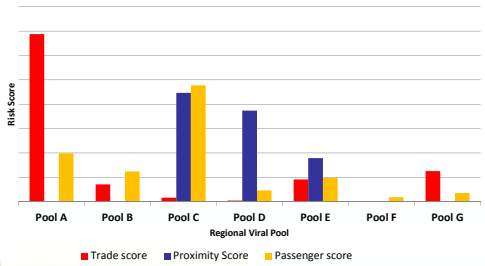
- **Illegal trade***: <u>Legal</u>* trade volume in 100 kg (2009) of animals and animal product (Eurostat)
- **Passengers**: data on air passenger arrivals into EU in 2009 (Eurostat)
- **Proximity:** score assigned to each regional pool

Proximity Scores:



Preliminary results

Risk Scores
(Score ≈ quantity * relative FMD prevalence)



Next steps

- Improve model:
  - Best data for:
    - Illegal trade (email discussion group)
    - FMD prevalence
  - Proximity score
  - Peer Review
- Present to Executive Feb, aim for adoption by General session

Summary

- Method **ranks viral pools** in terms of risk for being source of FMDV incursion to Europe
- Biggest risk from illegal imports – lack data and so use surrogates (Proximity, Legal trade, Passenger arrivals)
- Tool for vaccine bank decision makers, to be used in conjunction with other information
  - Circulating antigens
  - Vaccine matching
- Work in progress… & looking for input!

Acknowledgements:

Members of working group that met in UK in Sept 2010

Thank you!

Call for input!

- Further details on model
- Data
- Comments
- ETC

Incidence (median) (Sumption et al (2008))

Level	cattle	Pigs	sr
2 & 3	0.01	0.00	0.00
4	0.78	0.00	0.00
5	1.32	0.00	0.00
	Relative to levels 2 & 3		
2 & 3	1	1	1
4	91.10	-	110.50
5	154.55	-	-

Relative incidence scores: **level 2 & 3 = 1**
level 4 =100
level 5 = 150

Method: illegal trade

quantity *imports from pool * pool FMD* ***prevalence***

- FMD prevalence: Estimated prevalence (Sumption et al, 2008)
- Quantity:
  - *Legal* trade volume in 100 kg (2009) of animals and animal product (Eurostat)
  - data on air passenger arrivals into EU in 2009 (Eurostat)
- $risk_{trade}$ for each pool = $\sum (Qcountry_{trade} * Ic)_{country}$

Geographic proximity

*quantity imports from pool * pool FMD prevalence*

- *FMD rel* prevalence *rate*: Average of estimated prevalence of countries within each regional pool (Sumption et al, 2008)
- *Quantity*: Proximity score assigned to each regional pool

Welcome to the Disease BioPortal

Update on the Disease BioPortal project at UC Davis

Andres Perez

Associate Researcher and Associate Director
Center for Animal Disease Modeling and Surveillance,
School of Veterinary Medicine, University of California, Davis, CA 95616

UCDAVIS
UNIVERSITY OF CALIFORNIA

EUFMD meeting, Vienna, October 2010

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Disease BioPortal

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UCDAVIS
UNIVERSITY OF CALIFORNIA

Disease BioPortal

Conclusions

- Need for a global FMD surveillance system
- Role of epidemiological analysis
- International involvement

Disease BioPortal

Goal – Vision

To develop a web-based system for ***global animal disease modeling & surveillance***

Disease BioPortal

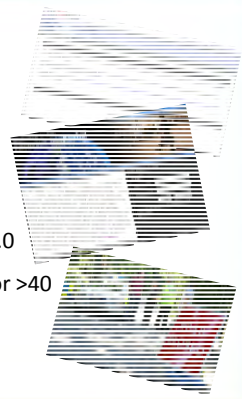
Attributes of the Disease BioPortal

- operational
- near real time surveillance
- multiple streams of information
- analytical capabilities and subject matter expertise –not just an IT system
- continue development
- genomic surveillance
- coordinated as a consortium

Disease BioPortal

Operation

- >600 users since becoming operational in January 2007
- 46 countries/organizations represented
- currently operating version 3.0
- private and public datasets for >40 diseases and syndromes



Operation

- Public datasets
  - ✓ FMD serotype data for samples submitted to the World Reference Laboratory, Pirbright since 1957
  - ✓ World organization for animal Helath since 2006
  - ✓ GenBank
  - ✓ PANAFTOSA
  - ✓ Other: eg: Vietnamese Animal Health Department

New release

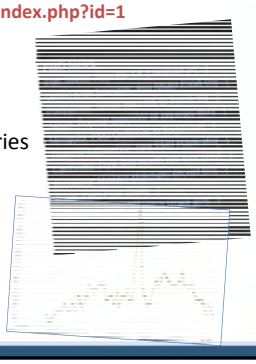


http://fmdbioportal.ucdavis.edu/

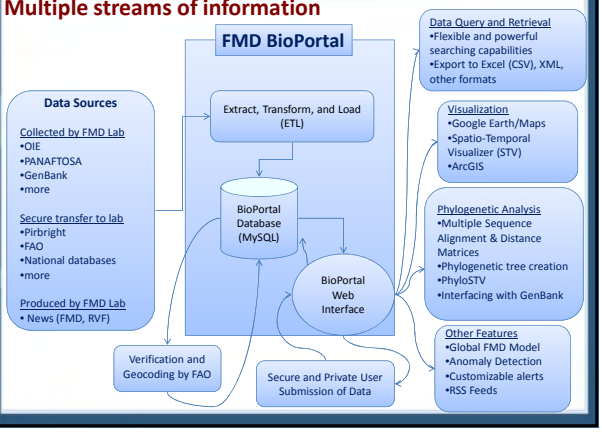
Near real time surveillance: FMD News

http://fmd.ucdavis.edu/index.php?id=1

- 459 subscribers from >50 countries
- 9542 items from 128 countries sent since 10-2004
- weekly, daily, and real time email



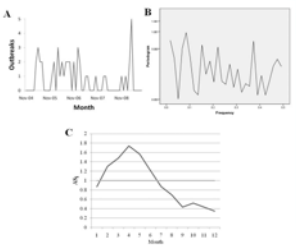
Multiple streams of information



Analytical capabilities

Models:

Global, US, Argentina, Colombia, Peru, Spain, Israel & Palestine, Turkey, Mongolia, Pakistan, and Iran



Time series analysis of serotype Asia 1 FMDv outbreaks in China

Continued development

- Techniques for detection of time-space clusters
- Web-service for automatic transfer of data with the FAO EMPRES-i system
- User submission of data
- Addition of new datasets and sources

What's next?

- Designation as FAO reference center in epidemiology
- GFRA mission
- Vaccination program in Pakistan (ARS, FAO)

Disease BioPortal

Consortium

This project is funded through grants, awards, and cooperative agreements from:

1. U.S. National Center for Medical intelligence
2. USDA – ARS / GFRA
3. University of California
4. UN/FAO EMPRES
5. DHS/CEEZAD, DHS/FAZD

Disease BioPortal

Thank you

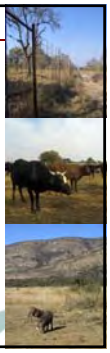
Custom-engineered chimeric FMD vaccine elicits protective immune responses in pigs

B. Blignaut, N. Visser, J. Theron, E. Rieder and F.F. Maree

Transboundary Animal Diseases

Onderstepoort Veterinary Institute
Agricultural Research Council

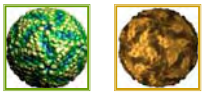
Introduction



- The financial impact of the 2009 FMD outbreak in South Africa was R25 million
- Certain FMDV are unsuitable as vaccine candidates
- Conventional cell adaptation is tedious and costly and may also alter the antigenic composition of the virus
- SAT types display high genomic and antigenic variation: serious implications for the control of the disease by vaccination
- Production of efficient vaccines necessitates the use of good vaccine strains: rapid growth in cells, high yields of stable antigen and appropriate immunological specificity
- Chimeric SAT type FMDV was engineered to produce conventional, chemically inactivated vaccine as an alternative control measure for FMD

Objectives

- Characterisation of phenotypic and antigenic properties
- Determination of the immune responses elicited in guinea pigs
- Comparison of the immune response evoked in pigs and the level of protection following challenge with live virus



vKNP/SAT2 KNP/196/91

Summary

FMDV genome can accommodate molecular manipulation: engineering of recombinant viruses to be used in vaccine production by introduction of the external capsid region of a field isolate

Chimera viruses retains properties of the parental virus

Recombinant viruses can be successfully propagated, inactivated, purified and formulated as vaccines with commercial oil-based adjuvant

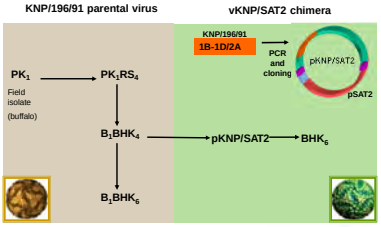
Similar immune responses induced in guinea pigs for both vaccines, indicating similar immunological profiles for the respective viral capsids

Vaccine containing chimera antigen induced significant immune responses in pigs to protect animals against homologous virus challenge

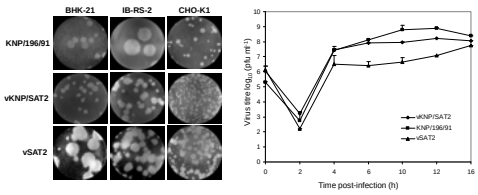
Formulated FMD recombinant vaccine was stable for 9 months

Results

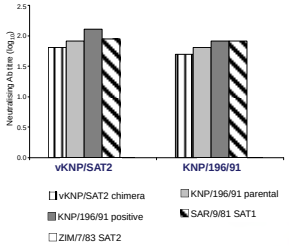
Generation of chimera and parental vaccine stocks



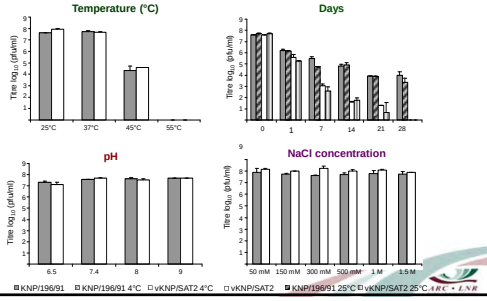
FMDV replication in cell culture



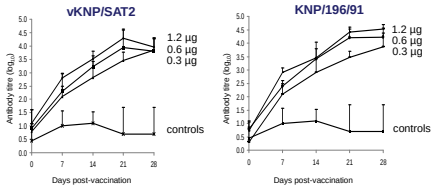
Antigenic profiles of vKNP/SAT2 and KNP/196/91 viruses



Biophysical properties of FMDV



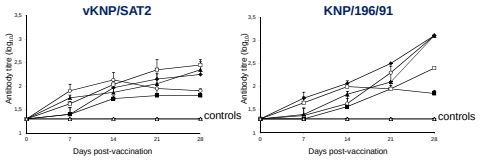
Guinea pig antibody titres in relation to vaccine dose



Chimera and parental vaccines: inactivated 146S antigens as double oil emulsions with Montanide ISA 206

Sera tested in a KNP/196/91-specific sandwich ELISA

Full potency test in pigs induces FMDV-specific antibody responses

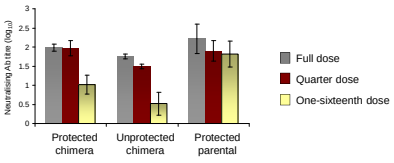


Potency test: Full dose 6 µg
Quarter dose 1.5 µg
One-sixteenth dose 0.375 µg

Sera tested in a KNP/196/91-specific solid-phase competition ELISA (SPCE)

Challenge with 10^4 PID_{50} FMDV at 28 dpv

Comparison of neutralisation titres and protection

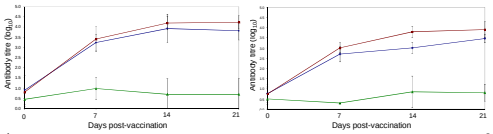


PD50 for vKNP/SAT2 chimera vaccine: >6.4

KNP/196/91 parental vaccine: >39.4

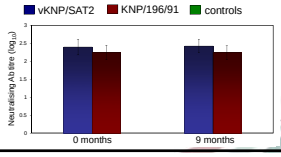
Vaccine stability evaluated in guinea pigs

Sandwich ELISA



0 months 9 months

VNT



Acknowledgements

J.J. Esterhuysen, W. Vosloo, H.G. O'Neill

Staff of the Transboundary Animal Diseases Programme, ARC-OVI

Funding from Intervet SPAH





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Thank you… Vielen Dank… Dankie



anses

FMD-DISCONVAC

Production of a safe EMCV-FMDV recombinant vaccine against FMD

Margot Carocci, Monique Guy, Sandra Blaise Boisseau , Stephan Zientara, Labib Bakkali Kassimi

UMR1161 (ANSES,ENVA,INRA) Maisons-Alfort, FRANCE

EnvA École nationale vétérinaire d'Alfort

eufmd

INRA

Plan

Introduction

Why a recombinant vaccine EMCV-FMDV?

Results

I. production and characterization of EMCVΔ2A

II. Virulence attenuation on human primary astrocytes

III. Virulence attenuation on mice

Conclusion

anses

Why a recombinant vaccine EMCV-FMDV?

- **A live attenuated vaccine**: fast and good protection

- **Easy to produce** less confinement, less expensive

- **Distinguish easily vaccinated and infected animals** : faster get back to a statue free from FMDV

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Why a recombinant vaccine EMCV-FMDV?

FMDV / EMCV

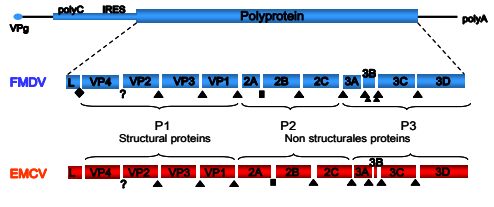
- *Picornaviridae* :
  - positive ssRNA, ~8kb
  - Structural and genomic organization similar
  - Encode a single polyprotein,
  - Similar viral cycle

They are the 2 Picornaviridae, of different genus (**Aphtovirus / Cardiovirus**), showing more similarity

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Why a recombinant vaccine EMCV-FMDV?

Genomic organization



Clivage sites

▲ $3C^{pro}$ ◆ L^{pro}

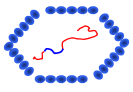
▮ 2A

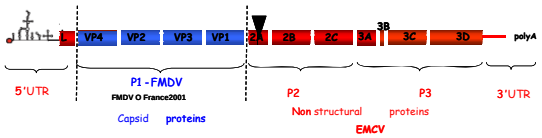
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Why a recombinant vaccine EMCV-FMDV?

Development of a chimera EMCV-FMDV virus :

Production of an hybrid genome :





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Towards a safe vaccine

EMCV : Encephalomyocarditis virus

Can infect many different animal species

Depending on the animal species and the viral strain, EMCV can cause
- Myocarditis
- Reproduction disorders
- Diabetes
- **Encephalitis**

=> Attenuation of EMCV virulence is needed

Plan

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II. Virulence attenuation on mice

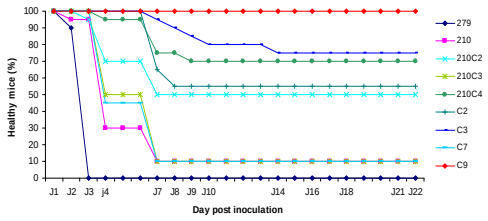
III. Virulence attenuation on human primary astrocytes

Conclusion

I. EMCVΔ2A production and characterization

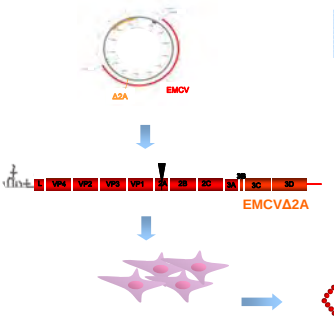
B279 strain 210 passed on BHK-21 : non-virulent for pigs
(P.Denis and F. Koenen Arch Virol 2003)

Test virulence on mice C57Bl/6



C9 clone => a **deletion** in the **2A protein** sequence.

I. EMCVΔ2A production and characterization



Incorporation of a deletion in 2A, using a reverse genetic previously developed in our lab (Bakkali et al., 2002).

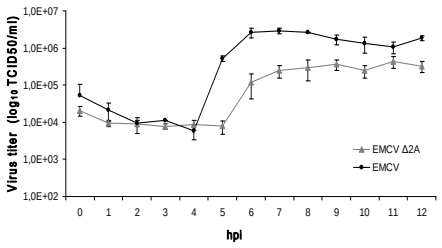
In vitro transcription

Transfection

EMCVΔ2A production

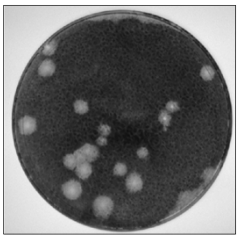
I. EMCVΔ2A production and characterization

One-step growth cycle

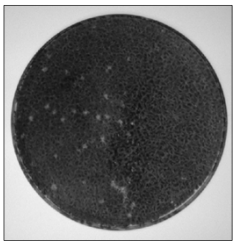


I. EMCVΔ2A production and characterization

Plaque assay on BHK-21



EMCV



EMCV Δ2A

Plan

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II. Virulence att. on human primary astrocytes

EMCV

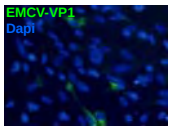
Able to infect a wide range of animal species.

Has been described to possibly infect and cause of head ache, vomiting, malaise, fever... In Human.
(Oberste et al, Emerg Inf Dis 2009)

Test infectivity of Human primary astrocytes by EMCV,
and the attenuation of EMCVΔ2A.

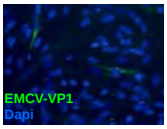
II. Virulence att. on human primary astrocytes

EMCV can infect and replicate on human primary astrocytes

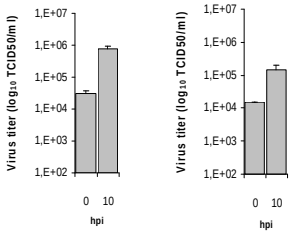


EMCV

Astrocytes 5hpi

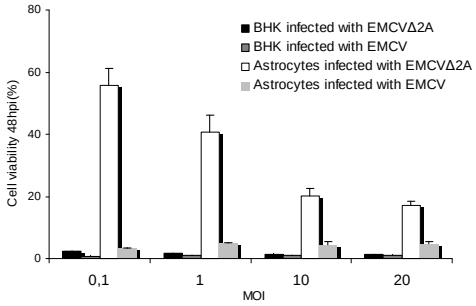


EMCVΔ2A



EMCVΔ2A is able to infect and replicate on human primary astrocytes but less efficiently.

II. Virulence att. on human primary astrocytes



EMCVΔ2A virus is less virulent than the wild type on Human primary Astrocytes

Plan

Introduction

Why a recombinant vaccine EMCV-FMDV?

Results

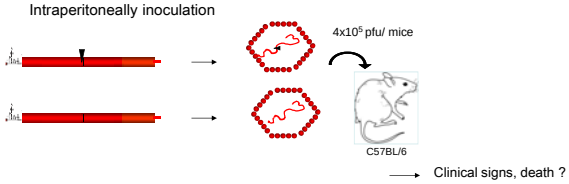
I. production and characterization of EMCVΔ2A

II. Virulence attenuation on human primary astrocytes

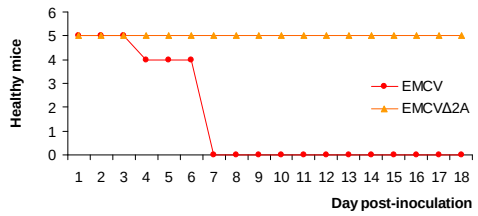
III. Virulence attenuation on mice

Conclusion

II. Virulence attenuation on mice



II. Virulence attenuation on mice

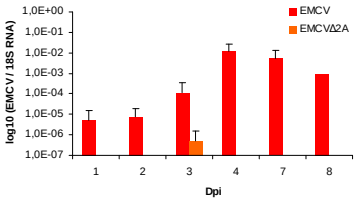


EMCV Δ2A **does not induce any clinical sign in C57BL/6 mice**

anses

II. Virulence attenuation on mice

Number of viral RNA copy in mice heart.

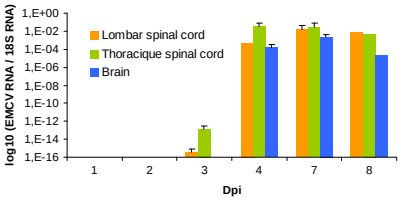


Detection of EMCV Δ2A only at 3dpi in one out of 5 mice heart.

anses

II. Virulence attenuation on mice

Number of EMCV RNA copy in mice CNS.



EMCV Δ2A **does not reach the mice CNS.**

anses

Conclusion

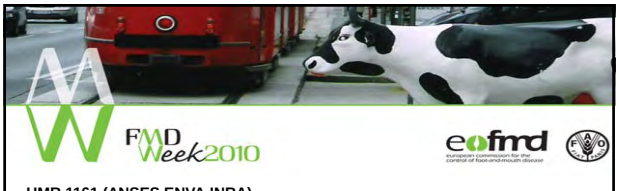
Virulence attenuation of EMCVΔ2A :

on BHK-21
on human primary astrocytes
(With first demonstration of human primary astrocytes sensitivity to EMCV.)
on mice

The EMCVΔ2A virus is an attenuated virus,

Which should be a safe base for a recombinant EMCV-FMDV vaccine.

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FMD Week2010

UMR 1161 (ANSES,ENVA,INRA)
Maisons-Alfort, FRANCE

Stephan Zientara,
Labib Bakkali Kassimi,
Sandra Blaise Boisseau,
Monique Guy.
Anthony Relmy,
Kamila Gorna,
Muriel Coulpier.

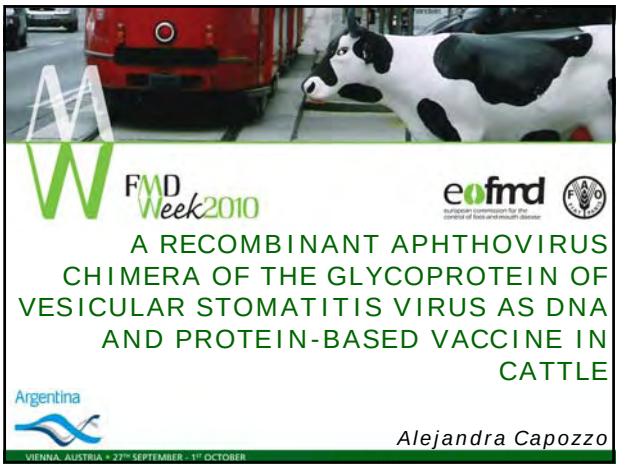
"The research leading to these results have received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 226556. "

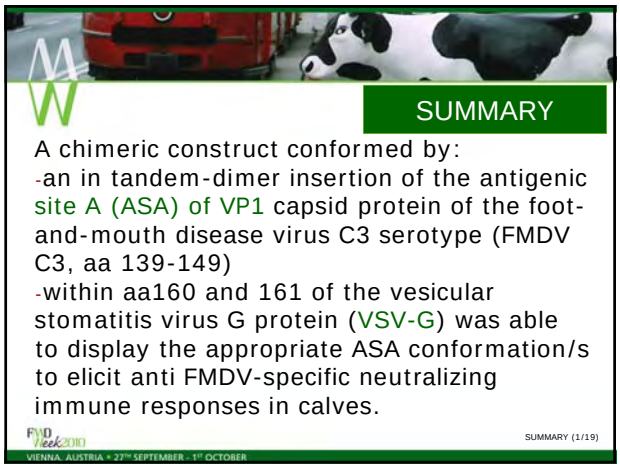
FMD-DISCONVAC

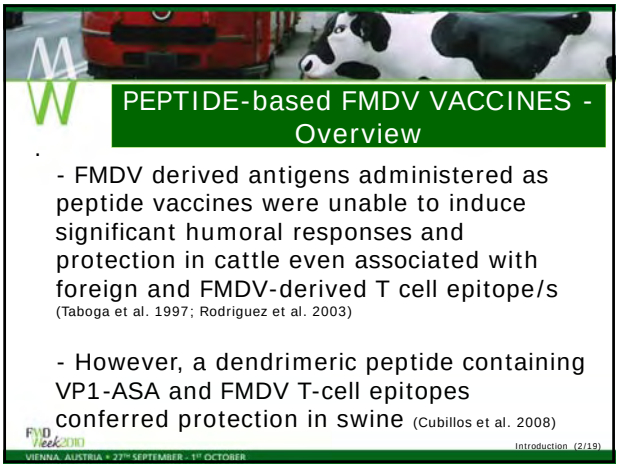
EnvA École nationale vétérinaire d'Alfort

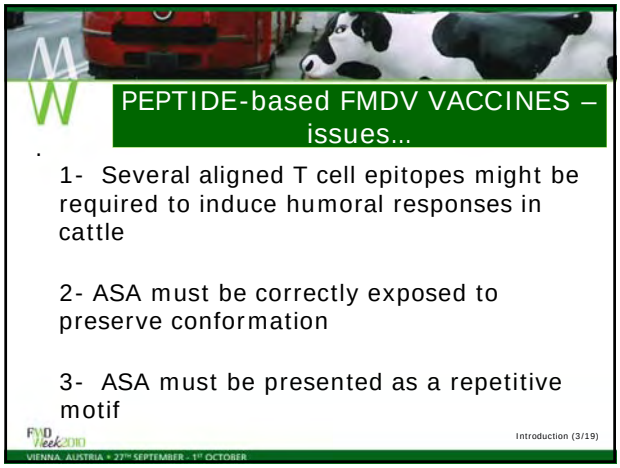
anses

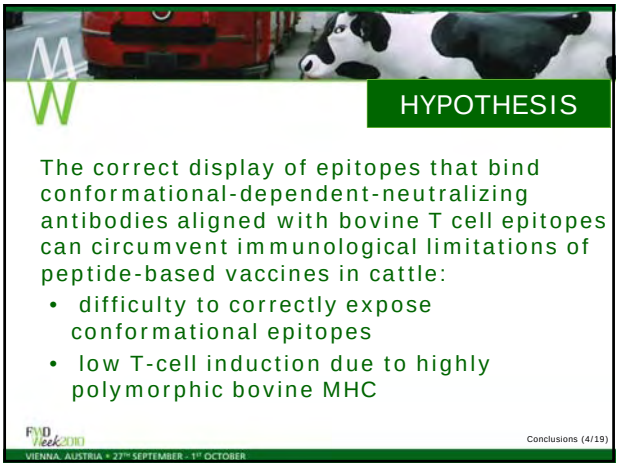
INRA

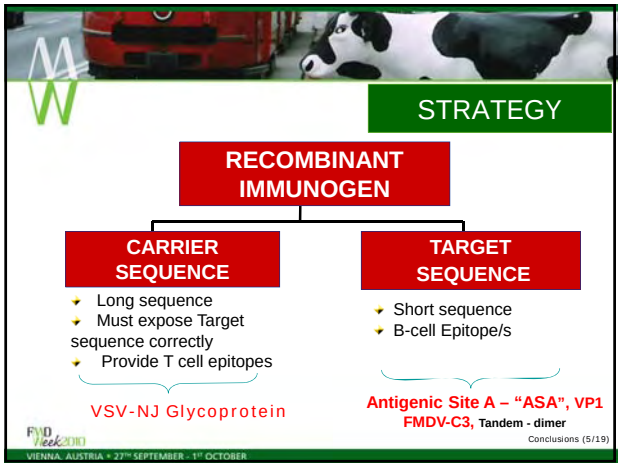

FMD Week2010
A RECOMBINANT APHTHOVIRUS CHIMERA OF THE GLYCOPROTEIN OF VESICULAR STOMATITIS VIRUS AS DNA AND PROTEIN-BASED VACCINE IN CATTLE
Argentina
Alejandra Capozzo
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


SUMMARY
A chimeric construct conformed by:
-an in tandem-dimer insertion of the antigenic site A (ASA) of VP1 capsid protein of the foot-and-mouth disease virus C3 serotype (FMDV C3, aa 139-149)
-within aa160 and 161 of the vesicular stomatitis virus G protein (VSV-G) was able to display the appropriate ASA conformation/s to elicit anti FMDV-specific neutralizing immune responses in calves.
SUMMARY (1/19)
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


PEPTIDE-based FMDV VACCINES - Overview
- FMDV derived antigens administered as peptide vaccines were unable to induce significant humoral responses and protection in cattle even associated with foreign and FMDV-derived T cell epitope/s (Taboga et al. 1997; Rodriguez et al. 2003)
- However, a dendrimeric peptide containing VP1-ASA and FMDV T-cell epitopes conferred protection in swine (Cubillos et al. 2008)
Introduction (2/19)
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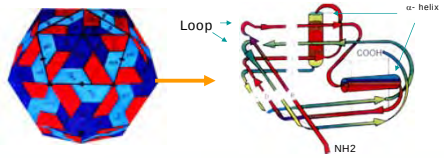

PEPTIDE-based FMDV VACCINES – issues...
1- Several aligned T cell epitopes might be required to induce humoral responses in cattle
2- ASA must be correctly exposed to preserve conformation
3- ASA must be presented as a repetitive motif
Introduction (3/19)
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HYPOTHESIS
The correct display of epitopes that bind conformational-dependent-neutralizing antibodies aligned with bovine T cell epitopes can circumvent immunological limitations of peptide-based vaccines in cattle:
• difficulty to correctly expose conformational epitopes
• low T-cell induction due to highly polymorphic bovine MHC
Conclusions (4/19)
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


STRATEGY
RECOMBINANT IMMUNOGEN
CARRIER SEQUENCE
Long sequence
Must expose Target sequence correctly
Provide T cell epitopes
VSV-NJ Glycoprotein
TARGET SEQUENCE
Short sequence
B-cell Epitope/s
Antigenic Site A – "ASA", VP1
FMDV-C3, Tandem - dimer
Conclusions (5/19)
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER

FMDV - VP1, Antigenic Site A: ASA

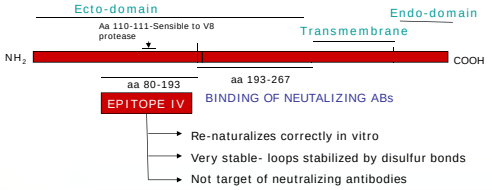
- Oligopeptide 11 aa long corresponding to VP1 sequence aa 139 y 149: ARRGDLAHLAT (Serotipe C).
- Antigenic site composed of several overlapping conformational epitopes reactive to neutralizing antibodies.



Binding of Neutralizing antibodies requires one or several conformations of ASA

CARRIER SEQUENCE : VSV-NJ/G

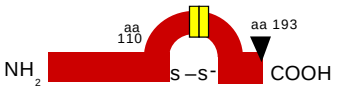
- Potent immunogen in bovines, rabbit, swine, mice; even without adjuvants
- Bovine T cell epitopes have been mapped along its sequence
- Deletion of c-terminal end brings more stability and facilitates refolding



VSV-NJ GLYCOPROTEIN G (VSV-G; 514 aa)

VSV-G/ASA chimera

ASA dimer



Region IV

T CELL EPITOPES

Recall-responses

Sample: whole blood
Method: BOVIGAM®

Status	Bovine #	Stimulating antigen: PWD	FMDV C3	DEL BAC	NIL
FMDV Vaccinated commercial vaccine	44	512,60	216,80	68,20	1,60
	47	421,60	315,40	30,60	0,60
	50	418,60	366,20	54,40	1,00
Naive	128	470,40	1,20	6,00	1,40
	146	512,60	0.40	3.80	0.40

IFN-γ ng/ml

VACCINE ANTIGENS

- BACULOVIRUS-expressed Protein: DEL BAC
- DNA VACCINE: pC DEL

IMMUNIZATION STUDIES

DEL BAC 30µg



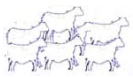
Route: i.m (Neck).
Vol/ dose: 3 ml.
Adyuvant: Marcol Montanide (w/o)
Schedule: 2 doses, 30 days.

Controls



- Commercial Vaccine (Frenkel-Multivalent)
- Empty plasmid
- Heterologous Baculovirus antigen +Ady

pC DEL 150µg

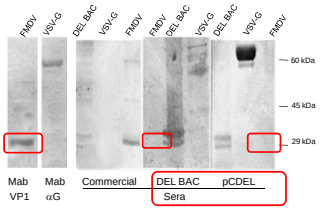


Route: i.m (Neck).
Vol/ dose: 3 ml.
Adyuvant: none
Schedule: 2 doses, 15 days.

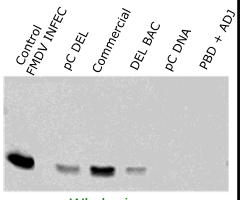
5 month-old calves

Reactivity with conformational ASA

WESTERN BLOT



IMMUNOPRECIPITATION

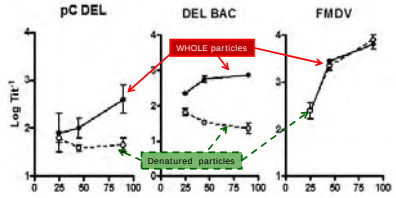


Whole virus
IP + WB (Mab anti VP1)

SERA FROM CHIMERA-VACCINATED ANIMALS STRONGLY REACT WITH WHOLE FMDV PARTICLES

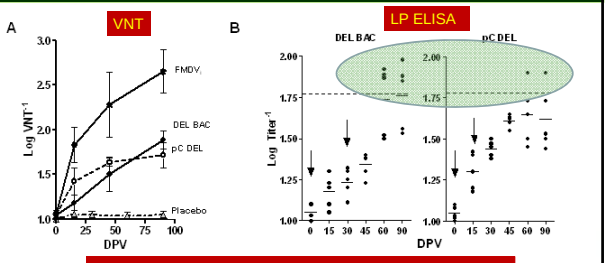
Reactivity with conformational VP1 epitopes

ELISA AGAINST WHOLE AND DENATURED VIRUS



SERA FROM CHIMERA-VACCINATED ANIMALS STRONGLY REACT WITH NATIVE FMDV

FMDV-SPECIFIC ANTIBODIES MEASURED BY VNT and Liquid Phase Blocking ELISA



INDIVIDUAL ANIMALS SURPASS PROTECTIVE LEVELS OF ABs (EPP >80%)

FMDV-specific IgG subtypes

Vaccine	DPV	IgG1 titer	IgG2 titer	RATIO IgG1/IgG2
pC DEL	15	1.62 +/- 0.32	1.54 +/- 0.42	1.05
	30	1.60 +/- 0.43	1.72 +/- 0.41	0.93
	45	1.64 +/- 0.14	2.08+/-0.16	0.78
	60	1.63+/-0.18	2.20+/-0.17	0.74
DEL BAC	15	ND	ND	ND
	45	1.54+/-0.38	1.35+/-0.31	1.14
	60	1.74+/-0.36	1.33+/-0.28	1.31
	90	1.90+/-0.37	1.34+/-0.29	1.42
Commercial $FMDV_i$ (Frenkel)	15	ND	ND	ND
	45	2.30+/-0.40	1.36+/-0.18	1.69
	90	2.20+/-0.23	1.38+/-0.19	1.59

CONCLUSIONS

- ✓ T-cell epitopes in the chimeric construct could induce T-cell activation in whole blood samples from commercially-vaccinated animals.
- ✓DNA-coded and baculovirus expressed forms of G-ASA induced strong humoral responses in cattle
- ✓Serum from all G-ASA vaccinated animals recognized conformational ASA in the native FMDV-140S particles
- ✓3 out of 5 animals had EPP% values (LP ELISA) above 80% and all DEL-BAC immunized calves showed high serum IgG1 titers, with values comparable to those recorded for protection with inactivated vaccines

CONCLUSIONS

The association of the FMDV-C3/85 ASA as a tandem dimer to VSV-G N terminal sequence could circumvent limitations of FMDV peptide-based antigens as effective vaccines for bovines


Argentina
MILSTEIN
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VIENNA. AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


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MILSTEIN
Thanks! (19/19)
VIENNA. AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER

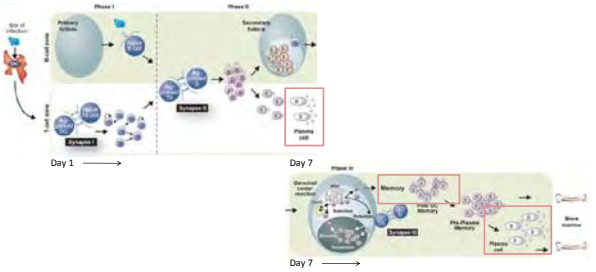
CHARACTERISATION OF THE T-DEPENDENT AND T-INDEPENDENT IMMUNE RESPONSES IN CATTLE.

Clare Grant, Veronica Carr, Helen Prentice, Bryan Charleston and Eric A Lefevre
Institute for Animal Health, Compton, UK

Summary

- T-dependent antigen response overview
- T-dependent and T-independent antigen study outline
- Study results
- Conclusions and future plans

T-dependent response to antigen



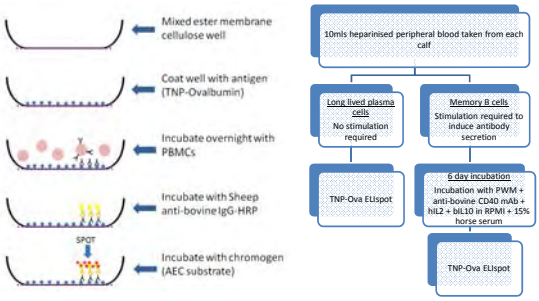
Short lived plasma cells:
Appear early in the response
Only produce low affinity IgM
Migrate towards specific niches (bone marrow, red pulp of spleen)

McHeyzer-Williams, L.J. & McHeyzer-Williams, M.G. Antigen-specific memory B cell development. *Annu Rev Immunol* 23, 487-513 (2005).

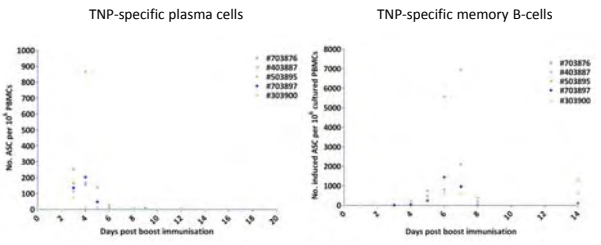
T-dependent and T-independent antigen study

- Ten 7 month old Holstein-Friesian calves
- 5 calves in each study group
  - TNP-Ficoll (T-independent) OR
  - TNP-CGG (T-dependent)
- Subcutaneous immunisation with 3mg of antigen and QuilA adjuvant.
  - Boost immunisation to be given at day 29
- 10mls peripheral blood taken at regular intervals to assess B and T cell responses
  - TNP-Ova ELIspot
  - TNP-Ova ELISA
  - TNP-Ova proliferation assay

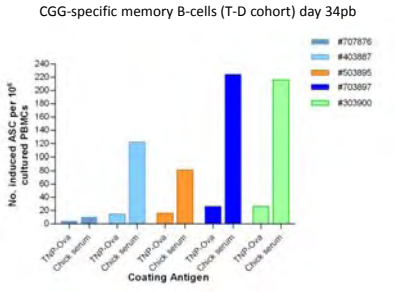
ELIspot & Sample workflow



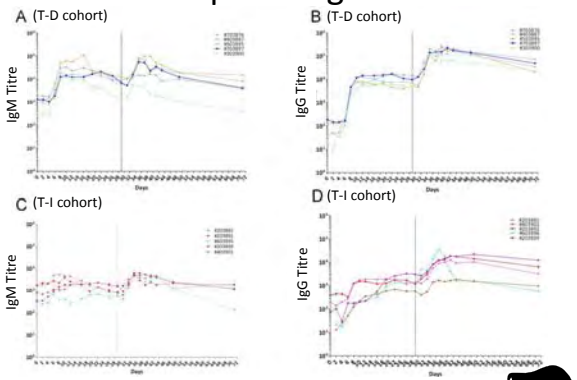
TNP-specific B-cells post-boost



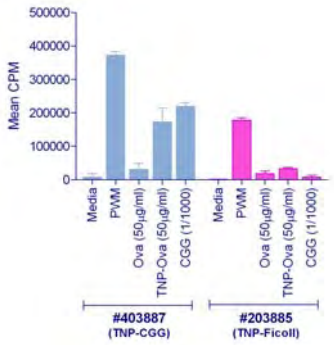
CGG-specific memory B-cells



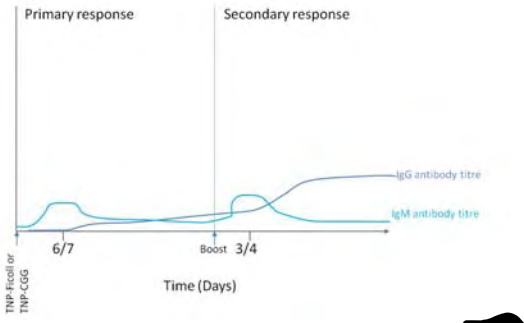
TNP-specific Ig titres



PBMC proliferative response to TNP and CGG (Day 7pb)



T-dependent B-cell response



Conclusions and further work

- T-D cohort demonstrated increased number of plasma and memory B-cells post-boost.
- Plasma cell burst was coupled with an early induction of TNP-specific IgM and IgG titres in the T-D cohort
- T-I cohort showed no detectable TNP-specific plasma or memory B-cell throughout the study.
- T-I cohort did generate an IgM and IgG response to TNP but at a much lower level as compared to T-D cohort.
- Investigate the effect of bystander stimulation upon TNP-specific B cell response in both cohorts.

Acknowledgements

- All in lab G2C, especially Helen Prentice and Veronica Carr.
- Supervisors: Bryan Charleston and Eric Lefevre
- Animal staff at Mayfield Rearing Unit
- Funders:

MERIAL BBSRC bioscience for the future

FREEZING OF FORMULATED FMDV VACCINES AND VACCINE BANKS: POTENTIAL FOR A FASTER RESPONSE IN EMERGENCY SITUATIONS

D.Goovaerts, N.Visser, P.Janssen, T.Jansen, G.Paul*

Intervet Schering-Plough Animal Health

Freezing and storage of formulated FMD vaccines at -20°C

1. Introduction and rationale
2. Effect on emulsion quality and microscopic appearance
3. Immediate effect on immunogenicity or potency
4. Prolonged effect on immunogenicity and stability

SPC: DO NOT FREEZE

W/O/W emulsions (Montanide ISA 206) or W/O emulsions should not be frozen (SEPPIC, France)

Oil adjuvanted vaccines stored at -20°C or -70°C show reduction in potency (Barnett PV Vaccine 20, (2002) 2060-2064)

Montanide ISA 206 can be snap frozen and kept at ultra low temperature (SACS vaccine concept) without detrimental effect on potency (Barnett PV Vaccine 20, (2002) 2060-2064)

Antigen and vaccine banks

Time and fast response are of crucial importance in case of an FMD outbreak.

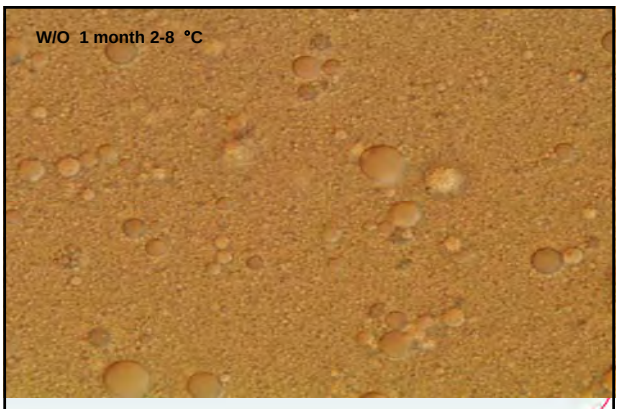
Antigen banks (-196°C) require immediate formulation (3-5 days) in case of an outbreak. Logistic challenge (minimal batch size, transport) and no final release testing (safety and potency) possible.

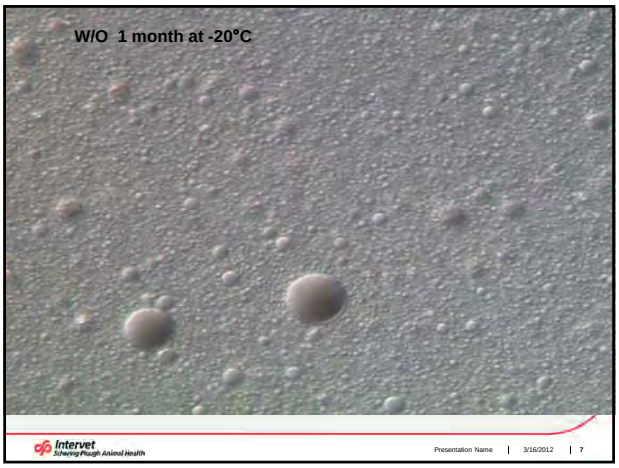
Vaccine banks (4°C) subject to declining stability of FMDV require frequent replenishment by fresh vaccine stocks.

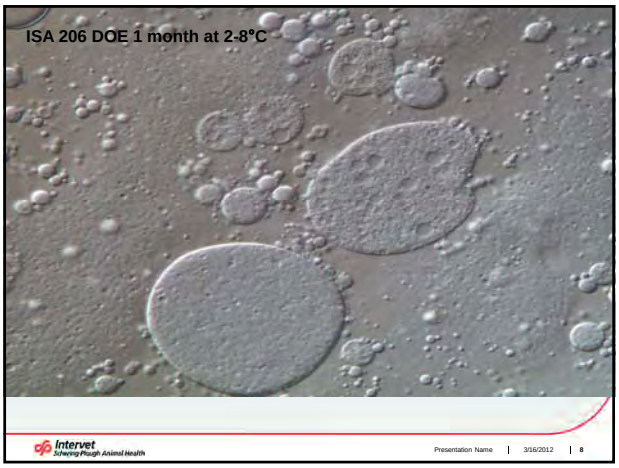
Despite the potentially much faster response of ready-to-use vaccine banks they are relatively costly and therefore compared to antigen banks their use currently is relatively limited.

Effect on emulsion quality, microscopic appearance and potency of -≤20°C freezing:

- Trivalent vaccines (O1Manisa, A Tur 20/06, Asia 1 Shamir)
- Standard antigen production, formulated at regular payloads
- Formulated with different adjuvants (Montanide ISA 206 and 2 types of W/O)
- 1 vial kept at 4°C, 1 vial frozen at -20°C using a regular compressor type house-hold freezer.
- Kept frozen at -20°C for 1 month
- I.M. vaccination in guinea pigs (n=6/group), serology 3 weeks after vaccination by VNT titres









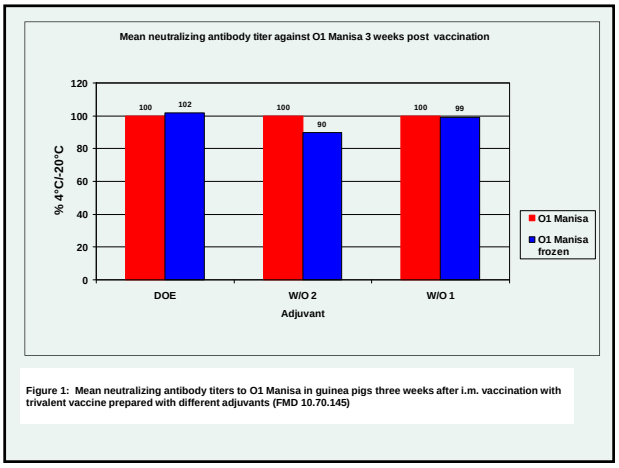


Figure 1: Mean neutralizing antibody titers to O1 Manisa in guinea pigs three weeks after i.m. vaccination with trivalent vaccine prepared with different adjuvants (FMD 10.70.145)

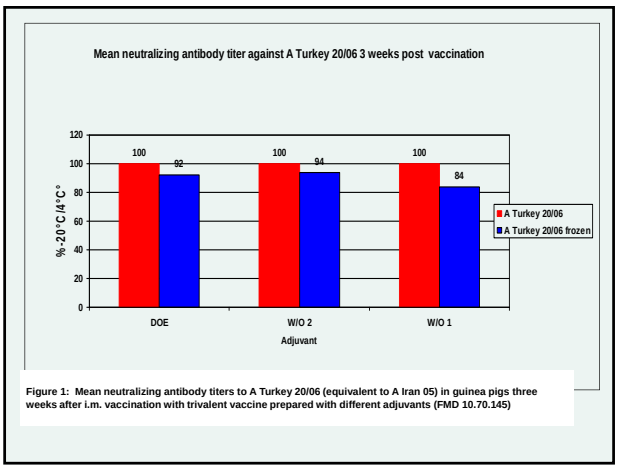


Figure 1: Mean neutralizing antibody titers to A Turkey 20/06 (equivalent to A Iran 05) in guinea pigs three weeks after i.m. vaccination with trivalent vaccine prepared with different adjuvants (FMD 10.70.145)

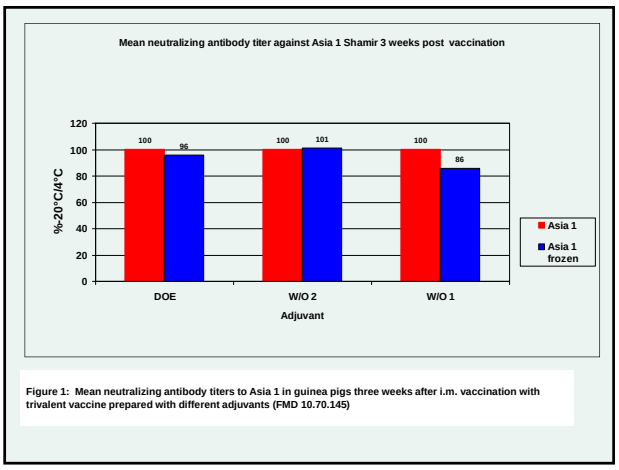


Figure 1: Mean neutralizing antibody titers to Asia 1 in guinea pigs three weeks after i.m. vaccination with trivalent vaccine prepared with different adjuvants (FMD 10.70.145)

Effect on stability: Materials and methods

- Monovalent, Bivalent and Trivalent vaccines (O1Manisa, A Tur 20/06, Asia 1 Shamir)
- Formulated with Montanide ISA 206
- 1 vial kept at 2-8°C, 1 vial frozen and kept at -20°C.
- Kept frozen at -20°C using a regular compressor type house-hold freezer for a period of **16 months**
- I.M. vaccination in guinea pigs (n=6/group), serology 4 weeks after vaccination by VNT titres
- 1 frozen trivalent vaccine containing (O1Manisa, A22 Iraq, Asia 1 Shamir) kept for a period of **41 months** and tested in guinea pigs.
- 1 frozen trivalent vaccine containing (O1Manisa, A22 Iraq, Asia 1 Shamir) kept for a period of **14 months** and tested in cattle

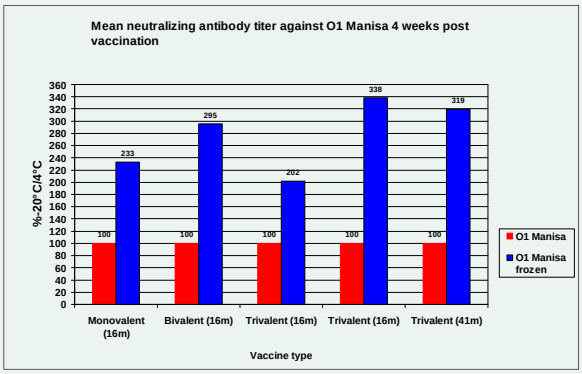


Figure 1: Mean neutralizing antibody titers to O1 Manisa in guinea pigs four weeks after i.m. vaccination (FMD 10.70.146)

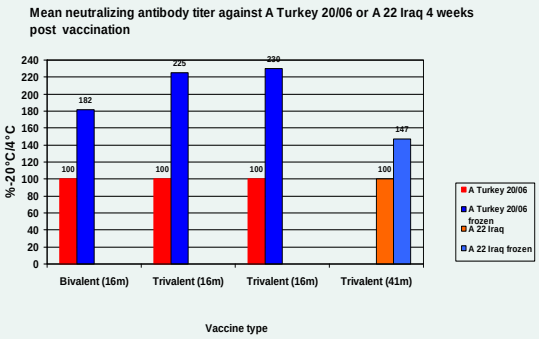


Figure 2: Mean neutralizing antibody titers to A Turkey 20/06 (equivalent to A Iran 05) or A 22 Iraq in guinea pigs four weeks after i.m. vaccination (FMD 10.70.146)

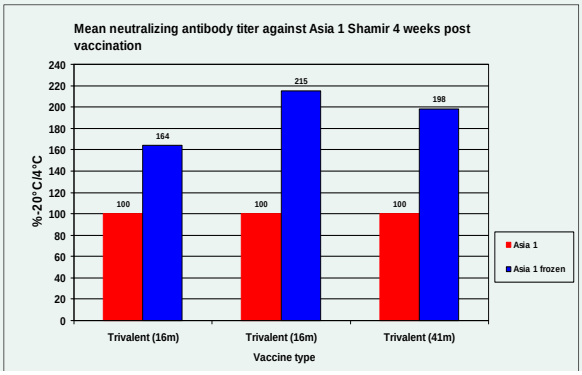


Figure 3: Mean neutralizing antibody titers to Asia 1 Shamir in guinea pigs four weeks after i.m. vaccination (FMD 10.70.146)

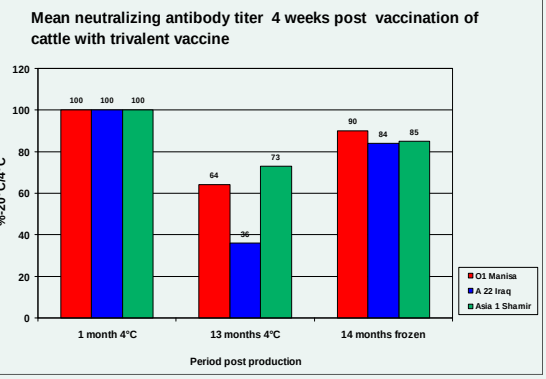


Figure 4: Mean neutralizing antibody titers in cattle four weeks after i.m. vaccination (FMC 07.70.000D, FMD 08.70.034, FMD 08.70.039)

Conclusions

1. In contrast to general prescriptions and perception, freezing of oil adjuvanted FMD vaccine is not necessarily detrimental to vaccine quality.
2. Although a (minimal) negative effect on the microscopic appearance of the emulsion can be observed, DOE and SOE formulated FMDV vaccines can be frozen at -20°C without an immediate negative effect on the potency of the vaccine and more importantly with improved shelf-life.
3. Freezing of ready to use FMDV vaccine greatly enhances the possibilities and use of vaccine banks like:
   - Frozen vaccine can be stored in the country of destination and at any quantity.
   - Vaccine is ready for immediate use, allowing for a faster response and emergency vaccination in case of an outbreak.
   - No "stand by" formulation production facilities needed, no minimum batch size.
   - Vaccine batches from the bank can be tested for vaccine and safety before release.

Thermostability and infectious properties of a Foot-and-Mouth Disease Type A Virus with a partially deleted VP1 G-H loop



Dr Veronica Fowler
Institute for Animal Health
EU-FMD 2010
Nick Knowles, John Bashiruddin, Bartek Bankowski, Sarah Cox and Paul Barnett
Graham Belsham, Carolina Stenfeldt and Anette Bøtner

Overview

Summary:
This presentation will outline the thermostability and infectious (*in vitro* and *in vivo*) properties of a Foot-and-Mouth Disease Type A Virus with a partially deleted VP1 G-H loop.

Conclusions:

- This is the first time that a virus lacking the RGD has been shown to be able to generalise in cattle.
- FMDV may be able to utilise more receptors than are currently defined.
- The identification of specific residues at the inter-pentameric interface may facilitate the development of more stable FMDV vaccines.

FMDV Capsid Assembly and Stability

Assembly of the capsid:

- Foot-and-mouth disease (FMD) virus capsid is composed of 60 copies each of four structural proteins (VP1-VP4).
- VP1, VP2, VP3 and VP4 associate to form a protomer,
- Five protomers associate to form a pentamer,
- 12 pentamers associate to form the icosahedral capsid.

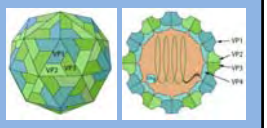
Stability of the capsid:

- FMD capsid stability is currently believed to be regulated by events at the inter-pentameric interface.

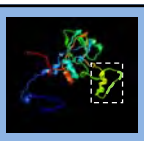
Capsids are disrupted by:

- temperatures above 56°C.
- pH's below 6.8.

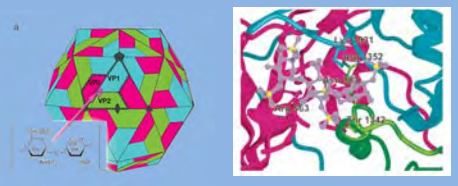
(Capsid dissociates into 12 pentamers)



Viral Receptors



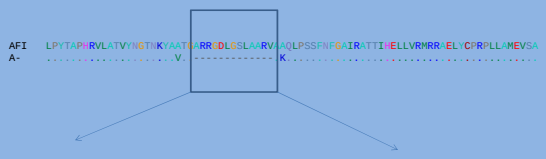
- Integrin (αvβ1, αvβ3, αvβ6, αvβ8) via the VP1 G-H loop.
- Heparin sulphate



- 'Third cellular receptor' (Baranowski *et al.*, 1998 and 2000).

Virus details

- Field isolate: A serotype virus vaccine with a native VP1 G-H loop
- A-: A serotype virus with a natural VP1 G-H loop deletion of 13aa

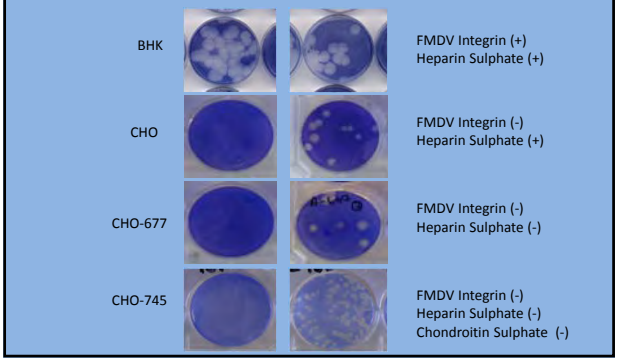


- VP1 139-152, including the RGD integrin-binding motif

Research Questions?

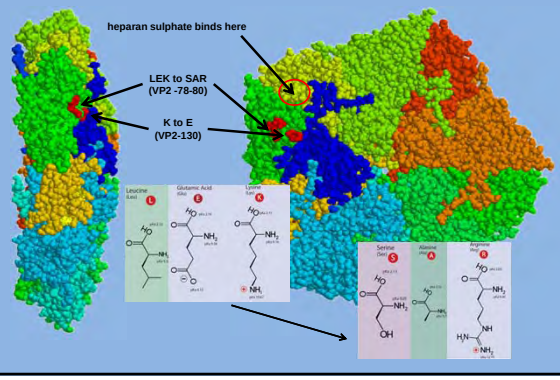
- Infectiousness *in vitro.*
- Infectiousness *in vivo.*
- Association between the above with sequence changes.

Infectiousness *in vitro*



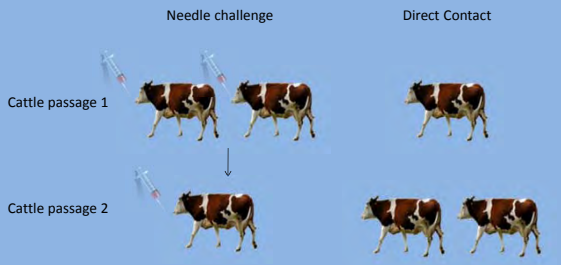
Amino Acid differences

Novel receptor site?



Infectiousness *in vivo*

Protocol



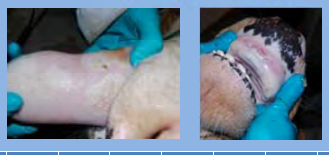
Infectiousness *in vivo*

Protocol

Needle challenge

Direct Contact

Cattle passage 1



X

Animal	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1 (13)	Serum	No Ct	22.69	21.81	38.74	No Ct	No Ct
	NS	No Ct	No Ct	31.75	37.48	42.65	No Ct
2 (14)	Serum	No Ct	21.59	19.78	46.89	No Ct	No Ct
	NS	No Ct	No Ct	37.36	31.43	No Ct	No Ct

Infectiousness *in vivo*

Protocol

Needle challenge

Direct Contact

Cattle passage 2

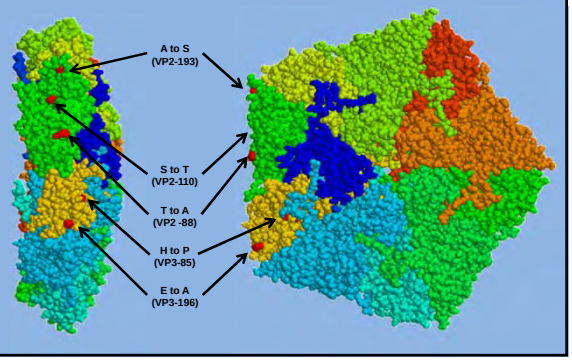


X

Animal	Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
3 (CP1)	Serum	No Ct	27.63	28.04	30.39	40.06	No Ct
	NS	No Ct	No Ct	No Ct	No Ct	48.25	No Ct

Amino Acid differences

Thermostability?



Thermostability

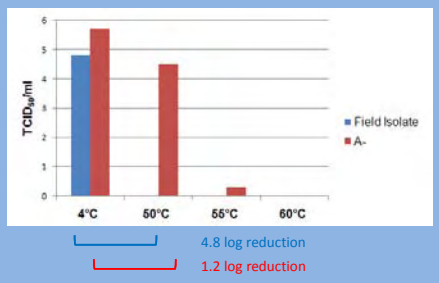


Figure 1. Virus titre and log_{10} reduction following heat treatment for 1h.

Summary

Infectiousness *In vitro*

- A- can replicate in cell lines deficient in FMDV specific integrins, HS and CS.
- This cell tropism was not apparent in the parent virus.
- The ability of A- to replicate in these cell lines may be associated with amino acid substitutions at VP2 78-80 (LEK-SAR).

Infectiousness *In vivo*

- Needle challenge of A- results in generalisation to the feet
- Needle challenge of A- results in RT-PCR + serum and nasal swabs
- A- appears unable to transmit to in contact cattle

Thermostability

- A- appears to be more thermostable than the field isolate.
- Sequencing of the capsid proteins revealed five amino acid substitutions (VP2 88, 110, 130, 193 and VP3 196) located close to the inter-pentameric interface.
- Two of the five sites have already been implicated acid (VP2 130) and thermostability (VP2 110) (Twomey *et al,* 1995 and Mateu *et al,* 2003).

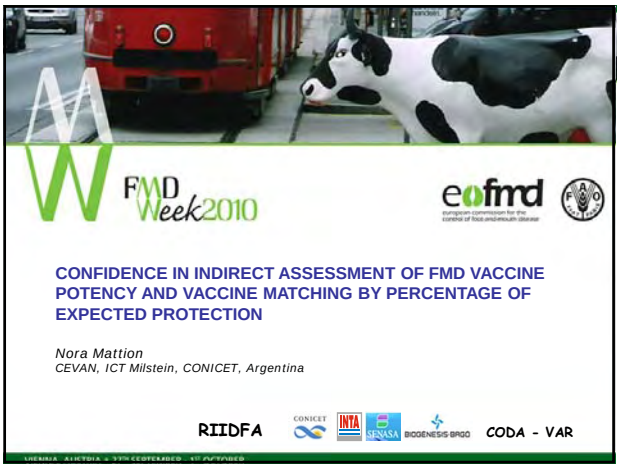
Significance of this work

- This is the first time that a virus lacking the RGD has been shown to be able to generalise in cattle.
- FMDV may be able to utilise more receptors than are currently defined.
- The identification of specific residues at the inter-pentameric interface may facilitate the development of more stable FMDV vaccines.

Acknowledgements

Graham Belsham, Carolina Stenfeldt and Anette Bøtner
(Lindholm, Technical University of Denmark)

Follow-on-fund and Seed fund



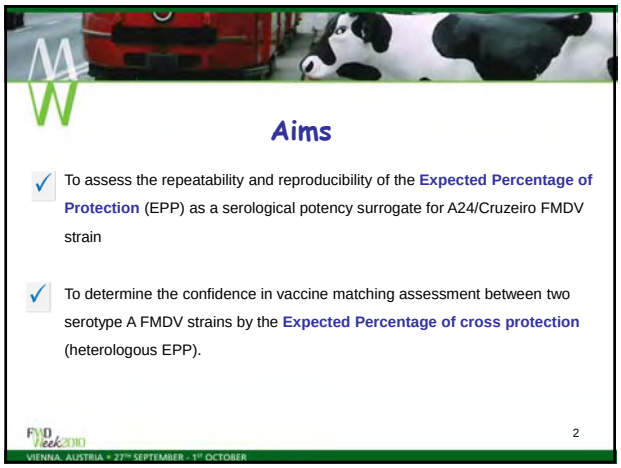
FMD Week 2010

eufmd

CONFIDENCE IN INDIRECT ASSESSMENT OF FMD VACCINE POTENCY AND VACCINE MATCHING BY PERCENTAGE OF EXPECTED PROTECTION

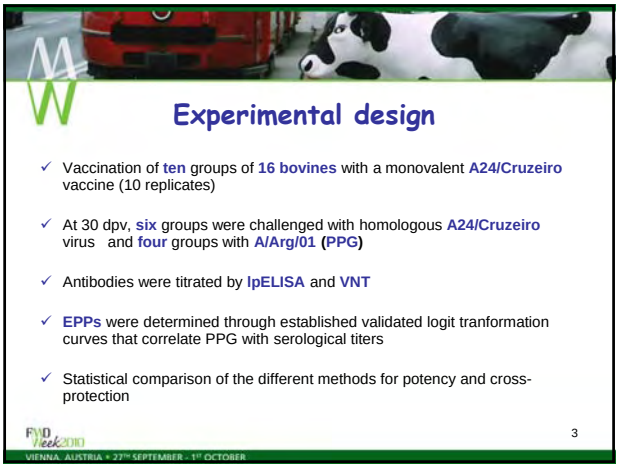
Nora Mattion
CEVAN, ICT Milstein, CONICET, Argentina

RIIDFA CONICET INTA SENASA BIOGENESIS BAGO CODA - VAR



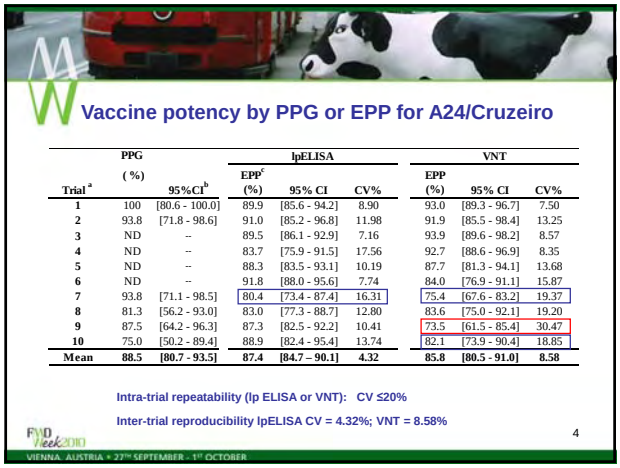
Aims

- ✓ To assess the repeatability and reproducibility of the **Expected Percentage of Protection** (EPP) as a serological potency surrogate for A24/Cruzeiro FMDV strain
- ✓ To determine the confidence in vaccine matching assessment between two serotype A FMDV strains by the **Expected Percentage of cross protection** (heterologous EPP).



Experimental design

- ✓ Vaccination of **ten** groups of **16 bovines** with a monovalent **A24/Cruzeiro** vaccine (10 replicates)
- ✓ At 30 dpv, **six** groups were challenged with homologous **A24/Cruzeiro** virus and **four** groups with **A/Arg/01** (**PPG**)
- ✓ Antibodies were titrated by **lpELISA** and **VNT**
- ✓ **EPPs** were determined through established validated logit tranformation curves that correlate PPG with serological titers
- ✓ Statistical comparison of the different methods for potency and cross-protection

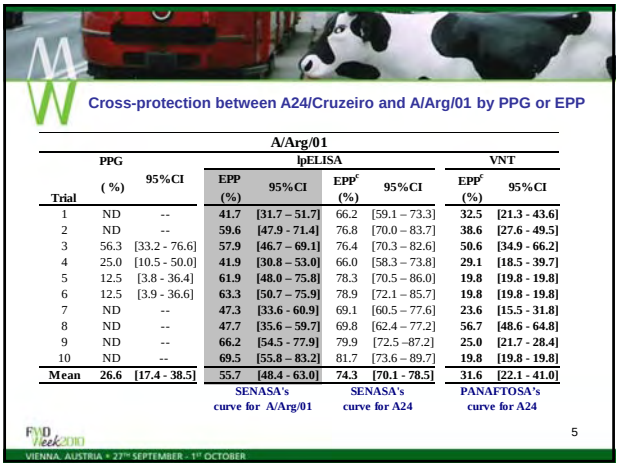


Vaccine potency by PPG or EPP for A24/Cruzeiro

Trial [a]	PPG (%)	95%CI [b]	lpELISA EPP [c] (%)	95% CI	CV%	VNT EPP (%)	95% CI	CV%
1	100	[80.6 - 100.0]	89.9	[85.6 - 94.2]	8.90	93.0	[89.3 - 96.7]	7.50
2	93.8	[71.8 - 98.6]	91.0	[85.2 - 96.8]	11.98	91.9	[85.5 - 98.4]	13.25
3	ND	--	89.5	[86.1 - 92.9]	7.16	93.9	[89.6 - 98.2]	8.57
4	ND	--	83.7	[75.9 - 91.5]	17.56	92.7	[88.6 - 96.9]	8.35
5	ND	--	88.3	[83.5 - 93.1]	10.19	87.7	[81.3 - 94.1]	13.68
6	ND	--	91.8	[88.0 - 95.6]	7.74	84.0	[76.9 - 91.1]	15.87
7	93.8	[71.1 - 98.5]	80.4	[73.4 - 87.4]	16.31	75.4	[67.6 - 83.2]	19.37
8	81.3	[56.2 - 93.0]	83.0	[77.3 - 88.7]	12.80	83.6	[75.0 - 92.1]	19.20
9	87.5	[64.2 - 96.3]	87.3	[82.5 - 92.2]	10.41	73.5	[61.5 - 85.4]	30.47
10	75.0	[50.2 - 89.4]	88.9	[82.4 - 95.4]	13.74	82.1	[73.9 - 90.4]	18.85
Mean	**88.5**	**[80.7 - 93.5]**	**87.4**	**[84.7 – 90.1]**	**4.32**	**85.8**	**[80.5 - 91.0]**	**8.58**

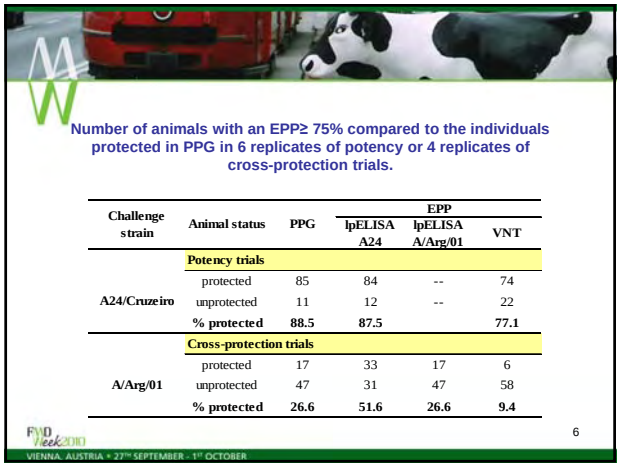
Intra-trial repeatability (lp ELISA or VNT): CV ≤20%

Inter-trial reproducibility lpELISA CV = 4.32%; VNT = 8.58%



Cross-protection between A24/Cruzeiro and A/Arg/01 by PPG or EPP

Trial	PPG (%)	95%CI	A/Arg/01 lpELISA EPP (%)	95%CI	EPP [c] (%)	95%CI	VNT EPP [c] (%)	95%CI
1	ND	--	**41.7**	**[31.7 – 51.7]**	66.2	[59.1 – 73.3]	**32.5**	**[21.3 - 43.6]**
2	ND	--	**59.6**	**[47.9 - 71.4]**	76.8	[70.0 – 83.7]	**38.6**	**[27.6 - 49.5]**
3	56.3	[33.2 - 76.6]	**57.9**	**[46.7 – 69.1]**	76.4	[70.3 – 82.6]	**50.6**	**[34.9 - 66.2]**
4	25.0	[10.5 - 50.0]	**41.9**	**[30.8 – 53.0]**	66.0	[58.3 – 73.8]	**29.1**	**[18.5 - 39.7]**
5	12.5	[3.8 - 36.4]	**61.9**	**[48.0 – 75.8]**	78.3	[70.5 – 86.0]	**19.8**	**[19.8 - 19.8]**
6	12.5	[3.9 - 36.6]	**63.3**	**[50.7 – 75.9]**	78.9	[72.1 – 85.7]	**19.8**	**[19.8 - 19.8]**
7	ND	--	**47.3**	**[33.6 - 60.9]**	69.1	[60.5 – 77.6]	**23.6**	**[15.5 - 31.8]**
8	ND	--	**47.7**	**[35.6 – 59.7]**	69.8	[62.4 – 77.2]	**56.7**	**[48.6 - 64.8]**
9	ND	--	**66.2**	**[54.5 - 77.9]**	79.9	[72.5 –87.2]	**25.0**	**[21.7 - 28.4]**
10	ND	--	**69.5**	**[55.8 – 83.2]**	81.7	[73.6 – 89.7]	**19.8**	**[19.8 - 19.8]**
Mean	**26.6**	**[17.4 - 38.5]**	**55.7**	**[48.4 - 63.0]**	**74.3**	**[70.1 - 78.5]**	**31.6**	**[22.1 - 41.0]**
			SENASA's curve for A/Arg/01		**SENASA's curve for A24**		**PANAFTOSA's curve for A24**	



Number of animals with an EPP≥ 75% compared to the individuals protected in PPG in 6 replicates of potency or 4 replicates of cross-protection trials.

Challenge strain	Animal status	PPG	EPP lpELISA A24	EPP lpELISA A/Arg/01	VNT
	Potency trials				
	protected	85	84	--	74
A24/Cruzeiro	unprotected	11	12	--	22
	% protected	**88.5**	**87.5**		**77.1**
	Cross-protection trials				
	protected	17	33	17	6
A/Arg/01	unprotected	47	31	47	58
	% protected	**26.6**	**51.6**	**26.6**	**9.4**

CONCLUSIONS (potency)

- ✓ Assessment of vaccine potency by EPP/lpELISA showed an excellent repeatability, reproducibility and concordance with PPG
- ✓ EPP/VNT falsely rejected the vaccine batch in one out of ten occasions
- ✓ The variation of EPP/lpELISA (4.32%) was lower than the variation of PPG (10.4%)
- ✓ The results strongly support the replacement of potency challenge tests by indirect serological assays, at least for A24/Cruzeiro FMDV strain.

CONCLUSIONS (cross-protection)

- ✓ The variability of cross-protection assays was high by all methods.
- ✓ Assessment of cross-protection by EPP (VNT titers) was closer to PPG
- ✓ EPP (lpELISA titers) was found dependent on the correlation curve (homologous or heterologous) was used for interpolation of the serum titers.
- ✓ In most outbreaks, a correlation curve for the emerging strain would not be available.

MILSTEIN
CEVAN

CONICET — *Blanca Robiolo, Cristina Seki, José La Torre, Nora Mattion*

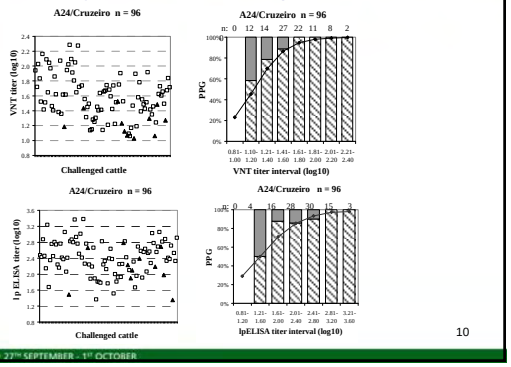
INTA — *Norberto Fondevila, Eduardo Palma*

SENASA — *Eduardo Maradei, Claudia Perez, Alejandro Perez*

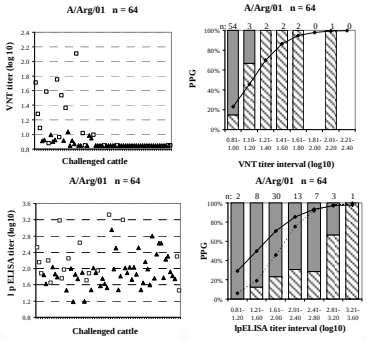
BIOGENESIS-BAGO — *Eliana Smitsaart*

CODA - VAR — *Nesya Goris, Kris De Clercq*

Potency: Distribution of VNT and lpELISA titers for A24 Cruzeiro



Cross protection: Distribution of VNT and lpELISA titers for A/Arg/01



IN VITRO ALTERNATIVES FOR FOOT-AND-MOUTH DISEASE VIRUS CHALLENGE IN THE PD_{50} VACCINE POTENCY TEST ARE SEROTYPE-DEPENDENT

Tom Willems[1] Dr. Vladimir Diev[2]
Dr. David Lefebvre[1] Dr. Vladimir Borisov[2]
Dr. Kris De Clercq[1] Dr. Guntram Paul[3]

[1] *Unit of Vesicular and Exotic Diseases, Department of Virology, CODA-CERVA-VAR, Groeselenberg 99, 1180 Ukkel, Belgium*
[2] *Biological and Technical Control Department, Federal Governmental Institution "Federal Centre for Animal Health", ARRIAH , 600901, Yur'evets, Vladimir, Russia*
[3] *Intervet International BV, Boxmeer, AA, The Netherlands*

Short summary

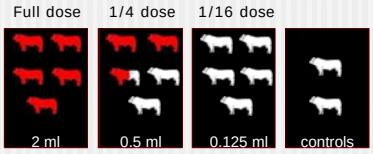
- Introduction
- *In vivo* PD_{50} results
- *In vitro* alternative PD_{50}potency test
- Is alternative serotype independent?
- Conclusions

Conclusions

- Indirect vaccine potency test based on serologic models
  → PD_{50} value
- Indirect vaccine potency test = valid alternative
  Model → underpredicts true PD_{50}
- Evaluation of serotype independence for model/lab

Introduction (1)

- Vaccinate-to-live policy → quality and suitability of vaccine
- Potency determination: PD_{50} test
- 1 PD_{50} = dose protects 50% of animals
- ↑ PD_{50} → ↑ protection



Introduction (2)

- Previouse Study → Precision
  - 10 replicate homologous PD_{50} tests
    - N=150
    - Vaccine / Challenge = FMDV O_1 Manisa
- Current Study → ≠ serotype
  - 5 replicate homologous PD_{50} tests
    - N=74 (1 died before challenge)
    - Vaccine / Challenge = FMDV A Iran 1996

In Vivo FMDV A Iran 96 Vaccine PD_{50} test

Trial	Vaccine Dose Group 1/16	1/4	Full	Animals #	PD50
1	4	4	5	15	18,37
2	1	5	5	15	10,55
3	5	5	4	15	24,24
4	5	5	5	15	31,99
5	4	4	5	14	24,24
Protected #	19	23	24	66	20,48
Animals #	25	24	25	74	

- In vivo PD_{50} range : 10,6 ~ 32,0
- Overall PD_{50} of 20.5 (95% CI: 14.9-27.4)
- Highly potent A Iran 1996 vaccine

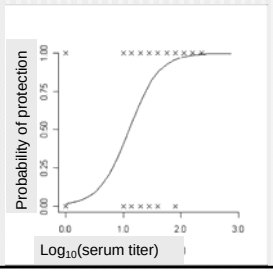
Serology – Materials and methods

- Sera
  - N = 74
  - PD_{50}
  - FMDV A Iran 96
  - 21 dpv
- Serological tests
  - VNT
  - LPBE

  Participating labs
  - VAR (Belgium)
  - Intervet (BHC, Germany)
  - FGI-ARRIAH (Russia)
- Sera 1:2 serially titrated

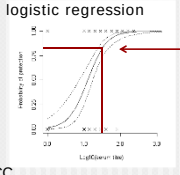
Logistic regression model (1)

- Model fit: Akaike Information Criterion (AIC)
- Somers' D rank correlation between t_i and protection status



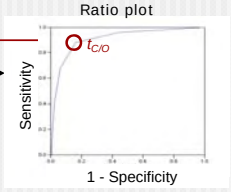
Logistic regression model (2)

logistic regression



ROC

True Positive-False Positive Ratio plot



- ACC
- $p_{C/O}$
- $t_{C/O}$
  - Max. predicting true protected
  - Min. predicting false protected
- $t_i \geq t_{C/O} \rightarrow$ protected
- $t_i < t_{C/O} \rightarrow$ not-protected

p_c	t_c	FPR	TPR	ACC (%)
0.406	1.00	0.634	0.991	67.8
0.547	1.15	0.439	0.981	77.1
0.687	1.30	0.317	0.880	78.1
0.795	1.45	0.220	0.824	80.2
0.876	1.60	0.098	0.676	78.9
0.928	1.76	0.024	0.500	73.8
0.958	1.90	0.024	0.315	64.5
0.976	2.05	0.000	0.194	59.7
0.986	2.20	0.000	0.065	53.2
0.992	2.35	0.000	0.028	51.4

Results VNT Model

VNT A96 prediction			/	VNT Model A96			
overall PD50	95% CI	Test		Model	EPP (%)	C/O (log10)	Accuracy (%)
20.5	14.9 - 27.4	-		In Vivo	-	-	-
11.1	9.2 - 19.1	ARRIAH		ARRIAH	88.5	1.34	78.7
10.2	8.4 - 19.1	VAR		VAR	93.8	1.53	82.5

- Model underpredicts overall PD_{50}

  9 /66 protected animals were negative for VN Ab!
- Overlap in 95%CI with *In Vivo*:

Results LPBE Model

LPBE A96 prediction			/	LPBE Model A96			
overall PD50	95% CI	Test		Model	EPP (%)	C/O (log10)	Accuracy (%)
20.5	14.9 - 27.4	-		In Vivo	-	-	-
6.0	5.0 - 11.2	ARRIAH		ARRIAH	94.7	1.34	72.6
14.7	12.5 - 21.1	VAR		VAR	96.4	1.73	81.8

- Both models underpredict overall PD_{50}
- ARRIAH is out of range of *In Vivo* results

  20 /66 protected animals were negative for Ab!
- VAR overlaps in 95%CI with *In Vivo*

Results VNT Model

VNT A96 prediction			/	VNT Model O1			
overall PD50	95% CI	Test		Model	EPP (%)	C/O (log10)	Accuracy (%)
20.5	14.9 - 27.4	-		In Vivo	-	-	-
5.8	5.0 - 14.0	ARRIAH		ARRIAH	73.6	1.51	75.8
8.7	6.9 - 14.6	VAR		VAR	87.0	1.68	76.7

VNT A96 prediction			/	VNT Model A96			
overall PD50	95% CI	Test		Model	EPP (%)	C/O (log10)	Accuracy (%)
20.5	14.9 - 27.4	-		In Vivo	-	-	-
11.1	9.2 - 19.1	ARRIAH		ARRIAH	88.5	1.34	78.7
10.2	8.4 - 19.1	VAR		VAR	93.8	1.53	82.5

- Both models underpredict PD_{50}

Results LPBE Model

LPBE A96 prediction / LPBE Model O1						
overall PD50	95% CI	Test	Model	EPP (%)	C/O (log10)	Accuracy (%)
20.5	14.9 - 27.4	-	In Vivo	-	-	-
2.1	1.7 - 3.1	ARRIAH	ARRIAH	81.2	1.65	73.6
13.1	11.2 - 20.5	VAR	VAR	83.4	1.95	84.2

LPBE A96 prediction / LPBE Model A96						
overall PD50	95% CI	Test	Model	EPP (%)	C/O (log10)	Accuracy (%)
20.5	14.9 - 27.4	-	In Vivo	-	-	-
6.0	5.0 - 11.2	ARRIAH	ARRIAH	94.7	1.34	72.6
14.7	12.5 - 21.1	VAR	VAR	96.4	1.73	81.8

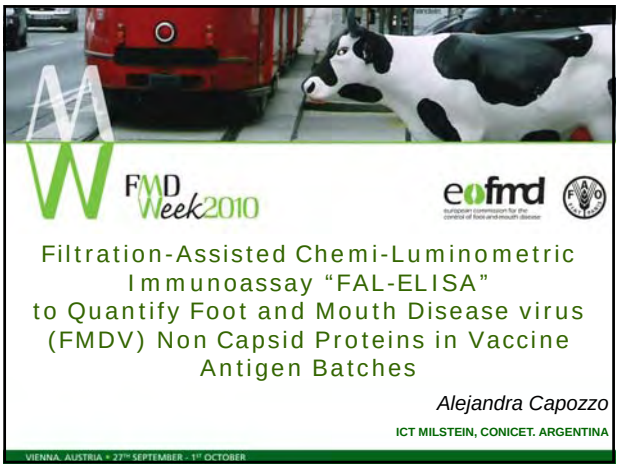
- Both models underpredict PD_{50}
- VAR models predict similar PD_{50}

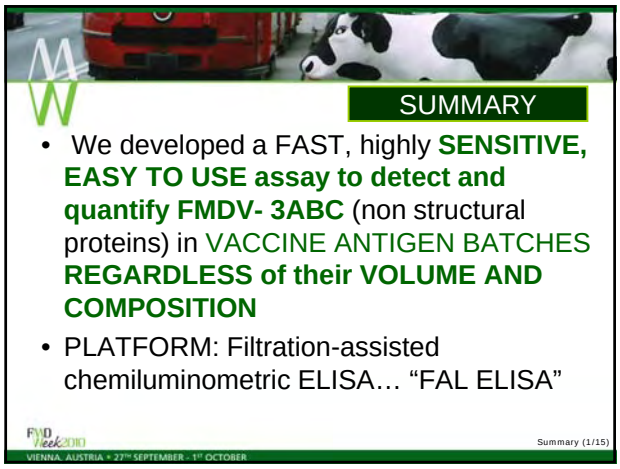
Conclusions

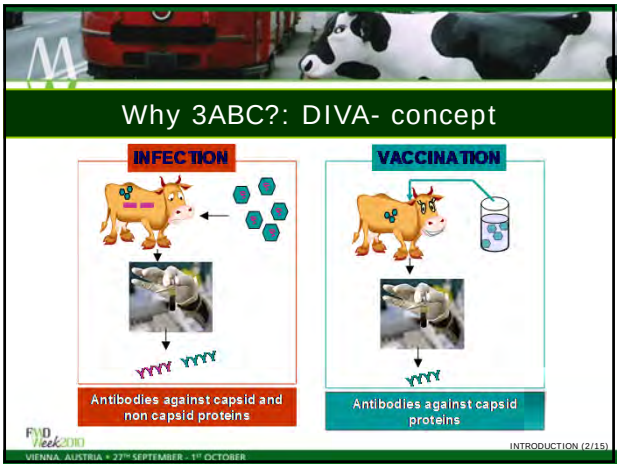
- Indirect vaccine potency test based on logistic regression + ROC
  → PD_{50} value
- Indirect procedure = valid alternative
  - Compared to *in vivo* PD_{50}
    Model → underpredicts true PD_{50}
- Evaluation of serotype independence for model/lab

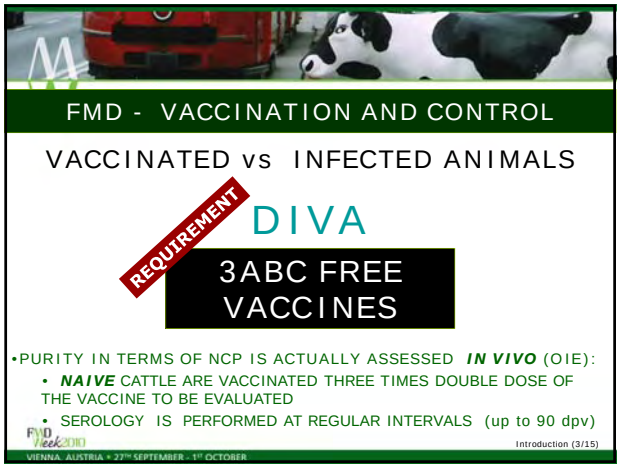
Thanks for your attention

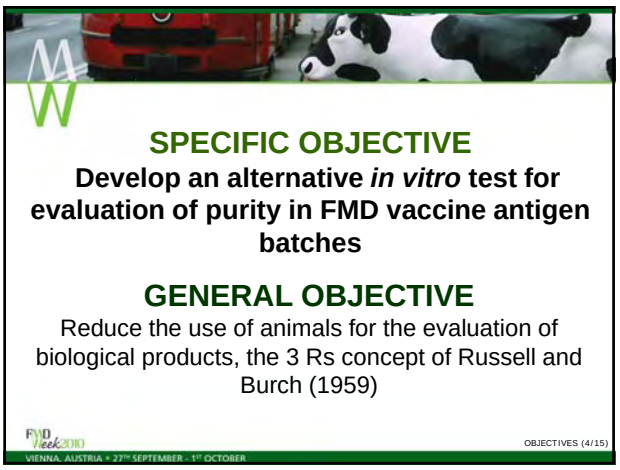


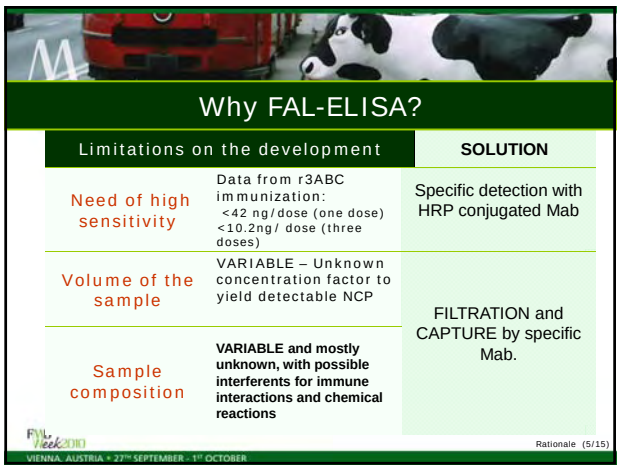

FMD Week2010
eufmd
Filtration-Assisted Chemi-Luminometric Immunoassay "FAL-ELISA" to Quantify Foot and Mouth Disease virus (FMDV) Non Capsid Proteins in Vaccine Antigen Batches
Alejandra Capozzo
ICT MILSTEIN, CONICET. ARGENTINA
VIENNA. AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


SUMMARY
• We developed a FAST, highly SENSITIVE, EASY TO USE assay to detect and quantify FMDV- 3ABC (non structural proteins) in VACCINE ANTIGEN BATCHES REGARDLESS of their VOLUME AND COMPOSITION
• PLATFORM: Filtration-assisted chemiluminometric ELISA… "FAL ELISA"
Summary (1/15)
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Why 3ABC?: DIVA- concept
INFECTION
VACCINATION
Antibodies against capsid and non capsid proteins
Antibodies against capsid proteins
INTRODUCTION (2/15)
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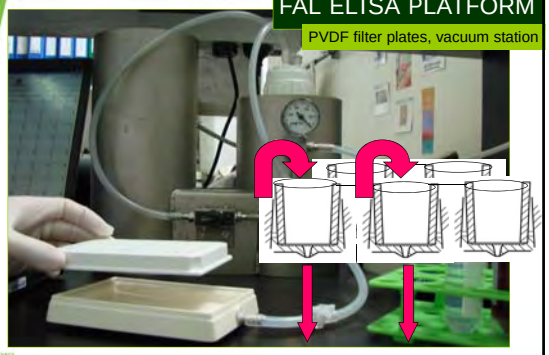

FMD - VACCINATION AND CONTROL
VACCINATED vs INFECTED ANIMALS
REQUIREMENT
DIVA
3ABC FREE VACCINES
•PURITY IN TERMS OF NCP IS ACTUALLY ASSESSED IN VIVO (OIE):
• NAIVE CATTLE ARE VACCINATED THREE TIMES DOUBLE DOSE OF THE VACCINE TO BE EVALUATED
• SEROLOGY IS PERFORMED AT REGULAR INTERVALS (up to 90 dpv)
Introduction (3/15)
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SPECIFIC OBJECTIVE
Develop an alternative in vitro test for evaluation of purity in FMD vaccine antigen batches
GENERAL OBJECTIVE
Reduce the use of animals for the evaluation of biological products, the 3 Rs concept of Russell and Burch (1959)
OBJECTIVES (4/15)
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Why FAL-ELISA?
Limitations on the development
SOLUTION
Need of high sensitivity
Data from r3ABC immunization: <42 ng/dose (one dose) <10.2ng/ dose (three doses)
Specific detection with HRP conjugated Mab
Volume of the sample
VARIABLE – Unknown concentration factor to yield detectable NCP
FILTRATION and CAPTURE by specific Mab.
Sample composition
VARIABLE and mostly unknown, with possible interferents for immune interactions and chemical reactions
Rationale (5/15)
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FAL ELISA PLATFORM

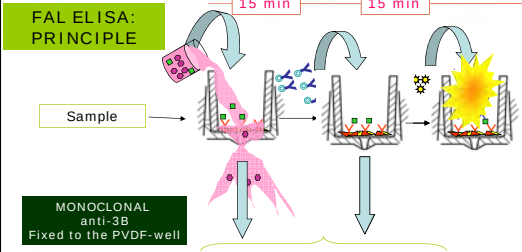
PVDF filter plates, vacuum station



Platform (6/15)

FAL ELISA: PRINCIPLE

SAMPLES — 15 min — DETECTION — 15 min — 40 min



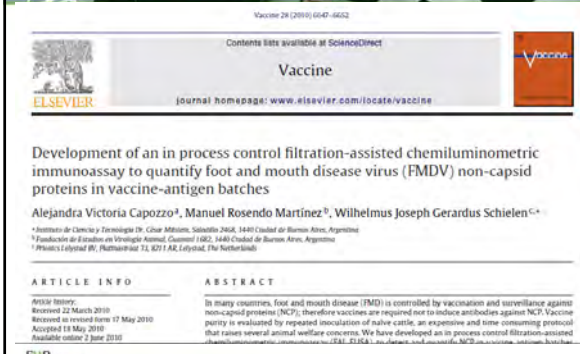
PRINCIPLE (7/15)

FAL ELISA PLATFORM

Photoluminometer



Platform (8/15)



Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Development of an in process control filtration-assisted chemiluminometric immunoassay to quantify foot and mouth disease virus (FMDV) non-capsid proteins in vaccine-antigen batches

Alejandra Victoria Capozzo[a], Manuel Rosendo Martínez[b], Wilhelmus Joseph Gerardus Schielen[c,*]

ARTICLE INFO

ABSTRACT

Platform (9/15)

FAL-ELISA to quantify FMDV-3ABC : VACCINE ANTIGEN BATCHES

SENSITIVITY: 2ng/well

Do vaccine components interfere with the reaction?

"SPIKING" VACCINE ANTIGEN BATCHES with known amounts of 3ABC

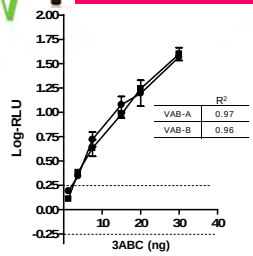
- TWO BATCHES:
  - 1x (filtered)
  - PEG concentrate



Platform (10/15)

FAL ELISA

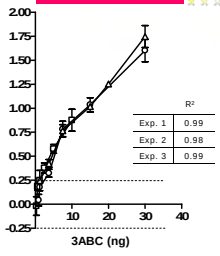
Spiked industrial vaccine antigen batches



CORRELATION ANALYSIS [3ABC]

	P value	r (Pearson)
VAB-A	0.0002	0.9880
VAB-B	0.0006	0.9792

Dilutions of Rec 3ABC



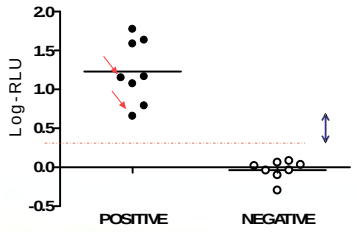
	P value	r (Pearson)
Exp. 1	0.0019	0.9640
Exp. 2	0.0014	0.9693
Exp. 3	<0.0001	0.9922

Results (11/15)

FAL ELISA - IPC

Application

- NCP negative VABs
- NCP spiked VAB
- One positive NCP-VAB (1:1 and 1:3)



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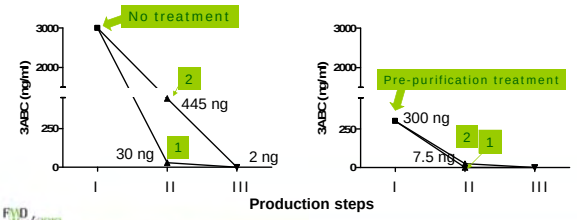
Results (12/15)

In Process Control

Application

FAL ELISA APPLIED TO FMDV VACCINE ANTIGEN PRODUCTION PROCESS

I- PRE-PURIFICATION TREATMENT	Unique
II- PARTIAL PURIFICATION TREATMENT	1 vs 2
III-FINAL PURIFICATION TREATMENT	Unique



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Results (13/15)

CONCLUSIONS

- FAL-ELISA analytical detection limit is ***more than two times below the desirable LOD***
- FAL ELISA is not interfered by components of antigen-batches from different manufacturers

FAL ELISA is now commercially available in a kit format

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Conclusions (14/15)

MUCHAS GRACIAS



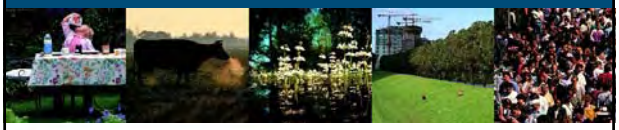
Alejandra Capozzo, Manuel Martínez, M. Ángeles Lavoria and ***Wim Schielen***

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Thanks-slide! (15/15)

EFFECT OF THIOMERSAL ON FMDV VIRION DISSOCIATION INTO 12S PARTICLES

Michiel Harmsen, Helmi Fijten, Douwe Westra, Jose Coco-Martin



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Talk Summary

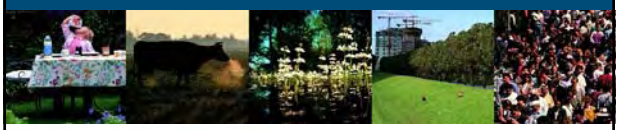
- Virion dissociation reduces vaccine efficacy
- ELISAs for measuring virion dissociation:
  - Based on (llama) single-domain antibody fragments
  - Specifically measuring 12S and 146S (O serotypes)
  - Based on measuring 12S before and after heating
- Effect of thiomersal on virion dissociation

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Conclusions

- Novel ELISAs usefull for measuring vaccine stability
- Thiomersal stimulates virion dissociation

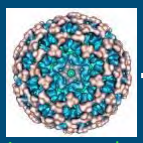
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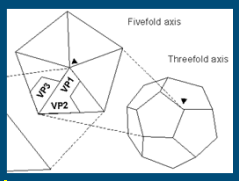
FMDV dissociation

146S → **12 x 12S**



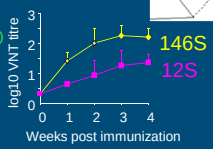
1 h 56°C

Acid treatment



Immunogenic

(Doel & Chong, 1982)



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FMDV O1 Manisa binding VHHs M3 and M170

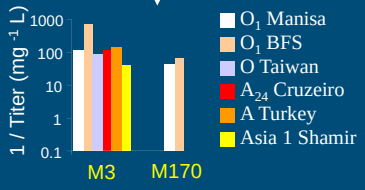


Llama heavy chain antibody

VHH (nanobody)

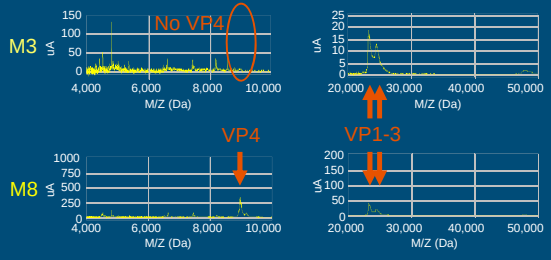
24 VHHs against FMDV O1 Manisa

(Harmsen et al., 2007, Vet Microbiol 120, 193-206)



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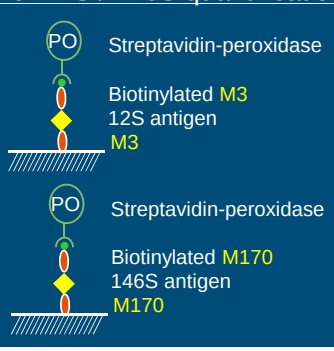
M3 binds viral particles lacking VP4



(Harmsen et al., 2010, Vaccine 28, 3363-3370)

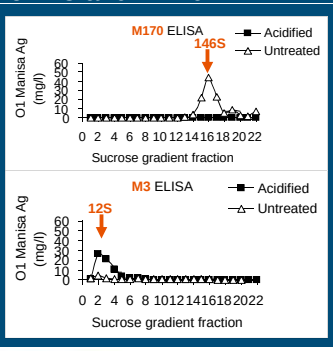
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DAS ELISAs for 12S / 146S quantification



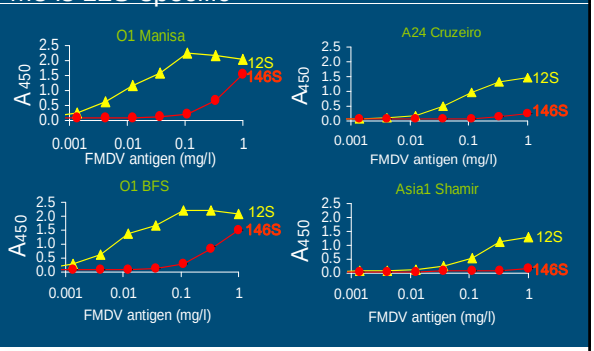
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Specificity of M3 and M170



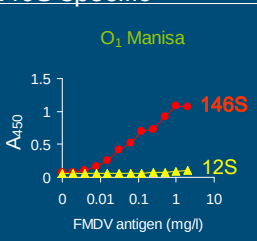
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M3 is 12S-specific



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M170 is 146S specific



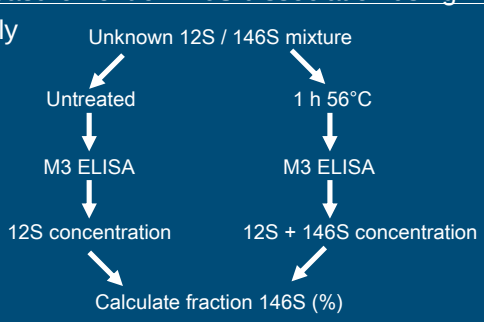
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Summary M3 and M170

Property	M3	M170
Serotypes recognized	O, A, C, Asia1	O
Particle specificity	12S	146S

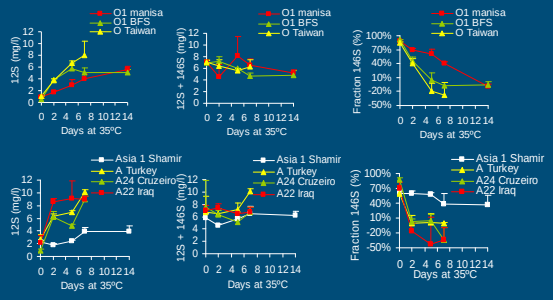
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Measurement of 146S dissociation using M3 only



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Stability of seven strains at 35°C



Thiomersal (thimerosal, merthiolate)

- Ethyl-mercury compound
- Preservative for multi-dose vaccines
- Decreases poliovirus immunogenicity
- Decreases reactivity in poliovirus whole virion specific ELISA

(Sawyer et al., 1994, Vaccine 12, 851-6)

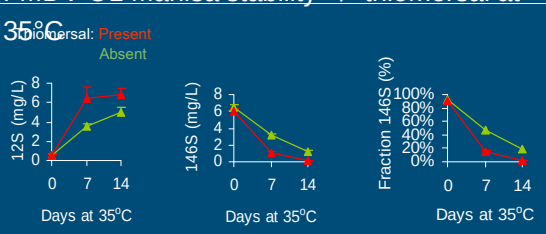
Thiomersal is also used in FMDV vaccines



COMPOSITION : Raksha Ovac Trivalent FMD Oil Adjuvant vaccine contains tissue culture virus strains " O, A and Asia1 " and inactivated with Aziridine compound. Mineral oil is added as an adjuvant. Thiomersal 0.01% w/v added as preservative

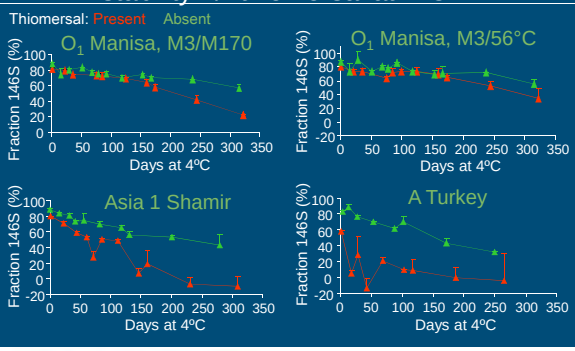
http://www.tradeindia.com/fp410135/Raksha-Ovac-Trivalent-Vaccine.html

FMDV O1 Manisa stability +/- thiomersal at 35°C



→ Measuring both 12S and 146S shows that decrease in 146S is not due to proteolysis but due to virion dissociation

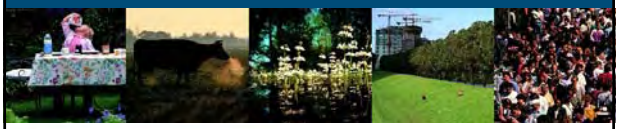
FMDV stability +/- thiomersal at 4°C



Conclusions

- Novel ELISAs usefull for measuring vaccine stability
- Thiomersal stimulates virion dissociation

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Policy and disease containment strategies in FMD: Living with uncertainty

29 September 2010 workshop

Universities of Liverpool and Lancaster, United Kingdom

Lost in Translation

A cross-disciplinary analysis of knowledge exchange and effectiveness in animal disease management

- Jonathan Wastling
- Sophia Latham
- Zoe Austin
- Maggie Mort
- Roger Pickup

Research Context

- Our project: collaboration between Liverpool and Lancaster Universities
- Our team: involves sociologists of health and science, human geographers, as well as veterinary, soil and water scientists
- Three year project – first phase: problem framing, developing shared expectations and common language. Initial consultation with an external advisory group

Project aims

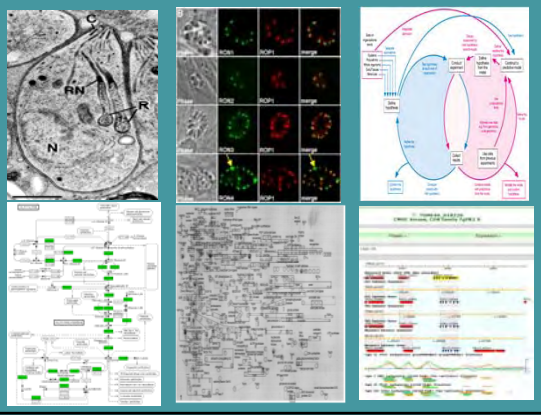
The project addresses two main issues:

- How we can understand better the issues of complexity and uncertainty in animal disease management strategies
- Why particular technical developments have been adopted and not others in the deployment of strategies of containment

Research strategy

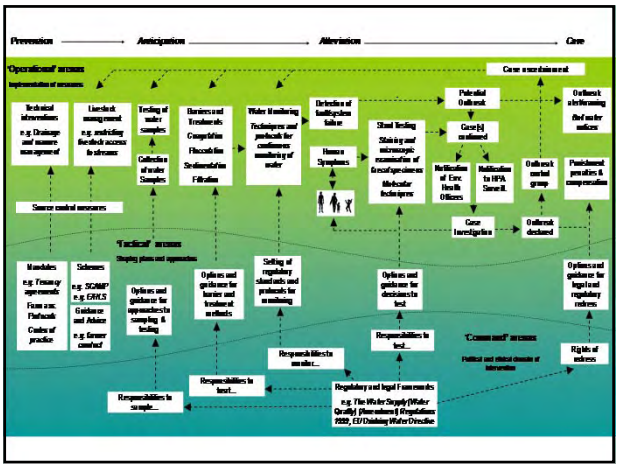
- Semi-structured interviews with experts at the strategic, tactical and operational levels (approx 50 interviews with stakeholders and experts)
- Disease specific workshops addressing areas of uncertainty identified from interview data
- Analysis of secondary data (existing archived material)

Complexity and uncertainty and the molecular and cellular level



A different form of complexity and uncertainty!







Is there a plan?

(Jeff Hammond – this meeting)

Contingency
Plan for
Exotic
Animal
Diseases
Framework
Response Plan
December 2008
www.defra

Science and technological uncertainties

Example focuses:

- Development and application of **modelling techniques** within surveillance and control of disease: FMD and AI
- Powerful tools (attractive to policy-makers)
- Appear to summarise a problem
- But can mask uncertainties and often poorly understood by non-modellers

Primary project focus is on making sense of uncertainties at the **operational** and **tactical** end of containment, and in particular, contexts in which the application of **science and technology** is an important area of innovation…

Foot and Mouth

Cryptosporidiosis

Avian Influenza

- What do S.o.Cs involve people doing and why do they do these things?
- Which aspects of S.o.Cs are perceived to work? And which aren't?
- What matters and what might change?
- What steps should be taken to realise more effective forms of disease governance?

Disease focus

• Cross-disease analysis with a focus on current and emerging practice in three disease areas:

– ***Avian Influenza***

– ***Foot and Mouth Disease***

– ***Cryptosporidiosis***

Workshop aims and objectives

- The workshops form an important stage in the process of the analysis, assessing:
  - The relevance of the identified themes
  - Gaps in the identified themes
  - Their validity, scope and completeness with reference to Avian Influenza
  - Relative importance/priorities for the future
  - Cross-disease containment strategies - the wider context.

Avian Influenza Workshop , Oxford, September 2010





Activity 1

- Examination of themes of uncertainty identified by experts
- Additional themes and comments

Process: Identifying cross disease themes

Step 1: Open coding
Read through interview transcripts marking 'significant' and recurrent issues and assign these into 'codes'

Step 2: Data refinement
Discuss and refine codes with research team, and group into substantiated 'themes'

Process: Identifying cross disease themes, steps 1 and 2: example

Quotes:
A number of quotes were identified relating to uncertainties and dispute surrounding the development of technologies for use within the animal disease strategy

Open codes:
Cost of developing new technology may be prohibitive

Lack of sharing of data/samples e.g. Difficulty obtaining samples from some parts of the world could decrease the speed of response to newly emerging strains

Ethical uncertainty and practical difficulties in ensuring that all countries benefit from isolate sharing

Theme:
"Development of new technologies"



Theme	Cryptosporidium	FMD	AI
Role and expertise of individual (T1)			
Media Coverage (T2)			
Political Will/Motivation (T3)			
Funding (T4)			
Priority given to Disease (T5)			
Changes in Regulation (T6)			
Research & Development (T7)			
Role of Industry (T8)			
Role of Animal and land Management (T9)			
Stakeholder Base (T10)			
Development of new technologies (T11)			
Use of new & existing technologies (T12)			
International Differences in regulation (T13)			
Human Behaviour (T14)			
Underlying objectives of outbreak response (T15)			

Rough data!



Activity 2

- Importance and likelihood of themes

Process: Identifying cross disease themes

Step 1: Open coding
Read through interview transcripts marking 'significant' and recurrent issues and assign these into 'codes'

Step 2: Data refinement
Discuss and refine codes with research team, and group into substantiated 'themes'

Step 3: Consultation
Themes discussed with stakeholders within disease specific **workshops.** Relevance and importance as well as missing elements are assessed.

Step 4. Final stages
Once themes have been reviewed and then the interview transcripts re-visited, relationships between themes can be established and assessed further

Programme

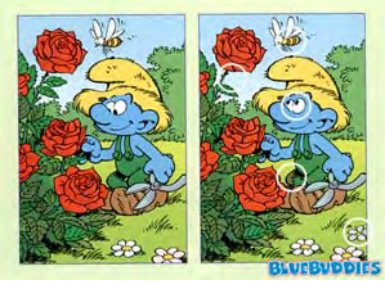
Time	Session
11.30	Group discussion on the relevance of themes and where there might be gaps
	Tea/coffee
12.30	Session 1. Critical review in groups of content of themes: Avian Influenza
13.15	*Lunch*
14.00	Session 2. Discussion in groups of relative importance of themes and scoring exercise
14.30	Revisit issues of uncertainty and technological development Concluding remarks. Cross-disease context and priorities for the future.
15.00	Close

Discussion: Identifying cross disease themes

- Are all of the themes relevant?
- Have we missed any?

Session 1. Critical review of content and importance of themes: Avian Influenza

Vaccine Matching Introduction



David Paton BBSRC

Summary

- Why need vaccine matching
- How done
- Issues and alternatives
- How done for other agents
- Gaps / Priorities

Immunity conferred by current vaccines

- Serotype-specific and relatively short-lived after a single course of vaccination
- Protection shown to correlate with capsid antibodies and CMI
- Early antibody response ~50:50 T cell dependent : T cell independent
- Strength and duration of antibody response depends upon vaccine potency and antigenic match
- Actual protection influenced by many other variables

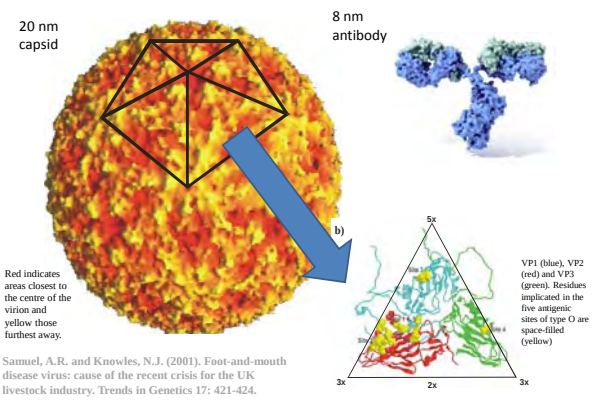


Need for vaccine matching for FMD

- Antigenic change due to mutation and recombination
- Some serotypes more antigenically diverse
- Experimental evidence of cross-protection and its lack
- Field evidence of importance
  - Major disease outbreaks in properly vaccinated animals?
  - Reduced efficacy of vaccination?
    - A Arg 2001 in A24 vaccinated cattle in S America
    - A Iran 05 strain cases in A Iran 96 vaccinated cattle in Middle East
    - SAT 2 outbreaks in Botswana despite use of a trivalent vaccine
    - O PanAsia2 in Turkey and Iran this year

Vaccine selection in practice

- Field work
  - to investigate outbreaks and collect samples
- Lab work
  - to determine the serotype, strain and vaccine match
- Vaccine producer
  - to produce and supply the vaccine
- Livestock industry / competent authority
  - to determine the vaccination policy and purchase vaccine for use / banks

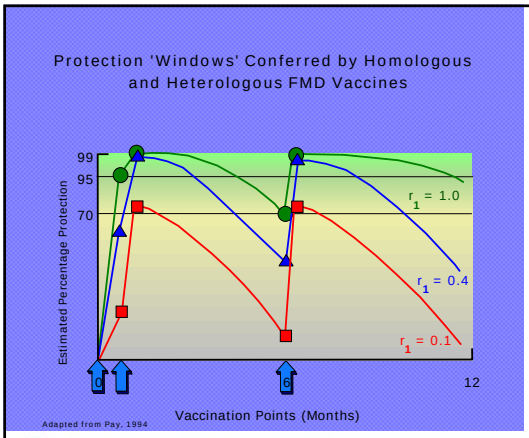


Mechanisms of antibody based protection

- Importance of phagocytosis in mouse model (McCullough et al., 1986; 1992)
  - antibodies protect in vivo at concentrations that are sub-neutralising in vitro
  - mAb-virus combinations that neutralise in vitro still kill in vivo if phagocytosis blocked
- Vaccine from a multiple mAb-resistant mutant virus (Dunn et al., 1998) in guinea-pig model
  - induced protective antibody to wild type virus associated with reduced in vitro neutralisation of wild type virus but not reduced binding in ELISA
  - implication that VNT may underestimate cross-protection due to effect of non-neutralising antibodies

Measuring antigenic match by serology

- Serological methods – CFT, VNT, ELISA
- Relationship (r) values between pairs of viruses
  - one way (r_1)
  - two way (r_2)
- Antigenic cartography for multiple comparisons
- How good a match is needed - cut-off value
  - related to vaccine potency and nature of challenge
- S. American approach to directly measure cross-protection from a given batch of tested vaccine



Slide courtesy of Tim Doel, Merial Animal Health


Vaccine (2008) 26, 1681–1687

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Vaccine

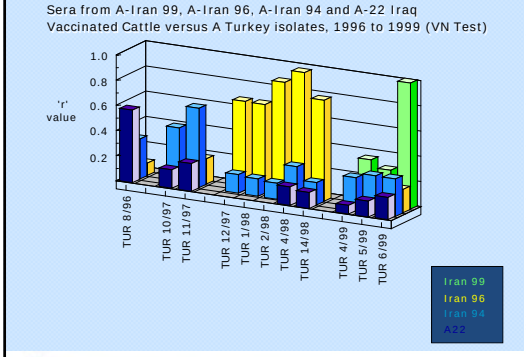
High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus

K.E. Brehm[a], N. Kumar[a], H.-H. Thulke[b], B. Haas[a,*]

KEYWORDS
Foot-and-mouth disease virus;
Protection by vaccines;
Serology

Summary In a series of three homologous and eight heterologous challenge experiments, it was shown that high potency vaccines against foot-and-mouth disease (FMD) serotype A can induce protection even against heterologous challenge infection with viruses that give low r-values with the vaccine strains.
The challenge virus specific neutralizing antibody response on the day of challenge (21 days post vaccination) generally correlated with protection.
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Vaccine matching (r values)



Slide courtesy of Tim Doel, Merial Animal Health


Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Recent Spread of a New Strain (A-Iran-05) of Foot-and-Mouth Disease Virus Type A in the Middle East

N. J. Knowles[1], M. H. Nazem Shirazi[2], J. Wadsworth[1], K. G. Swabey[1], J. M. Stirling[1], R. J. Statham[1], Y. Li[1], G. H. Hutchings[1], N. P. Ferris[1], O. Parlak[3], F. Özyörük[3], K. J. Sumption[4], D. P. King[1] and D. J. Paton[1]

Fig. 2. Map showing the locations of FMDV A-Iran-05 isolates from 2003 to 2008. Pink, 2003; purple, 2004; green, 2005; blue, 2006; red, 2007; yellow, 2008. Those markers that contain a dot indicate known locations while those without are not known and therefore placed in the approximate centre of the country of origin. The map was produced using Google Maps (©Google, 2008).

WRLFMD Ref. No.	Test	Antisera A Irn 96	A Irq 64	A Tur 06†
A/AFG/7/2007	VNT		0.33	
A/AFG/44/2007	VNT		0.32	
A/IRN/41/2003	VNT	0.35	0.33	
A/IRN/7/2004	VNT	0.14	0.67	
A/IRN/1/2005	VNT	0.06		1.00
A/IRN/4/2005	VNT	0.11	0.70‡	1.00
A/IRN/5/2005	VNT	0.17	0.58‡	
A/IRN/7/2005	VNT	0.05	0.31	0.86
A/IRN/7/2005	ELISA	0.12	0.79	
A/IRN/22/2005	VNT	0.05	0.45	
A/IRN/24/2005	VNT	0.13	0.83	
A/IRN/25/2005	VNT	0.15	0.93	
A/IRN/27/2005	VNT	0.23	1.00	
A/IRN/28/2005	VNT	0.05	0.41	
A/IRN/29/2005	VNT	0.05	0.45	
A/IRN/30/2005	VNT	0.26	1.00	
A/IRN/31/2005	VNT	0.13	0.85	
A/IRN/36/2007	VNT	0.14	0.21	0.39
A/IRN/39/2007	VNT	0.14	0.13	0.69

Issues with serological tests

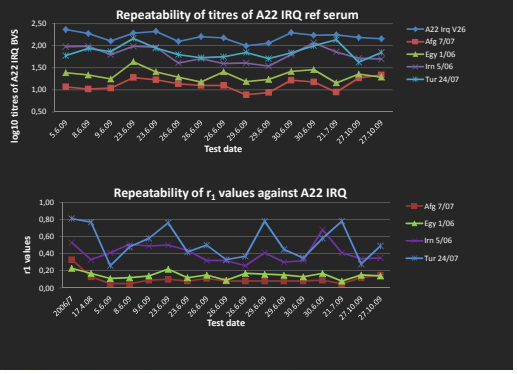
- Speed and effort
- Access to materials
- Reliability
  - Variability of vaccine antisera
    - animal differences
    - vaccine differences
  - Variability of tests
    - e.g. cells for VNT
    - e.g. antibody reagents for LPB ELISA

Contents lists available at ScienceDirect
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Some guidelines for determining foot-and-mouth disease vaccine strain matching by serology

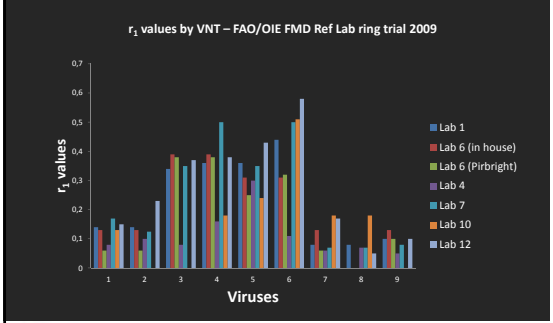
Nora Mattion[a,f,*,1], Nesya Goris[b,1], Tom Willems[b], Blanca Robiolo[a,f], Eduardo Maradei[c,f], Claudia Perez Beascoechea[c,f], Alejandro Perez[c,f], Eliana Smitsaart[e,f], Norberto Fondevila[d,f], Eduardo Palma[f], Kris De Clercq[b], José La Torre[a,f]

The results suggest that a suitable reference serum for vaccine matching r-value experiments might be a pool or a medium to high VNT or lpELISA titer serum



Slide courtesy of Yanmin Li, WRLFMD

Vaccine matching ring trials



Slide courtesy of Yanmin Li, WRLFMD

Matching for other viruses?

- Influenza A
  - H3N2 drift produces novel viruses every 2-5 years
  - HA protein structure and antigenic sites
    - homotrimer with distinct stalk and head
    - 5 antigenic sites comprised of 131 αα and ~13-19 key αα for antigenic predictions
    - cartography: ~2.9 αα changes / unit of antigenic distance
  - HI predicted antigenic distance in relation to vaccine effectiveness (VE) (Ndifon et al., 2009)
    - HI deduced distance measure ≥4 = divergent
    - importance of repeating serology to ensure meaningful results
    - r_2 data a better predictor of VE
  - Impact of immune escape on transmission dynamics (Park et al., 2009)
    - equine influenza model relating antigenic match to outbreak sizes
- Lyssaviruses
  - Antigenic Cartography of rabies and rabies-related viruses (Horton et al 2010)
    - sera from different species predict similar relationships
    - moderate correlation between αα changes and antigenicity

Gaps in knowledge

- The components of the polyclonal antibody response
  - what is the epitope repertoire?
  - What influences it?
  - what are the protective mechanisms?
- Which epitopes contribute to protection?
- Can this be modulated to improve cross-protection?

Alternative practical approaches

- More rigorous field evaluation
- In vitro alternatives
  - Use of monoclonal antibodies
  - Antigenic cartography
  - Sequence based matching
- Better use of existing approaches
  - Standardised methodologies
  - Improved access to materials
  - Better resourced testing regimes

Practical issues for providing best advice and making use of new approaches

- Process for recognising significant threats, making recommendations and changing vaccine strains
  - Clarity of roles and transparency of approach
  - More systematic risk based approach
- Checks by vaccine users – caveat emptor
  - Need for practical approaches
- Capacity to perform testing
  - IP issues in relation to vaccines
  - Funding issues in relation to antisera, testing and standardisation of assays
- Research into improved field and laboratory approaches

OIE/FAO Foot-and-Mouth Disease Reference Laboratories Network

International Network of FMD Vaccine Banks



1st Meeting IAH-Pirbright, 4-5th April, 2006

EU FP7 DISCONVAC PROJECT

2009-2012

Using antigenic data on the FMDV capsid for the selection of new vaccine candidates for the SAT types

B. Blignaut, J.J. Esterhuysen, A. Lukhwareni, T. Mlingo, J. Van Heerden and F.F. Maree

Transboundary Animal Diseases Program, ARC-OVI, Onderstepoort

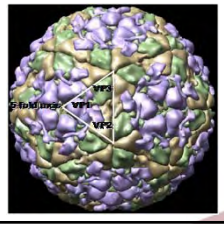
Introduction

- The prevalence of FMD in Africa, parts of Asia and South America, pose a constant threat to the rest of the world.
- Effective control is of global importance – barriers create the most efficient way of control:
  - Physical barriers, including fencing;
  - Immune barrier using vaccination.
- The control of FMD by vaccination is complicated by:
  - The SAT types display appreciably greater intratypic genomic and antigenic variation than the traditional "Euro-Asian" types
  - Vaccines unable to control multiple genetic lineages (and antigenic variants) of the virus that arises in different geographical regions.
- The ability to predict/meassure vaccine efficacy is avaluable tool in an effective control strategy

Introduction ...

Antigenic variation results from changes to the viral capsid.

- Single or multiple residue changes in surface exposed loops of the virion – antigenic drift.
- Changes that result in conformational changes (or recombination) – antigenic shift.

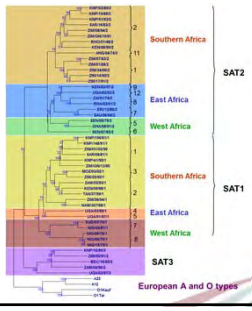


Summary

- Several topotypes exists for each serotype in Africa – molecular epidemiology indicates that large variation exist between topotypes within the SAT serotypes complicating control using vaccination.
- Limited antigenic studies indicate that each topotype within the SAT serotypes may need a vaccine strain – we provided the proof of principle that vaccine strains can be engineered for specific geographic regions.
- Assess the protection afforded by potential new SAT1 vaccine strains against a group of heterologous SAT1 viruses in southern Africa.
- Identified putative antigenic regions for SAT1 viruses using genetic and structural data.

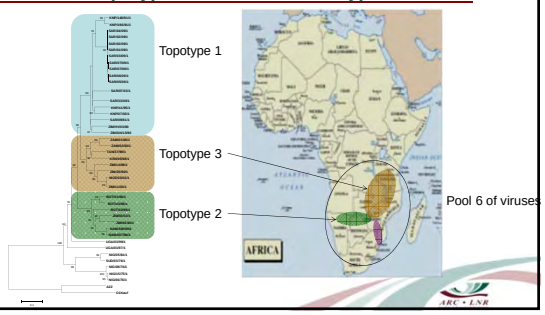
Phylogeny based on P1-coding region

Minimum evolution tree depicting gene relationships for P1-coding regions of SAT1, 2 and 3 viruses. Topotypes based on 1D phylogeny are indicated.



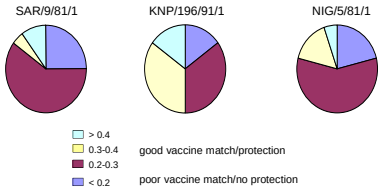
Jukes-Cantor distances and 1000 bootstrap replications were applied. Bootstrap support is indicated.

Topotypes within SAT1 serotype



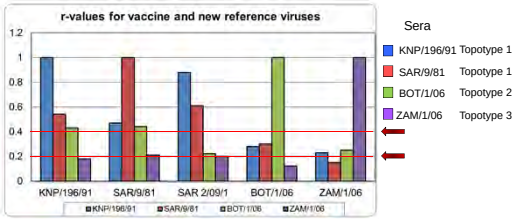
r_1-value distribution for SAT1 viruses

topotypes 1 and 7 (southern and western Africa)



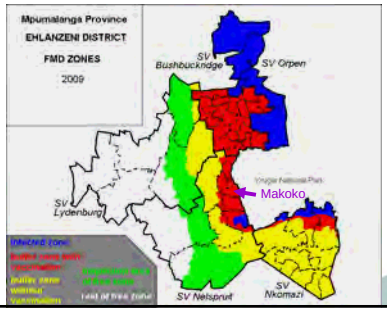
KNP/196/91/1 provides the best vaccine match/antigenic coverage

Cross-reaction of SAT1 reference viruses

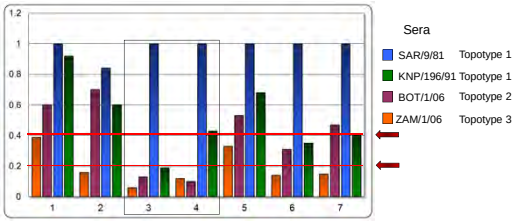


KNP/196/91 and SAR/9/81 vaccine have r-values of >0.4 for viruses from topotypes 1 and 2.

SAT1 outbreak in vaccination zone

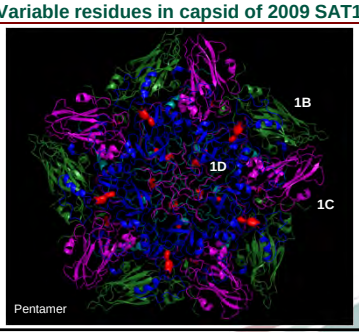


Antigenic match of 2009 SAT1 outbreak

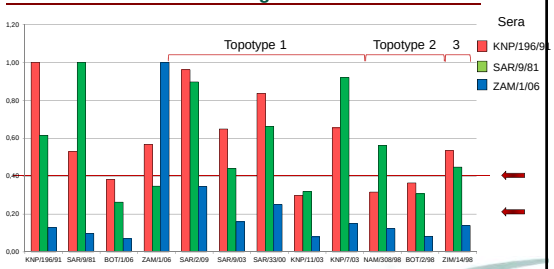


SAT1 outbreak viruses 2009

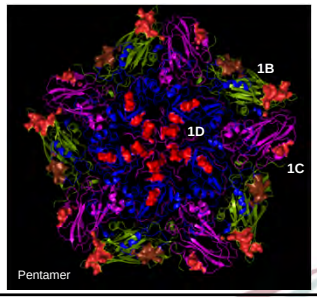
Variable residues in capsid of 2009 SAT1



r1-values of SAT1 viruses against reference strains



Variable residue on capsid surface exposed loops

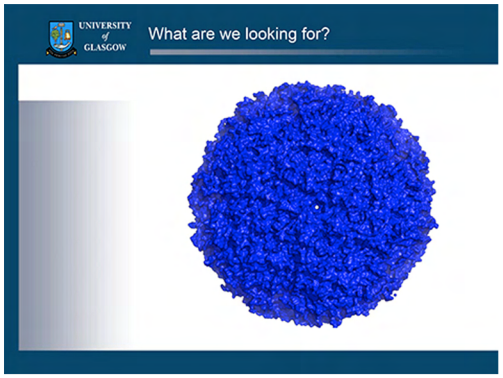


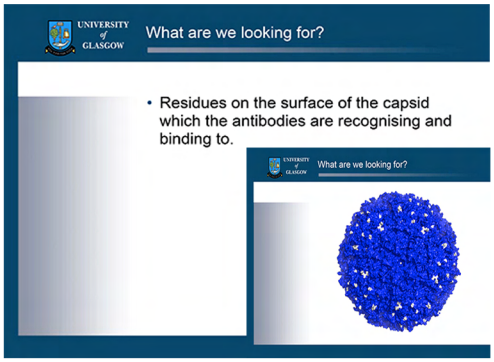
Acknowledgements

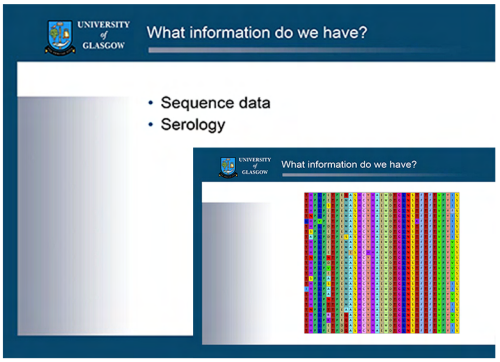
- Support personnel at TADP
- Project was funded by the FAO

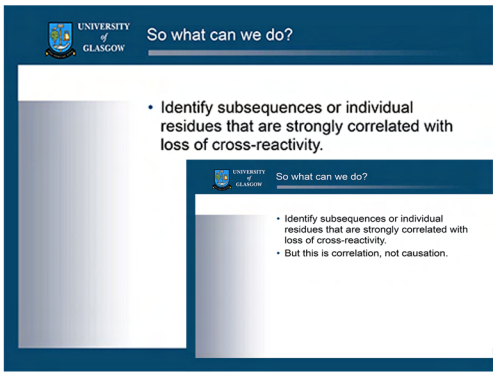
THANK YOU

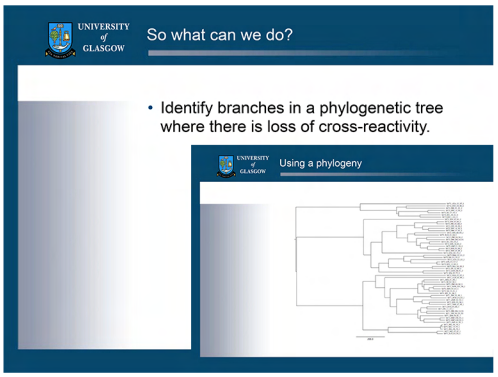








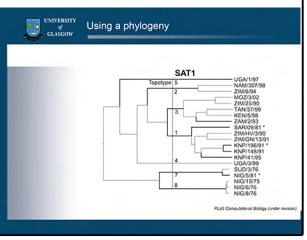




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Using a phylogeny

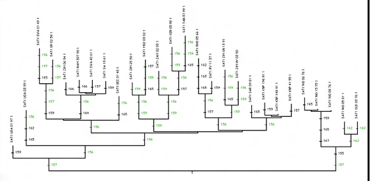
- We can identify the branches across which there is a drop in cross reactivity easily.



Using a phylogeny

- We can identify the branches across which there is a drop in cross reactivity easily.
- Now we no longer have correlation, but we don't have the information we wanted!
- How do we work out the residues responsible?

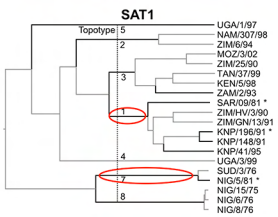
Using a phylogeny



Identifying epitopes

- We can identify the average drop in cross-reactivity associated with specific branches, and we know that underlying this are actual mutations.
- But if we have back mutations or parallel mutations in different parts of the tree, these effects will not just sum, they may cancel each other out.

Identifying epitopes



Identifying epitopes

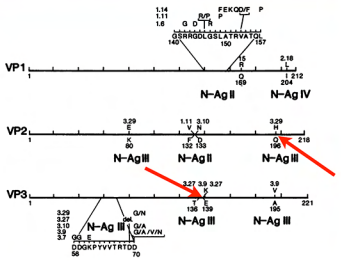
- Some specific mutation(s) must be causing the loss of cross-reactivity in any given branch.
- If the same epitope is being affected in two branches, this would cause an interaction between the cross-reactivity drops in both branches.
- So if we can identify sites that explain this interaction between the branches, we believe they must be associated with the epitopes.

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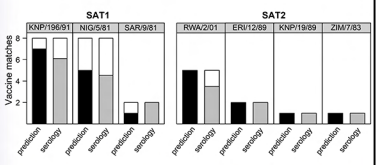
Identifying epitopes

- SAT1
- 3 epitopes
  - Conformational epitope (Site 3 on A10)
    - 5 loops
  - VP1 G-H loop
  - VP3 G-H loop (Site 3 on A12)
- 2 specific residues
  - Residue 138 on the VP3 E-F loop
    - 25% drop in r-value
  - Residue 198 on the VP2 H-I loop
    - 16% drop in r-value

Site 3 on A10

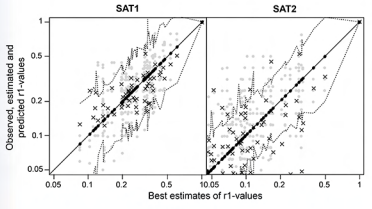


Vaccine matching



PLoS Computational Biology (under revision)

r-value prediction



PLoS Computational Biology (under revision)

Acknowledgements

- University of Glasgow
  - R. Reeve, L. Matthews, D.T. Haydon
- OVI
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  - E. Fry
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  - T.A.P. de Beer
- University of Pretoria
  - J. Theron
- Plum Island Animal Disease Center
  - E. Rieder
- Australian Animal Health Laboratory
  - W. Vosloo
- Northwestern University
  - H.G. O'Neill

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EVALUATION OF CROSS-PROTECTION BETWEEN O_1 MANISA AND O_1 CAMPOS IN CATTLE VACCINATED WITH O1 MANISA VACCINES

V.A.Srinivasan[1], **S.B.Nagendrakumar[1]**, M.Madhanmohan[1], S.Yuvaraj[1], S.Parida[2], Antenello di Nordo[2], D.J.Paton[2],*

1 Indian Immunologicals Limited, Rakshapuram, Gachibowli Post, Hyderabad 500 019, India

2 Pirbright Laboratory, Institute for Animal Health, Ash Road, Woking, Surrey GU24 0NF, UK

srini@indimmune.com

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Objectives

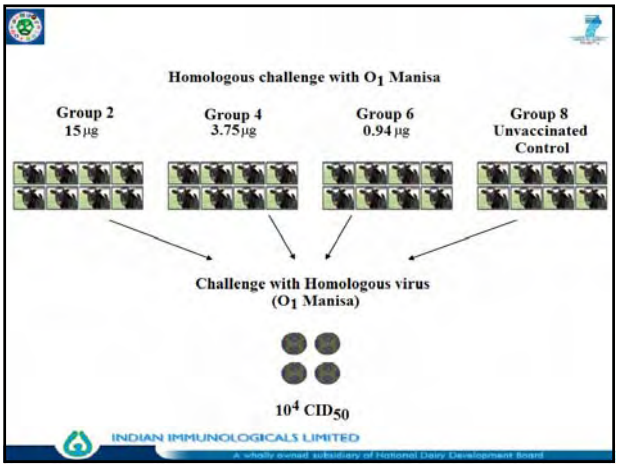
- Serology is used to predict vaccine induced protection against challenge with a heterologous strain of the same serotype of foot-and-mouth disease virus (FMDV)
- To evaluate the accuracy of such predictions the protection afforded to cattle vaccinated with different payloads of the O1 Manisa strain of FMDV against challenge with either a homologous (O1 Manisa) or a heterologous strain (O1 Campos) was compared (FP6 grant SSPE-CT-2003-503603).
- Serology by virus neutralization test (VNT) using O1 Manisa antiserum predicted an acceptable protection (r1 = 0.60) against such a challenge

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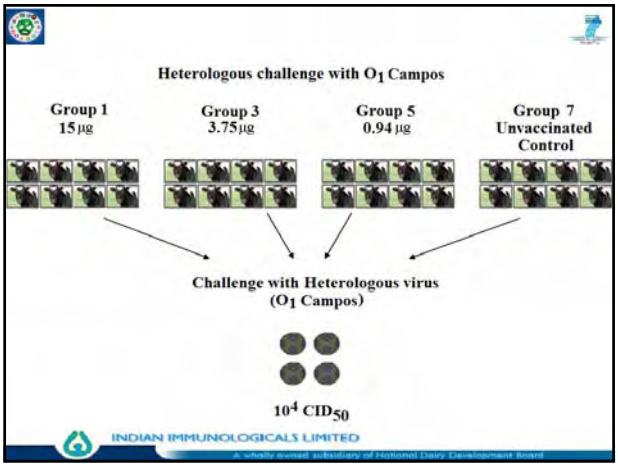
Experimental Design

- Oil adjuvant monovalent vaccines with O1 Manisa (2ml/dose) were prepared with the following payloads
  - 0.94 µg
  - 3.75 µg
  - 15 µg
  - 60 µg
- Preparation of cattle challenge viruses
  - O_1 Manisa
  - O_1 Campos
- Cattle challenge studies using modified potency test
  - Homologous potency test using blends 15, 3.75 and 0.94µg
  - Heterologous potency test using blends 60, 15, 3.75 µg

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Results of percentage protection against homologous challenge with O_1 Manisa on 21 dpv in cattle calves vaccinated with different payloads of O_1 Manisa vaccine

Groups	Payload (µg)	Experiment 1			Experiment 2		
		Number Challenged	Number Protected	Percentage Protection	Number Challenged	Number Protected	Percentage Protection
Group 2	15	8	8	100	5	5	100
Group 4	3.75	8	8	100	5	5	100
Group 6	0.94	8	7	88	5	1	20
Group 8	UV Control	8	0	0	2	0	0

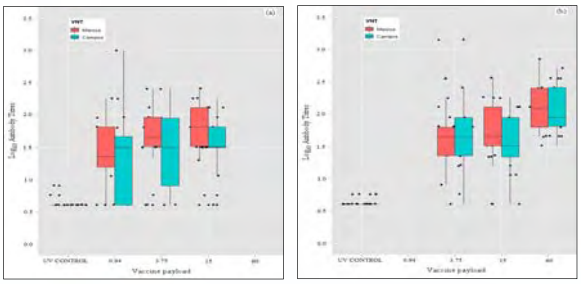
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Results of percentage protection against heterologous challenge with O_1 Campos on 21 dpv in cattle calves vaccinated with different payloads of O_1 Manisa vaccine

Groups	Payload (µg)	Experiment 1			Experiment 2		
		Number Challenged	Number Protected	Percentage Protection	Number Challenged	Number Protected	Percentage Protection
Group 1	60	8	6	75	5	5	100
Group 3	15	8	4	50	5	3	60
Group 5	3.75	7	2	29	5	0	0
Group 7	UV Control	8	0	0	2	0	0

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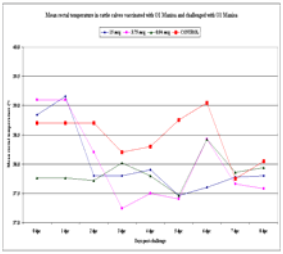
Serum antibody response towards O_1 Manisa vaccine in animals challenged with (a) O_1 Manisa and (b) O_1 Campos



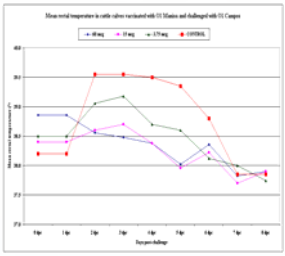
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Mean rectal temperature in vaccinated and control animals

Homologous challenge

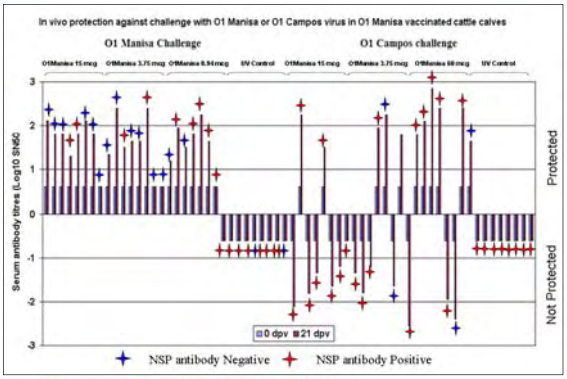


Homologous challenge



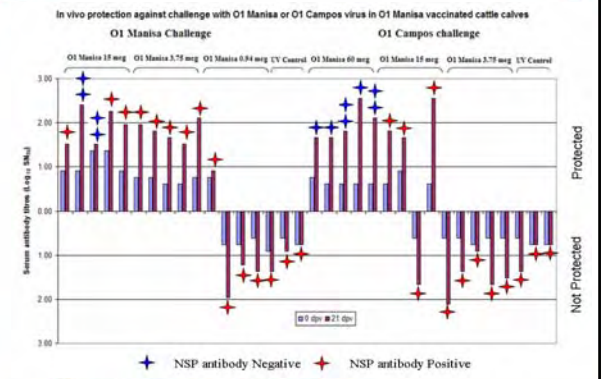
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Experiment 1



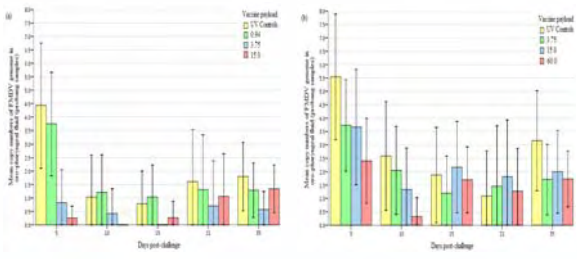
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Experiment 2



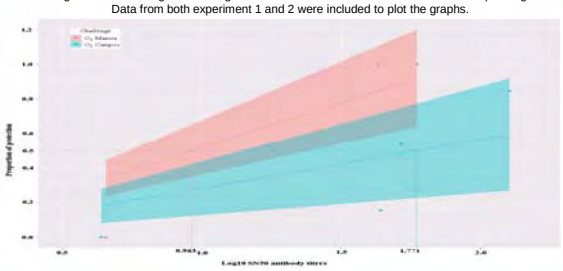
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Mean of copy numbers of FMDV detected at 5, 10, 15, 21 and 35 days post-challenge by quantitative RT-PCR with different Ag payloads used for homologous (a) and heterologous (b) challenge. Error bars represent 95% CI.



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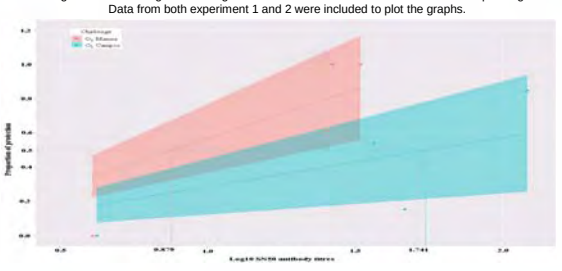
Scatter plot of O_1 Manisa serological responses at 21 days post-vaccination and proportion protected against homologous and heterologous challenge with fitted lines for the RTO models and corresponding 95% CIs. Data from both experiment 1 and 2 were included to plot the graphs.



PA50 for O_1 Manisa with O_1 Manisa VNT results: 0.963
PA50 for O_1 Campos with O_1 Manisa VNT results: 1.741

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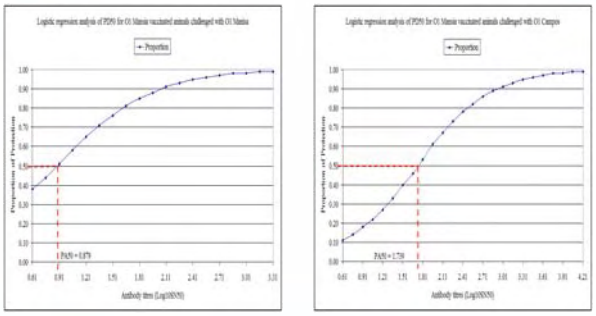
Scatter plot of O_1 Campos a serological responses at 21 days post-vaccination and proportion protected against homologous and heterologous challenge with fitted lines for the RTO models and corresponding 95% CIs. Data from both experiment 1 and 2 were included to plot the graphs.



PA50 for O_1 Manisa with O_1 Campos VNT results: 0.879
PA50 for O_1 Campos with O_1 Compos VNT results: 1.771

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Probit probability analysis of serum antibody titers for PA_{50} values

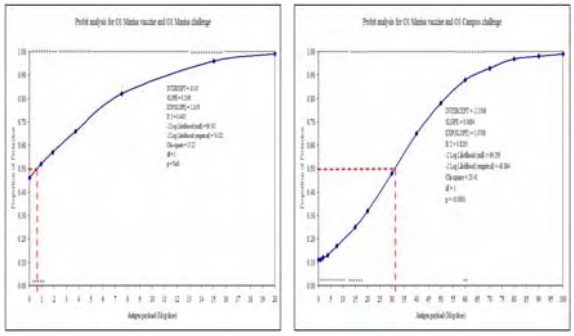


PA_{50} for O_1 Manisa: 0.879

PA_{50} for O_1 Campos: 1.739

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Probit probability analysis of antigen payload for PD_{50} values



PD_{50} for O_1 Manisa: 0.92 mcg/dose

PD_{50} for O_1 Campos: 32.2 mcg/dose

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B cell epitopes in 1D region

```
OMANISA69    TTSAGESADPVTATVENYGGETQVQRRQHTDVSFILDRFVKVTPKDQINVLDLMQTPAHT
O1CAMPOS55   TTSAGESADPVTTTVENYGGETQIQRRQHTDVSFIMDRFVKVTPQNQINILDLMQIPSHT

OMANISA69    LVGALLRTATYYFADLEVAVKHEGNLTWVPNGAPEAALDNTTNPTAYHKAPLTRLALPYT
O1CAMPOS55   LVGALLRASTYYFSDLEIAVKHEGDLTWVPNGAPEKGLDNTTNPTAYHKAPLTRLALPYT

OMANISA69    APHRVLATVYNGNCKYGDGTVANVRGDLQVLAQKAARALPTSFNYGAIKATRVTELLYRM
O1CAMPOS55   APHRVLATVYNGE-R- SRNAVP-VRGDLQVLAQKVVRTLPTSFNYGAIKATRVTELLYRM

OMANISA69      KRAETYCPRPLLAIHPDQARHKQKIVAPVKQLL
O1CAMPOS55     KRAETYCPRPLLAIHPTEARHKQKIVAPVKQTL
```

Examination of the B-cell epitopes in the 1D region show perceptible differences

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Sequence identities of the O_1 Manisa viruses used in vaccine production, cattle challenge test and neutralisation test

Capsid Protein	Amino acid Position	O1MANISA69 [AJ251477]	BHK7	BHKCZ	Cattle Passage 1	Cattle Passage 2	Cattle challenge virus	Affected cattle - 3930 Feet Epithelium
VP4	9	T	P	P	P	P	P	P
	40	D	D	D	E	E	E	E
	61	N	N	N	K	K	K	K
VP2	139	A	A	A	P	P	P	P
	163	S	S	S	P	P	P	S
VP1	57	P	R	R	P	P	P	P
	137	G	V	V	V	V	V	V

No changes were noticed in VP2 region

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Sequence identities of the O_1 Campos viruses used in vaccine production, cattle challenge test and neutralisation test

Capsid Protein	Amino acid Position	O1CAMPOS58 [AJ320488]	BHK5	BHK6	Cattle Passage 1	Cattle Passage 2	Cattle challenge virus	Affected cattle - 4167 Feet Epithelium
VP1	4	A	A	A	V	V	V	A
	13	T	T	A	A	A	A	A
	97	G	G	A	A	A	A	A
	156	V	V	A	A	A	A	A

No changes were noticed in VP4, VP2 and VP3 regions

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Comparison of r1 values for protection against heterologous challenge

r1 values	Protected	Not Protected	Total	% Protection
<0.19	2	2	4	50.00
0.20-0.39	2	1	3	66.67
0.40-0.70	5	4	9	55.56
0.71-1.00	4	3	7	57.14
>1	9	6	15	60.00
Mean Protection				57.87

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Comparison of serum antibody titres for protection against heterologous challenge

≤ Titres	Based on O1 Manisa titres			
	Protected	Not Protected	Total	% Protection
0.61	1.00	0.00	1.00	100.00
0.91	0.00	1.00	1.00	0.00
1.21	0.00	2.00	2.00	0.00
1.51	1.00	5.00	6.00	16.67
1.81	**7.00**	**6.00**	**13.00**	**53.85**
2.11	4.00	2.00	6.00	66.67
2.41	4.00	1.00	5.00	80.00
2.71	2.00	1.00	3.00	66.67
3.01	1.00	0.00	1.00	100.00
Mean protection				52.63

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Comparison of serum antibody titres for protection against heterologous challenge

≤ Titres	Based on O1 Campos titres			
	Protected	Not Protected	Total	% Protection
0.61	1.00	1.00	2.00	50.00
0.91	0.00	1.00	1.00	0.00
1.21	2.00	1.00	3.00	66.67
1.51	2.00	7.00	9.00	22.22
1.81	**4.00**	**5.00**	**9.00**	**44.44**
2.11	**2.00**	**2.00**	**4.00**	**50.00**
2.41	5.00	1.00	6.00	83.33
2.71	3.00	0.00	3.00	100.00
3.01	1.00	0.00	1.00	100.00
Mean protection				52.63

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Interpretations

- The two experiments produced almost similar results.
- Post-challenge generalization of the disease was noticed in all but one unvaccinated control animal.
- FMD-NSP antibodies could be detected from all the control animals until day 35 post challenge except in case of two animals in the homologous challenge group.
- Viral RNA/virus could be detected intermittently from the control animals up to day 35 post-challenge in most of the animals.
- Two control animals each in homologous and heterologous challenge did not show virus RNA/virus on day 35 post challenge.

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Interpretations

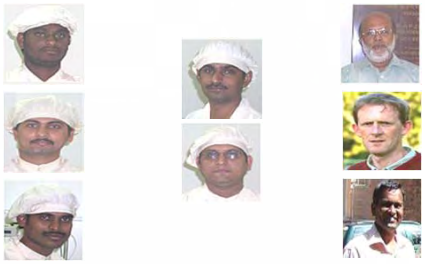
- Vaccine containing the highest payload of 60 µg could offer only partial protection (75%) in Expt 1 where as in Expt II it was 100%.
- Vaccines containing the payload of 15 µg could offer only 50-60% protection.
- Hence the results confirm that there is only partial cross protection for O1 Campos in animals vaccinated with O1 Manisa
- To achieve cross protection O1 Manisa pay load must be very high or possibly a repeat vaccination with the optimal payload may offer protection (to be confirmed by further studies)

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Interpretations

- NSP antibody response was noticed in most of the animals indicating virus replication in both the experiments
- Virus was not isolated from the probang samples in any of the vaccine groups.
- However, viral RNA could be detected from one animal in all the O1 Manisa challenged groups and one each in two O1 Campos challenged groups (60 and 3.75 µg) by qRT-PCR.
- The Log PA50 values for serum antibody tires for O1 Manisa was 0.879 while that for O1 Campos was 1.739
- The PD50 values for antigen payload for O1 Manisa was 0.92 mcg/dose while that for O1 Campos was 32.2 mcg/dose.

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Thank you

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Acknowledgements

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PREDICTING SITES OF ANTIGENIC IMPORTANCE OF SEROTYPE O FMD VIRUSES USING SEROLOGICAL AND CAPSID SEQUENCE DATA

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SUMMARY AND CONCLUSIONS

- Serological and sequence data generated for a range of type O viruses
- Linear mixed effects (LME) model applied to determine the best estimate r_1 values.
- Predictive LME model used to determine relationship between r_1 values and the changes within discrete surface exposed regions of type O capsid

Predictive LME model appears to more accurately predict antigenic relationships than serum neutralisation assay when compared to best estimate r_1 values for serotype O viruses

INTRODUCTION

- Currently available means for testing vaccine efficacy are time consuming, expensive and could lead to selection of sub optimal vaccines for outbreaks
- Previous studies using SAT viruses (Reeve *et al* PLoS Comp. Biol. under revision) have demonstrated the value of linear mixed effects modeling as a potential *in silico* method of determining antigenic relationships
- Work undertaken to determine if the same model can be applied to serotype O viruses

THE VIRUSES

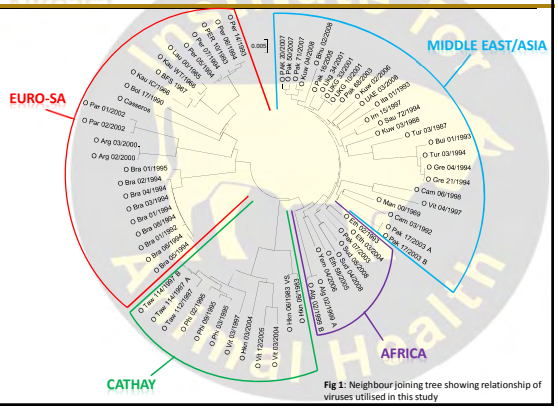


Fig 1: Neighbour joining tree showing relationship of viruses utilised in this study

MATERIALS & METHODS

- Pair wise virus neutralisation titres determined for the set of viruses listed above against 3 homologous systems, leading to the generation of a dataset containing 496 replicates
- P1 (Capsid) sequences generated for the same list of viruses
- Surface accessibility of each capsid residue determined by visual inspection of the molecular surface- surface exposed residues divided into 48 discrete regions varying in length from 1-40 residues

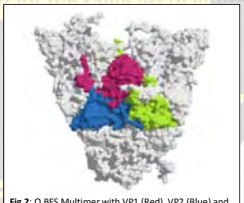


Fig 2: O BFS Multimer with VP1 (Red), VP2 (Blue) and VP3 (Green) highlighted on the central protomer

MATERIALS & METHODS (2)

- For each of the virus titres a linear mixed effects model was applied to generate a set of "best estimate" r_1 values.
- A predictive LME model was generated and each of the candidate surface exposed regions were tested within the model to correlate the significance of changes within these regions to the cross reactivity of viruses
- The model was then tested by using changes within these regions to try and predict a set of r values, these were then compared to both the individual serological r_1-values and the best estimate r_1 values

RESULTS

- After repeated runs of the linear model 4 regions were determined to be statistically significant at improving the model fit:
- The first region is located on VP1, residues 207-211
- Region 2 is located on VP1, residues 98-102
- Region 3 is located on VP2, residues 70-82
- Region 4 is located on VP3, residues 84-85***

RESULTS

- Two statistically significant regions are already known antigenic sites- remaining two are novel regions.

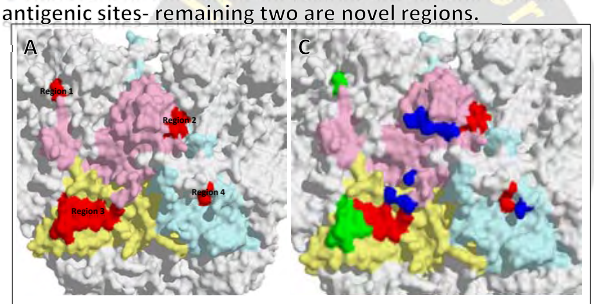


Fig 2: O BFS Multimers showing a comparison of surface location of regions statistically significant regions as determined by the linear mixed effects model (**A**- regions highlighted in red) with the known neutralising antigenic sites of serotype O (**B**- antigenic sites highlighted in blue). Image **C** where the two overlap this is coloured green. VP1 is coloured pink, VP2 is yellow and VP3 in cyan.

RESULTS

- 77.9% of r_1 values generated on day of test fall within 95% confidence limits of the best estimate r_1 values
- 88.5% of predicted r_1 values fall within 95% confidence limits of the best estimate r_1 values

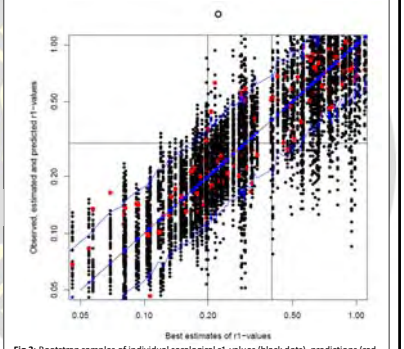


Fig 3: Bootstrap samples of individual serological r1-values (black dots), predictions (red dots) and matching best estimates and their confidence limits (Blue dots and blue line respectively) against best estimates for type O r1-values. Because of the log-normally distributed variance structure of the r1-values, data are plotted on a log scale. N=142.

CONCLUSIONS

It appears that potentially the linear mixed effects model can more accurately predict antigenic relationships than serum neutralisation assay when compared to best estimate r_1 values for serotype O viruses

However more work is needed to validate the results and refine the model

FUTURE PLANS

- The inclusion of at least one additional homologous serum for the generation of titres using the same list of viruses
- To progress on to more advanced analysis similar to that performed on the SAT viruses to identify the role individual residues in the antigenicity of serotype O
- Test the validity of the model using a reverse genetics system

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Intervet Schering-Plough
Nico Visser

Also thanks to all those in MCD and SAU, in particular Bob Statham, Anna Ludi, Jemma Wadsworth, Nick Knowles and Ginny Wilsdon.

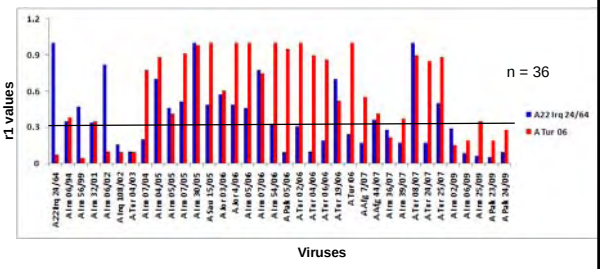
Funding
defra
BBSRC bioscience for the future
Intervet Schering-Plough Animal Health

Sequence based antigenic characterisation of serotype A FMD viruses from the Middle-East

Mana Mahapatra, S. Upadhyaya, G. Ayelet, D. King and D. Paton

Molecular Characterisation and Diagnostic Group
Institute for Animal Health
Pirbright

Antigenic relationship of type A isolates against the vaccine strains



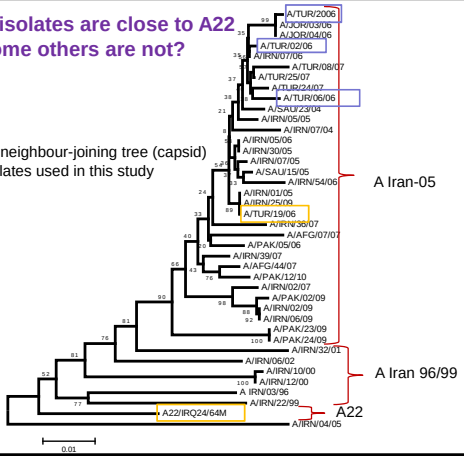
Values > 0.3 indicates a good match

Why??

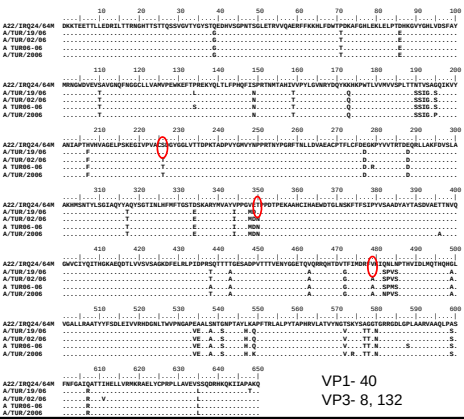
1. Why the isolates cross-reacted with A22 serum initially?
2. Why they stopped cross-reacting with A22?
3. Why some of the recent isolates are not cross-reacting with either of the vaccine strains?

Why some isolates are close to A22 whereas some others are not?

Fig. Amino acid neighbour-joining tree (capsid) of the type A isolates used in this study



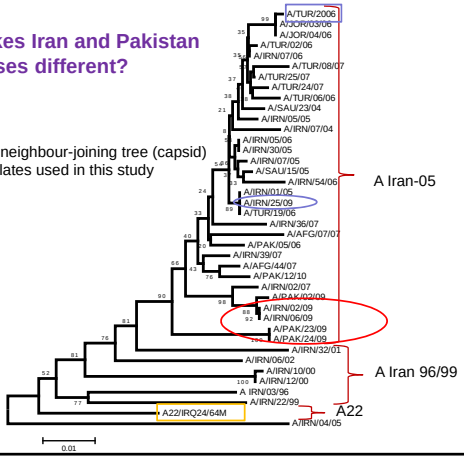
Why Tur 19/06 is close to A22 vaccine strain?



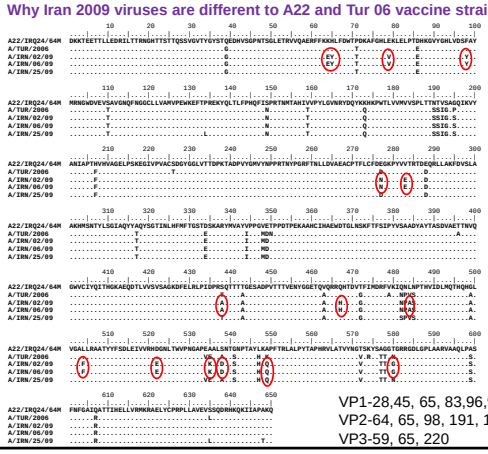
VP1- 40
VP3- 8, 132

What makes Iran and Pakistan 2009 viruses different?

Fig. Amino acid neighbour-joining tree (capsid) of the type A isolates used in this study



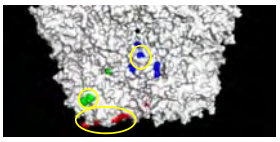
Why Iran 2009 viruses are different to A22 and Tur 06 vaccine strain?



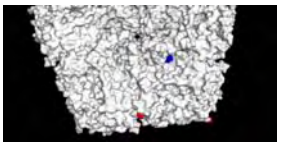
VP1-28,45, 65, 83,96,99,110,141
VP2-64, 65, 98, 191, 195
VP3-59, 65, 220

Why are Iran 2009 viruses different

(a) A22 pentamer outer surface



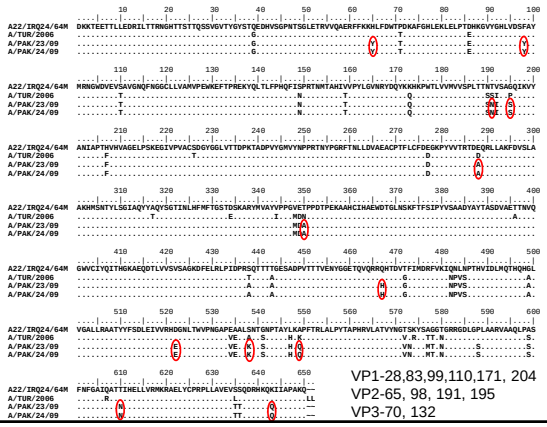
(b) A22 pentamer inner surface



VP1 28 and VP2 98 are not on the surface

VP1 –blue
VP2- red
VP3- green

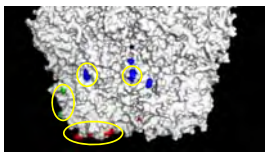
Why Pak 2009 viruses different to A22 and Tur 06 vaccine strain?



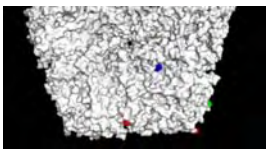
VP1-28,83,99,110,171, 204
VP2-65, 98, 191, 195
VP3-70, 132

Why are Pak 2009 viruses different?

(a) A22 pentamer outer surface



(b) A22 pentamer inner surface

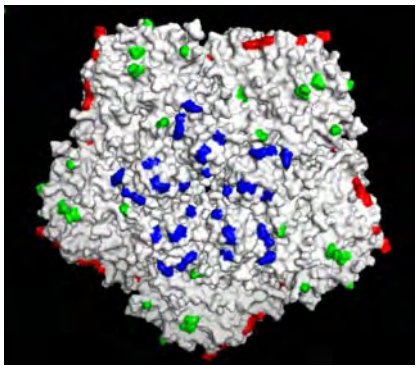


VP1 28 and VP2 98 are not on the surface

VP1 –blue
VP2- red
VP3- green

Why Iran/Pakistan 2009 viruses different?

VP1- 45, 65, 83,96,99,110, 141, 171, 204
VP2- 64, 65, 191, 195
VP3- 59, 65, 70, 132, 220



VP1 –blue
VP2- red
VP3- green

Conclusion

1. Tur 06 vaccine strain may provide protection
2. BAR 08 and AFG 07 sub-lineage may exhibit antigenic drift
3. Close monitoring of the out-break strains in this area is essential
4. Phylogenetic trees may not provide a true indication of the antigenic phenotype of the isolates.
5. Mutations in antigenic sites may be the cause of the change in antigenicity of the type A viruses
6. Some amino acid residues in the outer capsid may provide indication about the antigenic nature of the type A viruses

VP1- 40,45, 65, 83,96,99,110, 41, 171, 204
VP2- 64, 65, 134,191, 195
VP3- 8, 59, 65, 70, 132, 220

Future Plans

1. Include more recent isolates from ME countries
2. Analyse the data
3. Identified residues could be tested using a cDNA clone

ACKNOWLEDGEMENTS

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Cambridge Infectious Diseases Consortium

MAPPING THE ANTIGENIC VARIATION OF FMDV SEROTYPE A

A. Ludi*[1,2,3]

D. Horton[3,4], M. Mahapatra[1], D. King[1], N. Knowles[1], D. Paton[1], D. Smith[3], J. Wood ,[2] Y. Li[1], J. Hammond[1]

1. *Institute for Animal Health Pirbright Laboratory UK*
2. *Cambridge Infectious Disease Consortium, Univ. of Cambridge*
3. *Center of Pathogen Evolution, Univ. of Cambridge*
4. *Veterinary Laboratories Agency, Weybridge UK*

Summary

Antigenic cartography is being used to quantify and visualize antigenic relationships of FMDV Serotype A with the data then being compared to full capsid sequences.

Conclusion

Virus neutralization assays and capsid sequencing for 53 Serotype A viruses so far does not show a simple relationship between the antigenic and the genetic data. As well as increasing the data set further analytical work is currently being carried out.

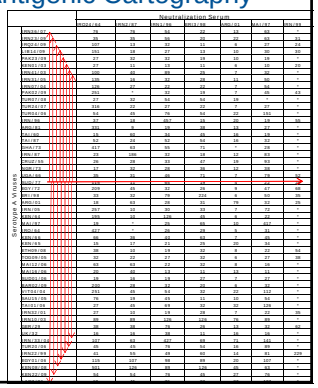
What is antigenic cartography?

Explanation of Antigenic Cartography

	Neutralization Value	r_1-values
Vaccine Virus: A IRQ/24/64	426	1
Field Virus: A SUD/77	115	0.27

Explanation of Antigenic Cartography

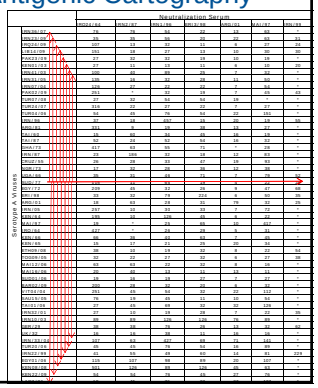
	Neutralization Values	r_1-values
A/IRQ/24/64	427	1
A/SUD/77	115	0.27



- Visualizing and quantifying antigenic relationships among viruses using serological data

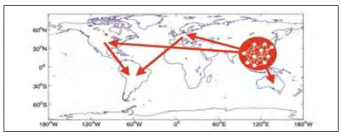
Explanation of Antigenic Cartography

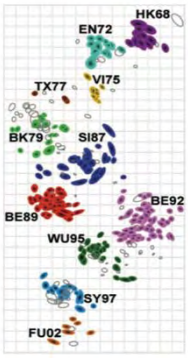
	Neutralization Values	r_1-values
A/IRQ/24/64	427	1
A/PAK/02/2009	115	0.27



- Visualizing and quantifying antigenic relationships among viruses using serological data
- Increased resolution as virus position not dependent on one serological test

Explanation of Antigenic Cartography





- Visualizing and quantifying antigenic relationships among viruses using serological data
- Increased resolution as virus position not dependent on one serological test

- A key component of vaccine selection of pandemic and seasonal flu*

*Smith, DJ, Mapping the Antigenic and Genetic Evolution of Influenza Virus. Science 2004, 305:371-376.

How are the maps constructed? A Vienna Example

Eugene Fritz McDonald





Prater Fair
1.86km

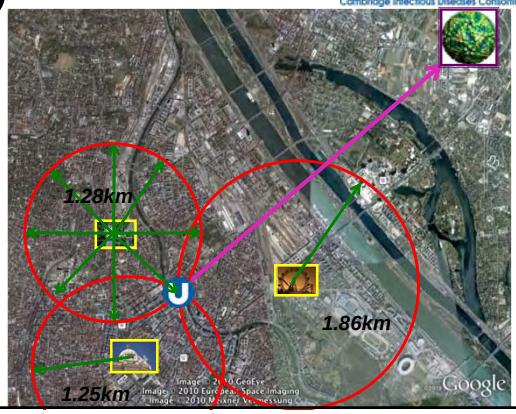


Hofburg Palais
1.25km



Palais Liechtenstein
1.28km

http://www.planetware.com/pictures-/vienna-a-w-vie.htm



Explanation of antigenic cartography

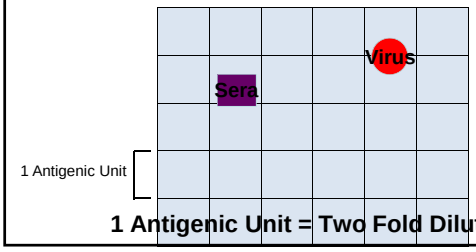
- Each neutralization value is converted to an antigenic unit

Equation

$$\frac{(\log_2 \text{ of highest neutralization titer})}{(\log_2 \text{ of field virus neutralization titer})}$$

Explanation of antigenic cartography

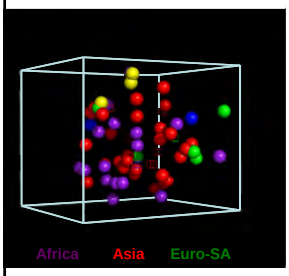
- A map is then constructed using these antigenic values as distances

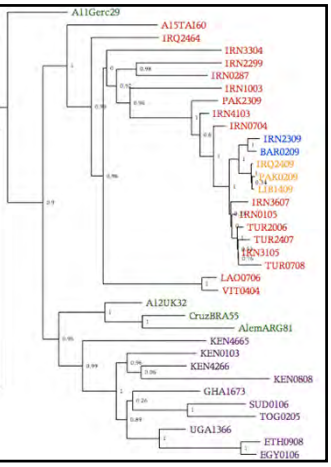


1 Antigenic Unit = Two Fold Dilution

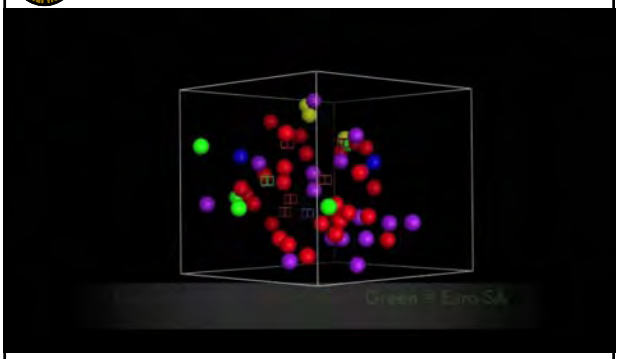
P1 Sequence

PhylML → TN93; aLRT

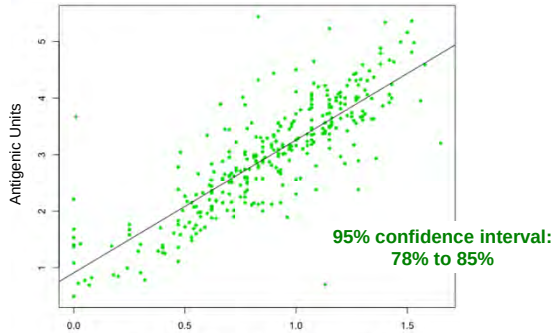




cidc
Cambridge Infectious Diseases Consortium



Comparison of neutralization values vs antigenic units



Diff. btw. highest neutralization value + the virus in question (log_{10})

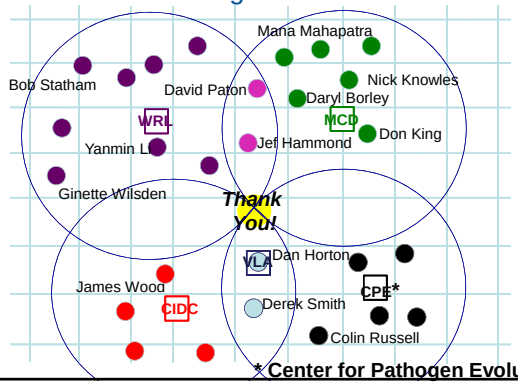
Conclusion

The antigenic data does not obviously reflect the genetic data. Current work focused on increasing the resolution and generating more data for comparison.

Future Work

- More in depth analysis of both sequence and antigenic data
- Addition of more serum to increase the resolution of antigenic maps
- Construct the antigenic map using LPBE
- How does pooled sera compare to individual serum?
- Does sera from different species produce the same antigenic map?

Acknowledgments



* Center for Pathogen Evolution

CLINICAL PROTECTION, SUB-CLINICAL INFECTION AND PERSISTENCE FOLLOWING VACCINATION WITH DIFFERENT PAYLOADS OF O_1 MANISA VACCINE AND CHALLENGE IN SHEEP AND GOATS

M.Madhanmohan,

S.B.Nagendrakumar, S.Yuvaraj, R.Kumar, J.Anil Kumar, K.Manikumar, V.A.Srinivasan

FMDV Laboratory, Research and Development Centre,
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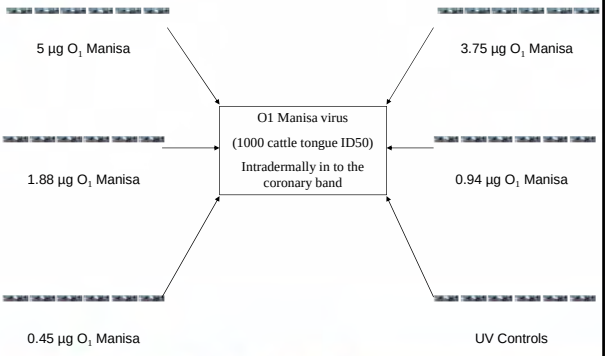
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Objectives

- Small ruminants play an important role in the epidemiology of Foot-and-Mouth Disease (FMD).
- Small ruminants are vaccinated with one-half or one-third of cattle dose of oil-based or aqueous vaccines respectively.
- The extinction antigen payload in vaccine for protection in small ruminants is poorly studied.
- The objective of this study was to determine the minimum antigen payload for serotype O for complete protection

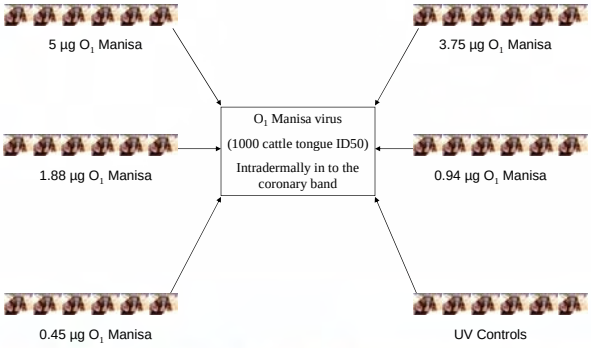
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Experimental Design – Sheep



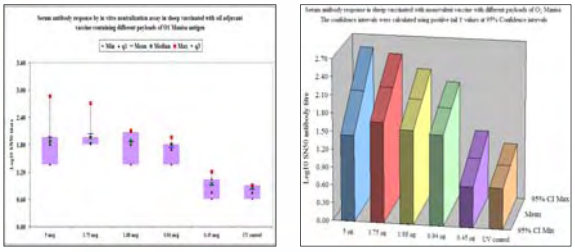
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Experimental Design – Goats



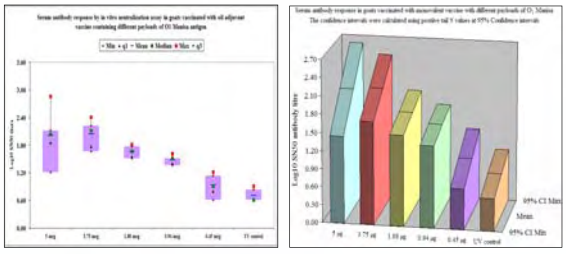
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Serology results - Sheep



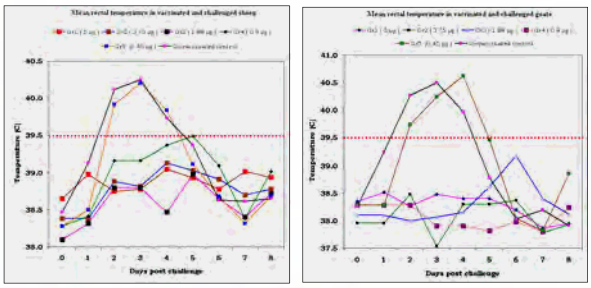
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Serology results - Goats



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Mean rectal temperature in vaccinated and challenged groups of sheep and goats compared with the unvaccinated controls



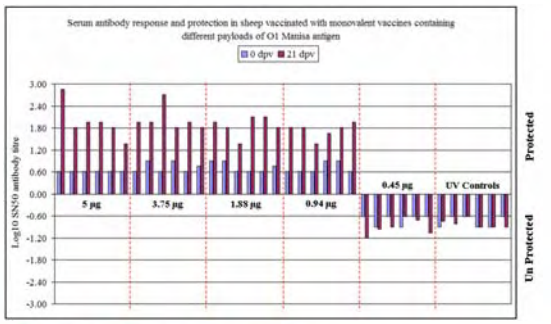
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Protection on challenge

Vaccine group	Sheep	Goat
5 µg payload	100%	100%
3.75 µg payload	100%	100%
1.88 µg payload	100%	100%
0.94 µg payload	100%	100%
0.45 µg payload	0 %	0 %
UV Control	0 %	0 %

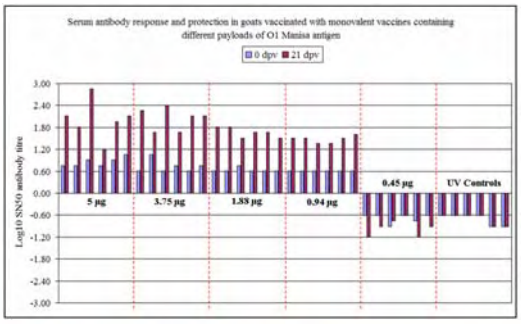
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Antibody response Vs Protection on challenge - Sheep



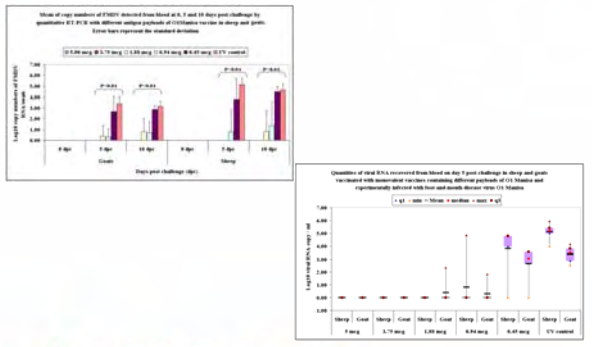
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Antibody response Vs Protection on challenge - Goat



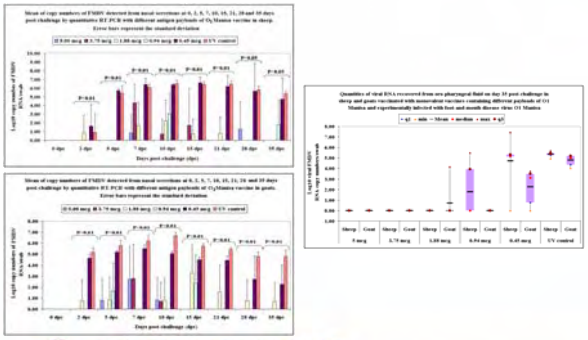
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Quantitation of FDMV RNA from blood



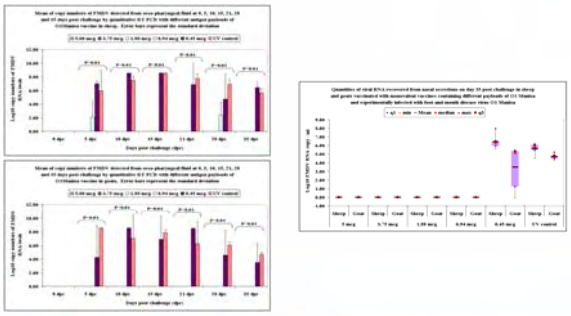
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Quantitation of FDMV RNA from nasal secretions



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Quantitation of FDMV RNA from probang samples



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Conclusions

- Optimal antibody titers were noticed in vaccine groups 5 and 3.75 µg
- Sub optimal titres were noticed in vaccine groups 1.88 and 0.94 µg groups
- Animals in vaccine group 0.45 µg showed poor response
- Sheep and goat vaccinated with 5-0.94 µg of O1 Manisa antigen were protected on challenge
- Animals vaccinated with 0.45 µg were not protected
- Unvaccinated animals showed generalized clinical signs of FMD

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Conclusions

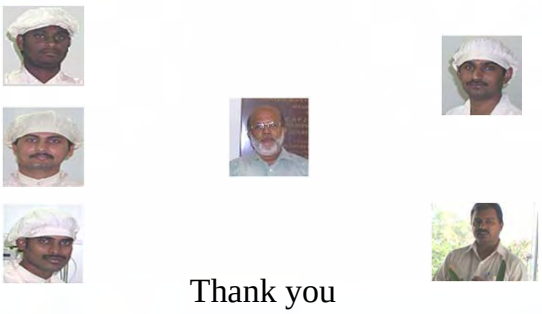
- Virus replication was evident in vaccine groups 1.88, 0.94 and 0.45 µg and in un vaccinated controls (P<0.01) in both sheep and goats
- Virus excretion from nasal secretions was higher in vaccine groups 1.88, 0.94 and 0.45 µg on all days until 10 dpc (P<0.01). Virus excretion from nasal secretions continued in vaccine group 0.45 µg and un vaccinated controls until 35 dpv (P<0.05)
- Sheep and goats in vaccine group 0.45 µg and un vaccinated controls harboured virus in the oro-pharyngeal region at least up to 35 dpv (P<0.01)

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Conclusions

- It was concluded that the current practice of administering half the dose of the oil adjuvant vaccine would be sufficient for clinical protection in sheep and goats.
- The final vaccine payload must be at 0.94 µg for complete protection.
- A final vaccine payload of 1.88 µg would offer sterile immunity.

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Thank you

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Animal Health Service

Progressive control pathway and serological surveys: methodological approach to sampling

G. Ferrari, M. Hussain, E. Khan, A.H. Nawroz, A. Aslami, S. Murvatulloev, A. Tatov, M. Eranov

AGAH/EMPRES
FAO

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Animal Production and Health Division

Animal Health Service

Summary

- The FAO regional project on Trans-boundary animal diseases is assisting Afghanistan, Pakistan, Tajikistan, Turkmenistan and Uzbekistan to progress along the FMD Progressive Control Pathway in the framework of the West Eurasia regional roadmap;
- One important component of the monitoring activities to shift from stage 0 to 1 is serology;
- The overall crude data of 2009 are presented along with some highlights on the results obtained;
- Serologic surveys aimed at estimating proportions at individual level (in addition to herd level) are complex (multi-stage cluster sampling design) and have important implications mainly due to the higher variance of the estimates if compared with a simple random sampling design;
- Wider confidence intervals determine a loss of statistical power;
- The approach originally adopted has been reviewed in an attempt to better address the above issues.

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Conclusions

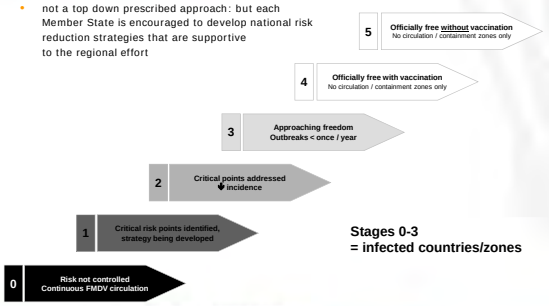
- Individual countries when embarking themselves into the progressive control pathway face the challenges of designing complex serological surveys;
- Those type of surveys have important methodological implications which must be understood;
- Training is needed to ensure that those methodological implications are duly taken into consideration;
- It is proposed the creation of a network of epidemiologists in the context of regional roadmaps to standardize procedures for sampling and analysis of data (to work in parallel and close collaboration with the laboratory diagnosticians).

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FAO Progressive Control Pathway – Risk reduction approach

- not a top down prescribed approach: but each Member State is encouraged to develop national risk reduction strategies that are supportive to the regional effort



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Progressive Control pathway

- Afghanistan, Pakistan, Tajikistan, Turkmenistan and Uzbekistan have been assisted (FAO project GTFS/INT/907/ITA) to shift from stage 0 to stage 1 of the PCP;
- Framework: West Eurasia regional roadmap for the progressive control of FMD



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Progressive Control pathway

- Serology is an important component of the overall monitoring/surveillance system;
- Through serology countries are supposed to generate more and more complex information as they move along the pathway:
  - Assess the level of virus circulation as may be determined through NSP antibodies detection both at herd and individual level;
  - Contrast different sub-groups to assess efficacy of health interventions (such as vaccination for instance) or detect different level of risk;
  - Assess vaccine coverage.

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West Eurasia Regional Roadmap



Status of the member countries as self-assessed in Shiraz 2008

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West Eurasia Regional Roadmap



Status of the member countries as determined during the Regional Meeting held in Istanbul, October 2009

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Progressive Control pathway (serological sampling approach)

- Cross-sectional approach;
- Two-stage cluster sampling design with the first stage being the epidemiological unit of concern (villages or individual farms);
- Within each epidemiological unit a fixed number of random individual samples (second stage) were collected (n=16) for each of three age classes (from 0 to 1 year old; from 1 to 2 years old and more than 2 years) making a total of 48 samples in each primary unit;

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Progressive Control pathway

- The main target was the "subsistence farming system" with a minimum of 60 villages to be sampled (Primary Units – PUs);
- Additional farming systems (i.e. commercial dairy, mixed, etc..) have been sampled with the purpose of contrasting the prevalence of NSP antibodies against the subsistence farming system utilized as reference group;
- In 2009 a total of 16,920 blood samples have been collected and tested.

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Progressive Control pathway

- Summary of the overall (crude) results at village level

Country	Age group 1		Age group 2		Age group 3	
	tested	p	tested	p	tested	p
Afghanistan	1015	54.3%	1027	50.9%	1117	58.9%
Pakistan	1279	15.3%	1280	24.2%	1280	42.1%
Tajikistan	948	43.9%	942	43.7%	998	46.9%
Turkmenistan	499	36.5%	659	40.7%	1078	55.8%
Uzbekistan	963	9.3%	957	6.8%	960	8.2%

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Progressive Control pathway

- Breakdown of the overall results (some highlights):
  - Afghanistan: a significant 2.25 higher risk of being NSP positive in animals less than one year old at village level vs individuals kept in commercial farms;
  - Pakistan: the risk of becoming NSP positive once entered into a peri-urban farming system has been estimated as high as 78.3% in two-months period;
  - Uzbekistan: no difference between villages within the buffer zone (vaccination zone) vs villages in the non-vaccination zone.

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Progressive Control pathway (main methodological implications)

- The simple random selection of the PUs may not be satisfactory in terms of geographic coverage;
- Data on the animal populations (at least at the lower administrative level) are unlikely to be available in developing countries and expedients to increase in advance the precision of the estimates at individual level (stratification, etc,..) may not be used. The minimum dataset needed to post-adjust the estimates must be collected at the time of blood sampling (for example the number of the individuals eligible for being sampled);

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Progressive Control pathway (main methodological implications)

- The standard error of the sample mean under a cluster design can be much higher if compared with a simple random sampling scheme (with the same number of individual samples tested). In the cross-sectional approach the statistical power (when comparing different sub-groups) is rapidly eroded;
- The relative efficiency of the cluster design vs simple random sampling is measured through the Design Effect (Deff).

$$s^2(p) = (1 - f_1)\frac{s_b^2}{h} + \frac{f_1}{h^2}\sum_{\alpha=1}^{h}\frac{(p_\alpha)(q_\alpha)}{(m_\alpha - 1)}(1 - f_{2\alpha})$$

between

within

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Progressive Control pathway (reviewed approach)

- The simple random selection of the PUs has been replaced with a systematic sampling scheme where PUs are sampled on average every k square Km;
- The number of PUs to be sampled can be further adjusted if the total number of PUs (villages) is available at lower administrative level increasing the intensity of sampling in more dense areas;
- Sampling at individual level will be restricted only to one age-class (n=16 from individuals from 6 to 18 months of age).

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Progressive Control pathway (reviewed approach)

- Sample size to compare sub-groups where different level of risk are assumed to be present will be estimated according to the procedure proposed by Hayes RJ and Bennet S (International Journal of Epidemiology 1999:**28;**319:326) for cluster randomized trials through which the number of clusters needed can be more easily estimated;
- The procedure (in addition to the usual assumption to be made regarding the magnitude of the outcome of interest and the size of the effect to be detected) requires only a guessed estimate of the coefficient of variation of the proportions between clusters.

$$c = 1 + (Z_{\alpha/2} + Z_{\beta})\frac{\left[\frac{p_1(1-p_1)}{n} + \frac{p_2(1-p_2)}{n} + k^2(p_1^2 + p_2^2)\right]}{(p_1 - p_2)^2}$$

coefficient of variation

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Progressive Control pathway (geographical coverage)

- As an example in a country like Tajikistan, having established a minimum sampling density of 1 PU every ~2,000 SqKm, the number of villages that will be sampled in the next round is 84 (which gives a satisfactory level of precision) with a total number of individual samples up to 1,344;
- The minimum sampling density is 1 PU every ~2,000 SqKm (in less densely populated areas) and 1 PU for every ~706 SqKm (in more densely populated area).

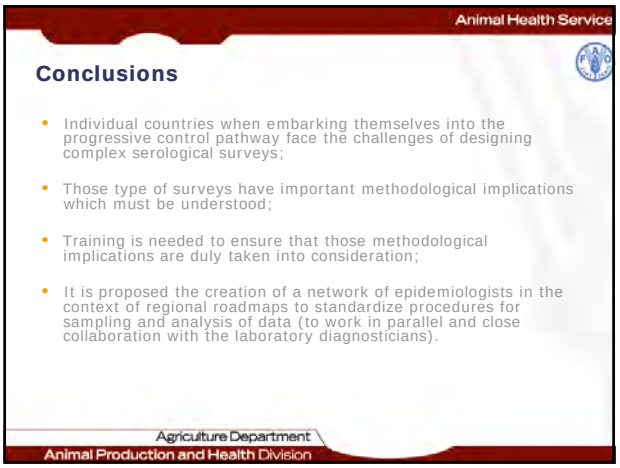
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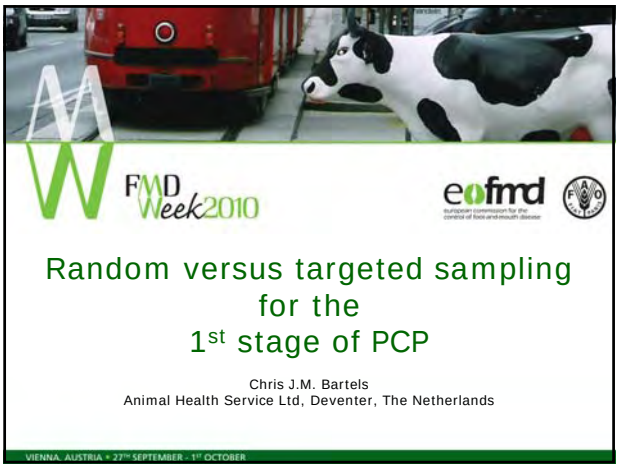
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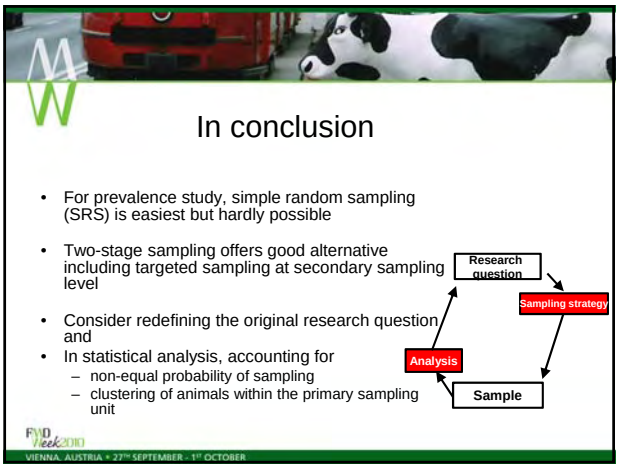
Progressive Control pathway (... to summarize ...)

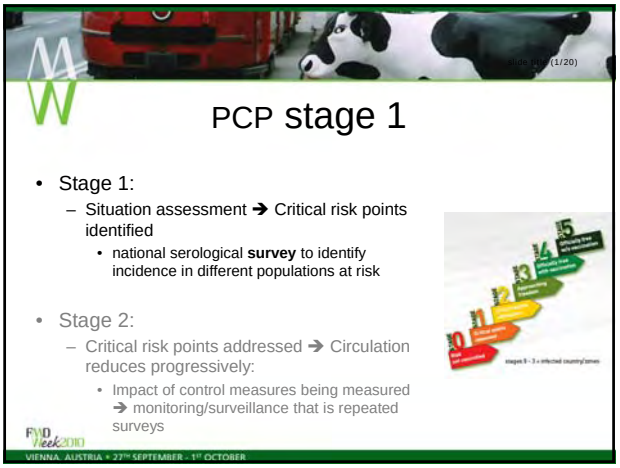
- A baseline country-wide survey (targeting only one age-group – from 6 to 18 months);
- Ad hoc surveys aimed at contrasting different farming systems;
- Ad hoc surveys aimed at contrasting different sub-populations (within the same farming system) which may differ because of being or not being target for specific control programs;

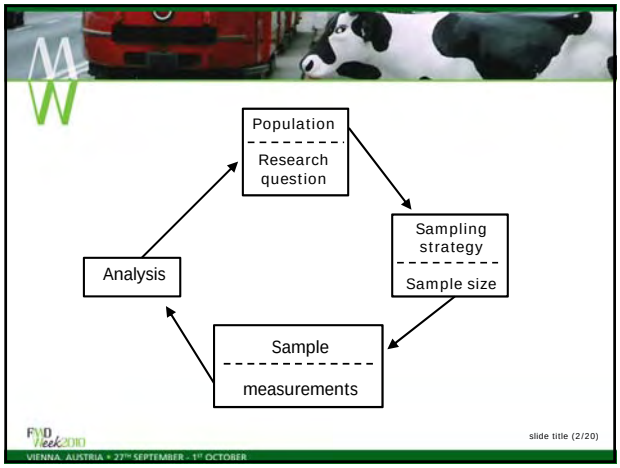
Agriculture Department
Animal Production and Health Division

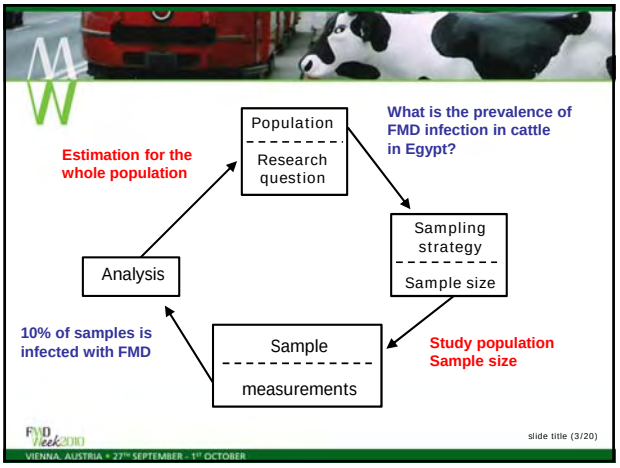

Animal Health Service
Conclusions
Individual countries when embarking themselves into the progressive control pathway face the challenges of designing complex serological surveys;
Those type of surveys have important methodological implications which must be understood;
Training is needed to ensure that those methodological implications are duly taken into consideration;
It is proposed the creation of a network of epidemiologists in the context of regional roadmaps to standardize procedures for sampling and analysis of data (to work in parallel and close collaboration with the laboratory diagnosticians).
Agriculture Department
Animal Production and Health Division

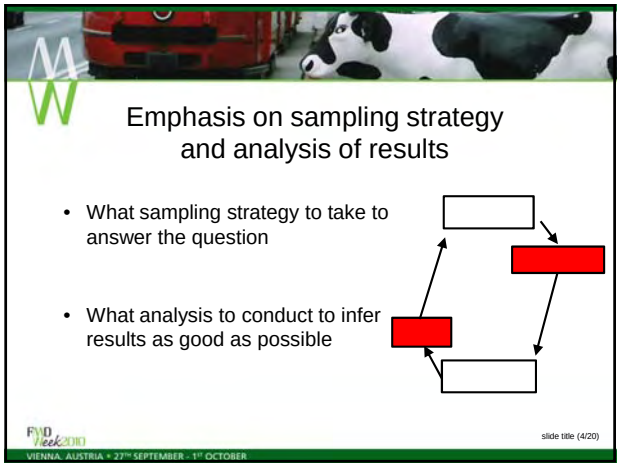

FMD Week2010
eufmd
Random versus targeted sampling for the 1st stage of PCP
Chris J.M. Bartels
Animal Health Service Ltd, Deventer, The Netherlands
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


In conclusion
• For prevalence study, simple random sampling (SRS) is easiest but hardly possible
• Two-stage sampling offers good alternative including targeted sampling at secondary sampling level
• Consider redefining the original research question and
• In statistical analysis, accounting for
– non-equal probability of sampling
– clustering of animals within the primary sampling unit
Research question
Sampling strategy
Analysis
Sample
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PCP stage 1
• Stage 1:
– Situation assessment ➔ Critical risk points identified
• national serological **survey** to identify incidence in different populations at risk
• Stage 2:
– Critical risk points addressed ➔ Circulation reduces progressively:
• Impact of control measures being measured ➔ monitoring/surveillance that is repeated surveys
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


Population
Research question
Sampling strategy
Sample size
Sample
measurements
Analysis
slide title (2/20)
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


Population
Research question
Estimation for the whole population
What is the prevalence of FMD infection in cattle in Egypt?
Sampling strategy
Sample size
Analysis
10% of samples is infected with FMD
Sample
measurements
Study population Sample size
slide title (3/20)
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


Emphasis on sampling strategy and analysis of results
• What sampling strategy to take to answer the question
• What analysis to conduct to infer results as good as possible
slide title (4/20)
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Types of sampling

Non-probability
- No formal process
  - Judgement
  - Purposive
  - Convenience
- Pilot or analytical study

Probability
- Every element in population has a **non-zero** probability of being included
- Need for **formal process** of random selection
  - Sampling frame: list of all elements

slide title (5/20)

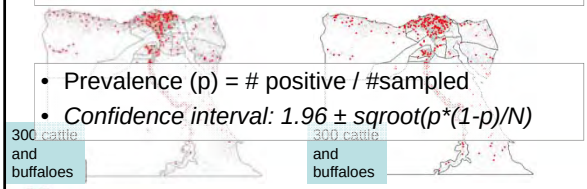
Probability sampling

- **Simple random sampling**
  - **Complete list of subjects required**
- Systematic random sampling
- Stratified random sampling
- Cluster sampling
- **Multistage sampling**
  - **Primary and secondary sampling unit**
- **Targeted (risk based) sampling**
  - **Focus on high-risk groups**

slide title (6/20)

Simple random sampling

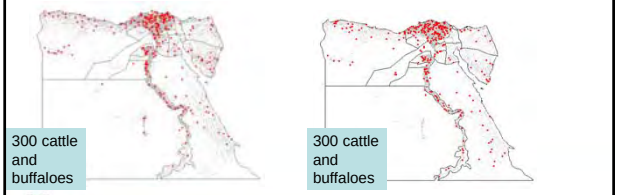
- Each animal has equal probability to be sampled using a formal process of random selection



- Prevalence (p) = # positive / #sampled
- *Confidence interval: 1.96 ± sqroot(p*(1-p)/N)*

slide title (7/20)

Simple random sampling



slide title (7/20)

Non-equal probability

- If equal probability sampling is not possible: because no list of sampling units (I&R) available
- Then two-stage sampling:
  - Primary level: herds/villages/districts
  - Secondary level: cattle



slide title (8/20)

Concept

- Cattle in Egypt are often herded on communal grounds. In addition, biosecurity measures at owner's place are minimal. Thus transmission of infection between premisis happens easily.
- Statistically this translates into dependency of observations results on individual cattle)
  ➔ clustering of infection



slide title (9/20)

Two stage
Primary sampling unit: villages

- Random selection of villages
  - Number of villages required calculated based on
    - Expected prevalence (75%)
    - Margin of acceptable error (5%)
    - Confidence level (95%)
  - ➔ **need to sample 289 villages**
- List of villages
- Random number selector
  - Excel or statistical program

Sample size within villages

- Based on a **qualitative assessment** of situation within village: presence or absence of FMD infection
  - Design prevalence: 20%
    - assuming when FMD has gone through village, a minimum of 20% of animals have become infected
  - Confidence level: 95%
  - Population size in village: 200

➔ need to sample 14 animals per village



Design prevalence (X%)

- If all samples test seronegative, one can say with 95% confidence that prevalence is not equal or above X%
- When assuming a higher design prevalence, less samples are needed
  - 10% prevalence: 30 animals
  - 20% prevalence: 14 animals
  - 40% prevalence: 6 animals
- **If one or more samples test seropositive, the proportion seropositives = x/n**
- **However, lower sample size means less precise estimates**

Targeted sampling

- Stratification of source population based on one (or more) characteristics which are thought to be associated with the probability of disease/infection occurrence
- Visual observation of animals with clinical signs
- Mostly used to improve the chance to detect disease/infection when present

➔ **reducing the number of samples required**

Redefinition of primary research question

- What is the percentage of villages with FMD-infected cattle?
- Knowing this, we will get a clear picture of regional differences
- Secondary research questions:
  estimation of within-village animal prevalence and overall animal prevalence

Consequences for analysis

Correction for non-equal probability of being sampled

- Sampling weights: Not all animals have same probability of being selected from the study population

  ➔ point estimate and SE may differ from SRS

- Sampling weight is inverse of probability of selectioni reflecting the number of cows that each of sampled individual is representing

- Need to know N (total # village in population) and M (total # animals within each village)

Correction for clustering

- Clustering: cattle within villages are more alike than cattle between villages

- Standard errors are underestimated
  - Use 'robust' in STATA
  - Use of mixed models (multilevel logistic regression analysis)

Correction for targeted sample

- Risk ratio of characteristic
- Fraction of population with characteristic

- Uncertainties around these estimations are accounted for (Monte Carlo simulations, bootstrap sampling).

- Alternatively, no correction but redefinition of primary research question and thus inference for population

Example: FMD nsp-antibodies

- **2938 tested, 920 positive**

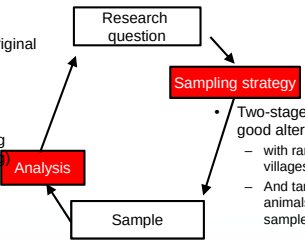
	Prop	Sd	Effect
Simple Rrandom Sample	0.3131	0.00855	
Sampling weight	0.3088	0.00855	Lower prevalence
Clustering	0.3131	0.01745	Larger Sd
Regression	0.3089	0.01670	Lower prevalence + larger Sd

In PCP stage 1, insight into the FMD virus circulation: prevalence study.

Random sampling is the easiest strategy.

However, this is often impossible due to lack of information (I&R)

- This may affect the original research question,
- this needs to be accounted in the analysis (sampling weights and clustering)
- when inferring for the target population



- Two-stage sampling is a good alternative
  - with random selection of villages/holdings/herds
  - And targeted sampling of animals, reducing the sample size

With acknowledgements to
Giancarlo Ferrari, Carsten Potzsch, Melissa McLaws, Wim Swart, Kees van Maanen





INCORPORATING DESCRIPTIVE EPIDEMIOLOGY AND MARKETING NETWORK ANALYSIS INTO THE PCP

Melissa McLaws & Nick Taylor

VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER

Summary

- Risk analysis in market networks:
  - generates list of risk hotspots and proposals for risk reduction measures
  - Knowledge gain through consultation and engagement of stakeholders
  - Data collection iterative and progressive
  - Part of suite of PCP Activities: complementary and synergistic

Background: PCP & Risk

- Progressive Control Pathway Principle:
  - Optimisation of scarce resources: Target control measures where have most impact
  - "Risk based"
- How to determine where risk is?
  - Time
  - Space
  - Production sector

Value Chain Analysis

- Is being combined with risk analysis concepts and applied in control of HPAI, also FMD (*work by FAO, RVC, VEERU,ILRI*)

Marketing network
+
Consideration of **why** the network is as it is:
Economics, Incentives, Governance

Value chains = contact networks
with *opportunities for transmission* of disease

PCP Stage 1:

Marketing network
Transmission opportunities
+
National Husbandry characteristics
Seasonality; Geographical distribution/movement patterns
+
Descriptive epidemiology
Susceptible populations; Circulating strains

Identify "Risk Hotspots": Points in the value chain where the *combined* effect of the probability and the consequences of FMD entry/spread are greatest.

PCP Stage 1:

Marketing network
Transmission opportunities

Descriptive epidemiology
Susceptible populations; Circulating strains

National Husbandry characteristics
Seasonality; Geographical distribution/movement patterns

Identify "Risk Hotspots": Points in the value chain where the *combined* effect of the probability and the consequences of FMD entry/spread are greatest.

Critical Control Points

- At each risk hotspot: conduct risk assessment to identify **critical control points** (risk pathway analysis is useful)
- 3 criteria for CCP:
  1. FMD poses a significant risk *and*
  2. Control measures exist and their implementation is feasible *and*
  3. The control measures should reduce the risk to an acceptable level.

PVP activities are *connected* and *synergistic*

Critical control point criteria	PCP core activity
FMD poses a significant risk	•Value chain analysis •Surveillance/Monitoring
Control measures exist and their implementation is feasible	•Value chain analysis •Laboratory capacity
The control measures should reduce the risk to an acceptable level.	•Laboratory capacity •Surveillance/monitoring

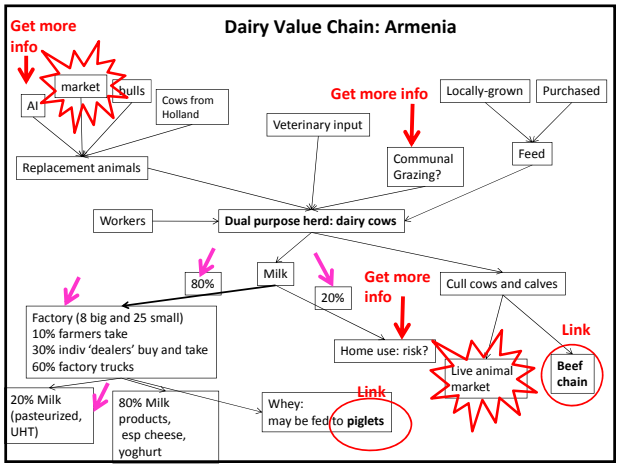
NT1

PCP in practice: Armenia

- 1.5 day workshop, July 2010
- Stakeholders from cattle, small ruminants, swine sectors
- Market network analysis, seasonal calendars, mapping...



Dairy Value Chain: Armenia



- Outcome: List 8 candidate risk hotspots:
  1. Mixing at seasonal pastures and subsequent return to villages
  2. Pigs consuming contaminated feed
  3. Free-ranging domestic swine can contact other susceptible species and FMDV-contaminated products, and return to village.....
- Useful discussion tool; Not an end point:
  - Highlight interconnectedness of stakeholders
  - Identify knowledge gaps
  - Start to consider feasibility of alternate control measures
- Next steps:
  - Data gaps
  - Risk analysis at each hotspot to focus/elaborate

Conclusions

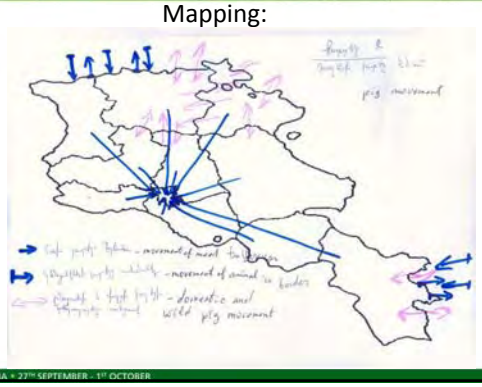
- PCP activities :
  - Risk analysis in market chains generates list of risk hotspots and proposals for risk reduction strategy
  - Parts of a whole: Mutually informative, Synergistic
- Non-prescriptive approach:
  - Knowledge gain through consultation and engagement of stakeholders
- Data collection iterative and progressive
  - Highlights areas where we don't know things, data are needed most; cost effective data collection

Acknowledgements

- RVC: Jonathan Rushton
- FAO: Nicoline DeHaan, Julio Pinto, Anni McLeod

Thank you for your attention

Mapping:



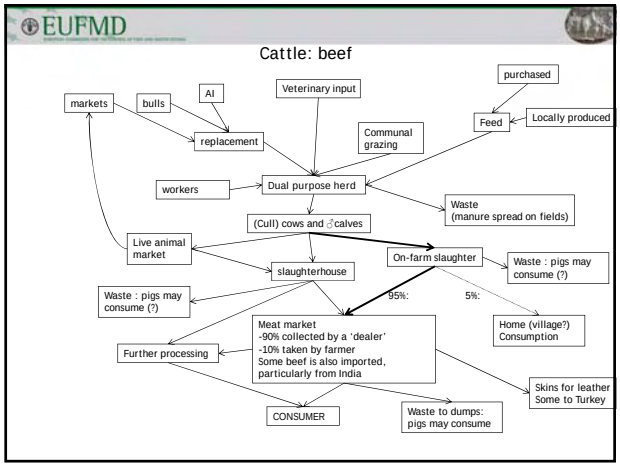
- PCP Stage 1:
  - descriptive epidemiology and market network analysis
  - monitoring, information systems
  - enhance laboratory capacity,

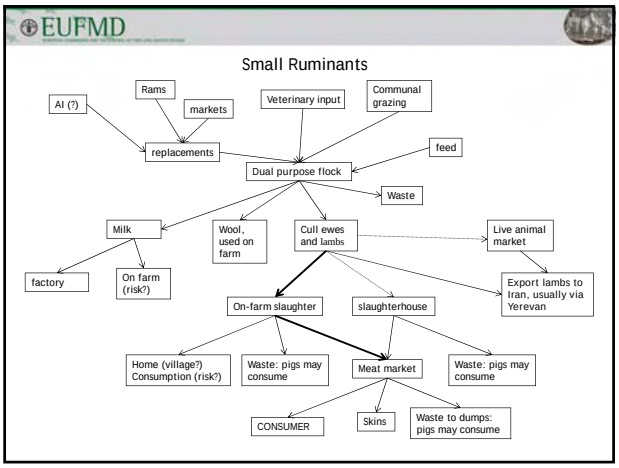
FMD control strategy based on *risk*

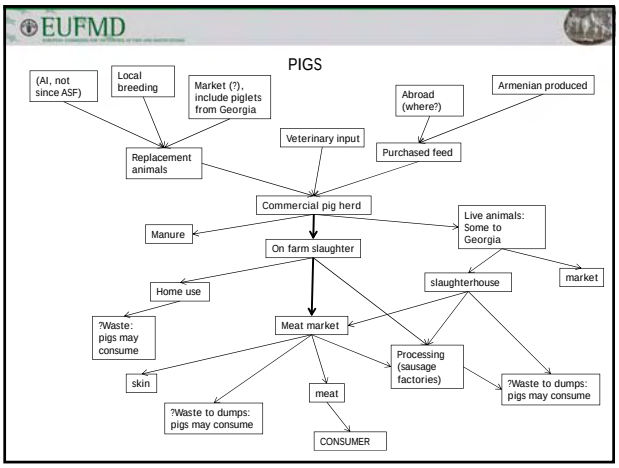
(developed in stage 1... implemented in stage 2)

What did we learn in Armenia?

- Engagement participation
- Illustration of complexity…chains, different stakeholders
- Iterative…need national committment
- Highlights areas where we don't know things (pigs)
- List of risk hotspots/tables/next steps




EUFMD
Small Ruminants
AI (?)
Rams
markets
Veterinary input
Communal grazing
replacements
feed
Dual purpose flock
Waste
Milk
Wool, used on farm
Cull ewes and lambs
Live animal market
factory
On farm (risk?)
Export lambs to Iran, usually via Yerevan
On-farm slaughter
slaughterhouse
Home (village?) Consumption (risk?)
Waste: pigs may consume
Meat market
Waste: pigs may consume
CONSUMER
Skins
Waste to dumps: pigs may consume


EUFMD
PIGS
(AI, not since ASF)
Local breeding
Market (?), include piglets from Georgia
Abroad (where?)
Armenian produced
Veterinary input
Replacement animals
Purchased feed
Commercial pig herd
Manure
Live animals: Some to Georgia
On farm slaughter
Home use
slaughterhouse
market
?Waste: pigs may consume
Meat market
Processing (sausage factories)
skin
meat
?Waste to dumps: pigs may consume
?Waste to dumps: pigs may consume
CONSUMER

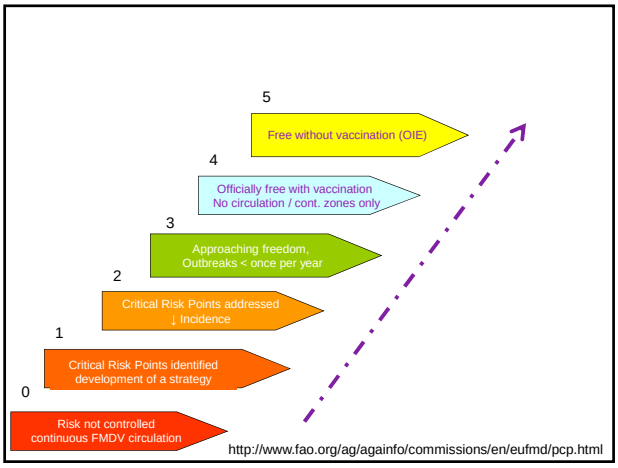
RVC Royal Veterinary College University of London

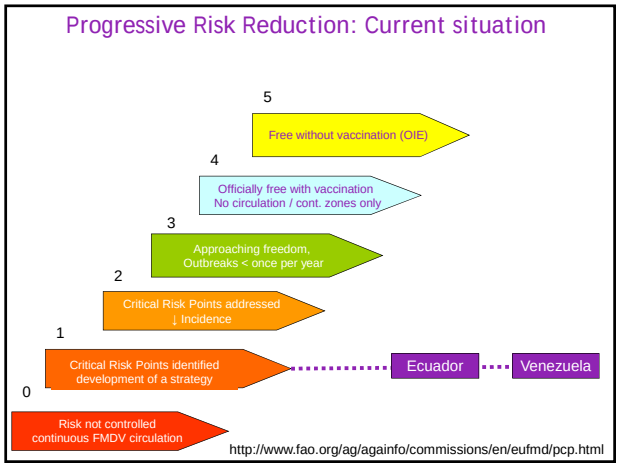
Evaluation of the impact of FMD for smallholders of the Andean region

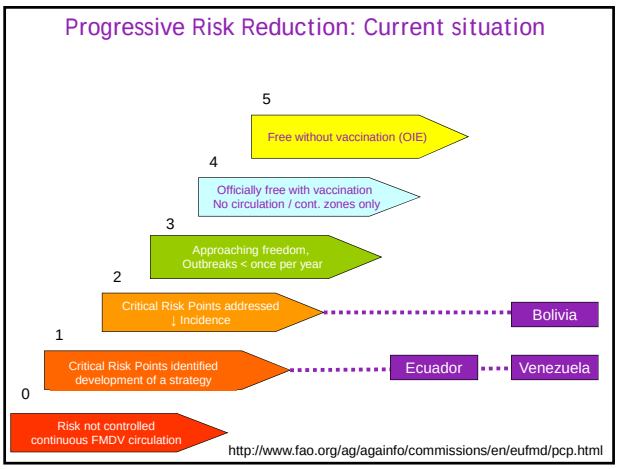
Javier Guitián, Ana Riviere, Alejandro Rivera, Jonathan Rushton, Georgina Limon

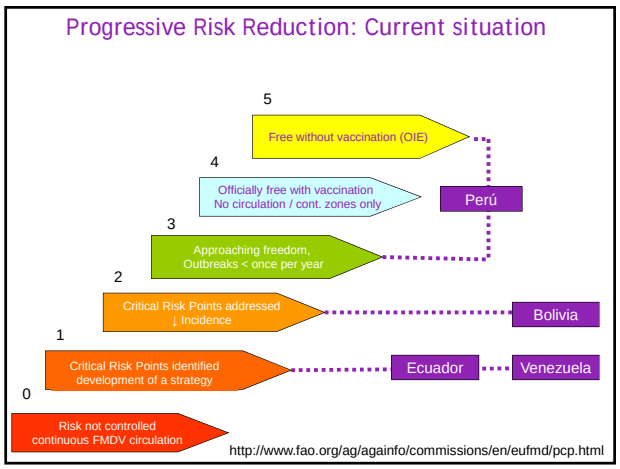
CONTROL PROGRESIVO DE LA FIEBRE AFTOSA

GCP/RLA/178/SPA GTFS/RLA/172/ITA

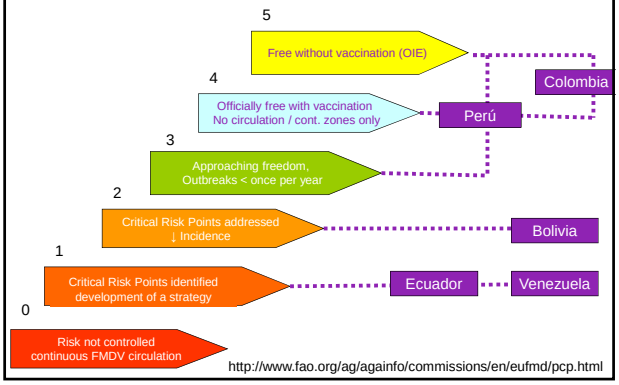








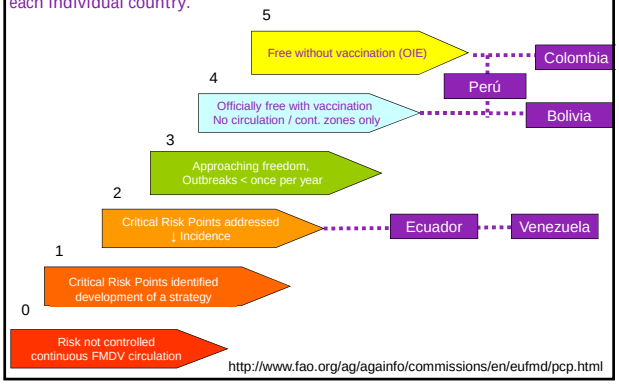
Progressive Risk Reduction: Current situation



http://www.fao.org/ag/againfo/commissions/en/eufmd/pcp.html

Progressive Risk Reduction: Current situation

Risk reduction strategies will be implemented through national programs by each individual country.



http://www.fao.org/ag/againfo/commissions/en/eufmd/pcp.html

Background

risk reduction strategies

vaccination, risk-based surveillance, movement control

RVC

Background

risk reduction strategies

vaccination, risk-based surveillance, movement control



smallholders

Andean Region: Around 100 million animals of the susceptible species, mostly keep by smallholders

RVC

Background

risk reduction strategies

vaccination, risk-based surveillance, movement control



smallholders

Andean Region: Around 100 million animals of the susceptible species, mostly keep by smallholders

RVC

Background

"If FMD control is to be achieved there must be an obvious benefit to the smallholders in order to gain their cooperation and positive involvement..."

Gleeson & Ozawa (1999)

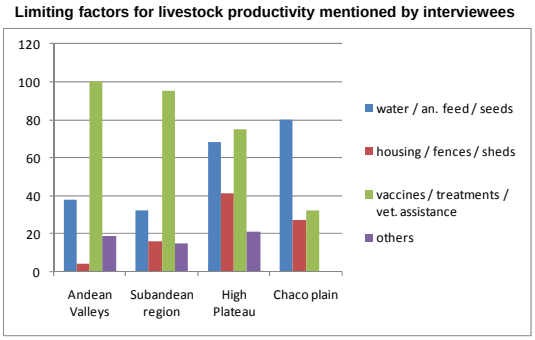
RVC

Background

Areas	FMD	
	Ha oído hablar de la enfermedad	
	Has previously heard of FMD	Believe their animals currently have FMD
Chaco plain	98%	2%
High Plateau	94%	–
Andean valleys	100%	–
Sub-Andean	96%	–

Background

"Smallholders" are a diverse group



Background

passive

% of households that, in the event of a new severe disease affecting the household herd/flock would report to:



Background

passive

% of households that, in the event of a new severe disease affecting the household herd/flock would report to:



active

Overall, 60% of households reported not having ever been visited (large variations between areas)

The question

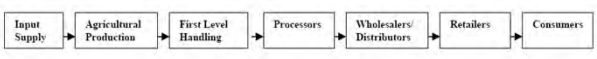
- What is the impact of risk reduction strategies for smallholders?
- What are the incentives of livestock keepers and their likely behaviour when specific risk-reduction strategies are implemented?

Challenging because of:

- "smallholders" are a diverse group
- Livestock make very diverse contributions to livelihoods
- Will require combination of quantitative and qualitative data

The strategy (1)

- Value chain mapping: qualitative description of actors at all stages of production, distribution, processing... with a focus on relationships and dynamics between actors.



RVC

The strategy (1)

- Value chain mapping: qualitative description of actors at all stages of production, distribution, processing... with a focus on relationships and dynamics between actors.
  - Identification of actors who would be directly or indirectly affected by risk reduction strategies
  - Evaluation of how the value chain is likely to react to changes introduced as a result of the strategies

  "who may be affected and how they may behave in response"

RVC

The strategy (2)

- Facilitate / promote the consideration of the value chain and likely reactions from actors when risk reduction strategies are decided

RVC

The strategy (2)

- Facilitate / promote the consideration of the value chain and likely reactions from actors when risk reduction strategies are decided

  e.g. risk based surveillance → identification of high risk subpopulations,
  - high risk subpopulations may change in reaction to implementation of strategies, value chain analysis could permit consideration of likely changes
  - value chain analysis could highlight subgroups that would have more / less incentive to report occurrence of disease

RVC

The strategy (3)

- Quantitative assessment of impact at household level using data from household surveys
- Qualitative assessment of impact from field interviews
- Combination of both

RVC

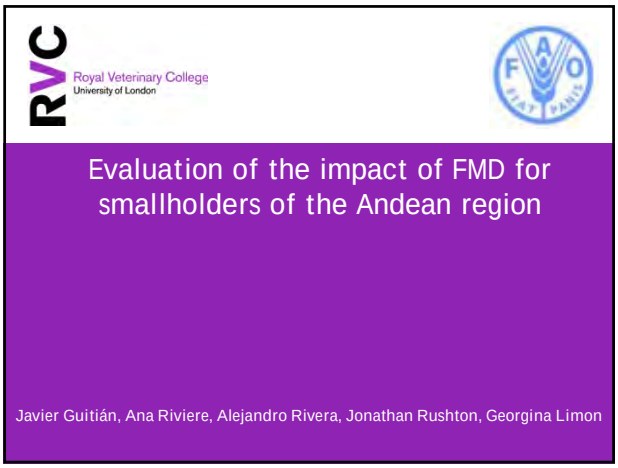
- Impact of strategies on smallholders and incentives should be considered across the progressive control pathway
- Challenging
- Value chain mapping + quantitative / qualitative study of impact at household and community level

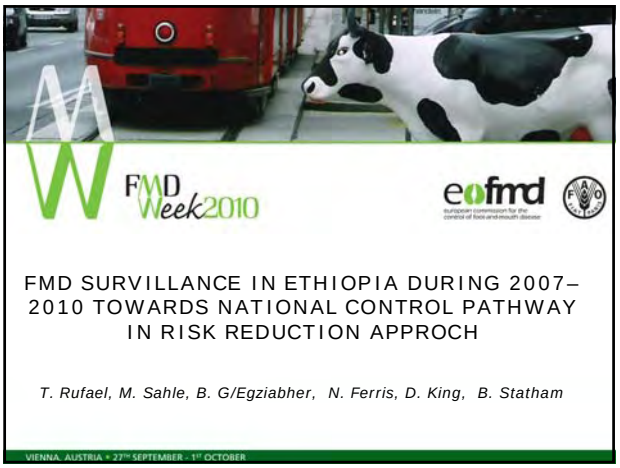
RVC

Acknowledgements

- RVC: Jonathan Rushton, Georgina Limon
- FAO: Ana Riviere, Alejandro Rivera

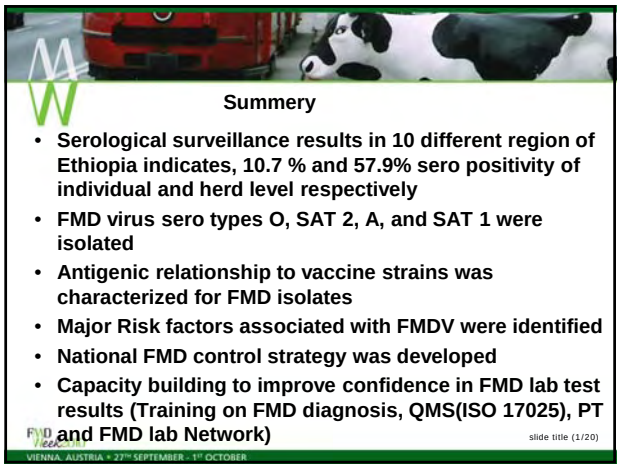
RVC


RVC
Royal Veterinary College
University of London
Evaluation of the impact of FMD for smallholders of the Andean region
Javier Guitián, Ana Riviere, Alejandro Rivera, Jonathan Rushton, Georgina Limon



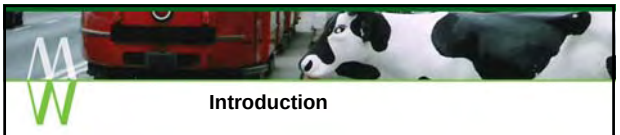
FMD SURVILLANCE IN ETHIOPIA DURING 2007–2010 TOWARDS NATIONAL CONTROL PATHWAY IN RISK REDUCTION APPROCH

T. Rufael, M. Sahle, B. G/Egziabher, N. Ferris, D. King, B. Statham



Summery

- **Serological surveillance results in 10 different region of Ethiopia indicates, 10.7 % and 57.9% sero positivity of individual and herd level respectively**
- **FMD virus sero types O, SAT 2, A, and SAT 1 were isolated**
- **Antigenic relationship to vaccine strains was characterized for FMD isolates**
- **Major Risk factors associated with FMDV were identified**
- **National FMD control strategy was developed**
- **Capacity building to improve confidence in FMD lab test results (Training on FMD diagnosis, QMS(ISO 17025), PT and FMD lab Network)**

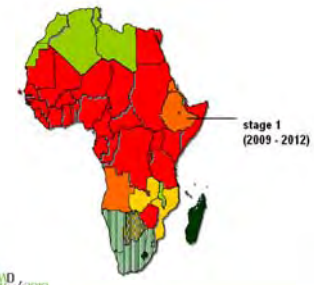


Introduction

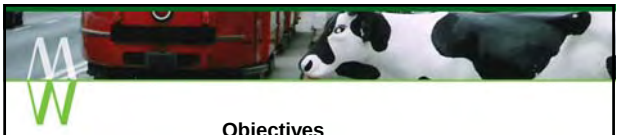
- Ethiopia is FMD endemic country and categorized in Pool 4
- The country has the largest livestock population in Africa that export live animal and its product to North Africa and Middle East directly or indirectly
- Ethiopia is currently at stage 1 of PCP according to regional Roadmap for FMD progression to 2020.



Ethiopia is currently at stage 1 of PCP according to Regional Roadmap for FMD progression to 2020.

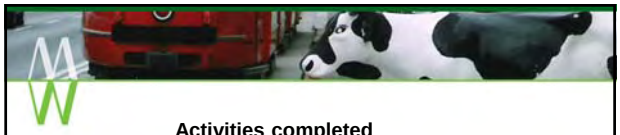






Objectives

- To improve the save livestock trade for our customers countries and other in the long run by reduction of risks of FMD transmission
- To develop livelihood of livestock producers and traders

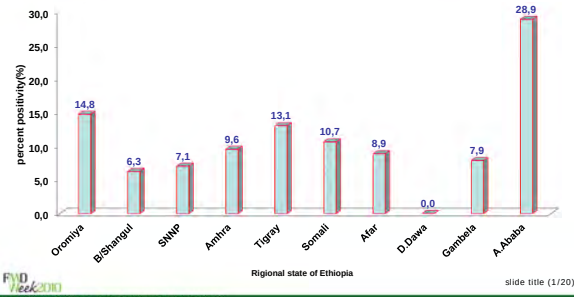


Activities completed

I. Sero-survillance



Sero prevalence of FMD in different regions of Ethiopia from 2008- 2009



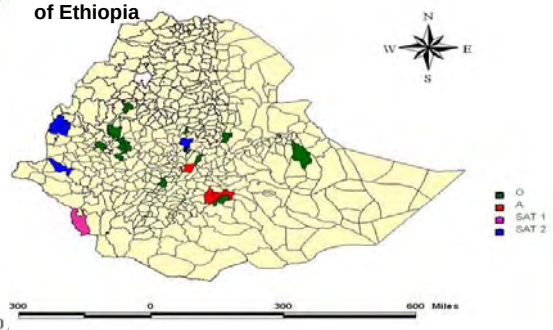
II. Outbreak investigation



Sero types of FMDV isolated in Ethiopia during 2007-2009

Year	No of samples	Virus isolation in cell culture /ELISA					NDV	RT-PCR for FMD virus	
		FMD virus serotypes						+ve	-ve
		O	A	C	SAT1	SAT2			
2007	28	4	1	-	4	1	18	8	20
2008	12	3	-	-	-	-	9	4	8
2009	40	15	2	-	-	6	12	26	9
Total	70	22	3	-	4	7	39	38	37

FMD virus serotypes distribution in different districts of Ethiopia



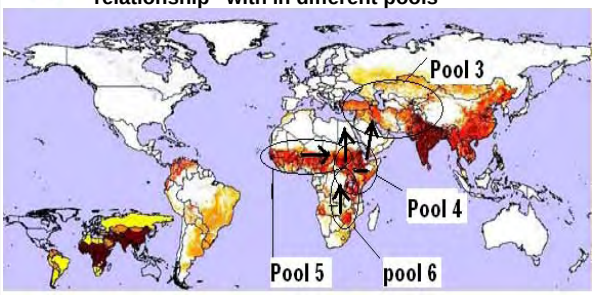
Genetic relationship of circulating FMD virus serotypes isolated from 2007- 2010

FMD Virus isolates	Areas affected in Ethiopia	Genetic relationship	Isolated year	Topotypes	Vaccine matched
O	Amahara, oromia and SNNP	Kenya, Sudan	2004	EA-3.	O Manisa
		Yemen	2004		
A	Oromia	Ethiopia	2007, 2008 & 2009	Africa lineage	A Eri 98 & A Tur 06
		Egypt	2006		
SAT 2	Benishangul, Gambella & Oromia, Around Addis Ababa	Ethiopia	2007, 2008	East African XIII	SAT2 Eri 3218 & SAT 2 Zim 7/83
		Sudan	2008		
SAT1	SNNP	Ethiopia	2007	Not designated	poor
		Nigeria	1976		

FMD virus serotypes distribution in different districts of Ethiopia and vaccine matched

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		Sudan	2008		
SAT1	SNNP	Ethiopia	2007	Not designated	poor
		Nigeria	1976		

The FMD virus serotypes in Ethiopia and Genetic relationship with in different pools



slide title (1/20)

III. Risk associated with FMD was identified



slide title (1/20)

1. Complex marketing chains (cattle from primary market through middle men to secondary market from one corner of the country and animals move to highlands areas for fattening and finally to the terminal live animal markets or exported from other corner



slide title (1/20)

2. Transport mode of live animal from secondary market to the final market



slide title (1/20)

3. Production systems : (pastoralism, agro-pastoralism, sedentary) and recurrent drought taht allow seasonal movement.



slide title (1/20)

4. Breeds of cattle affected and



slide title (1/20)

5. Interactions between domestic and wild ruminants



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IV. FMD control strategy was developed



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Short and long term FMD control strategy

Short term	Long term
SPS measures • inspection, testing, vaccination, quarantine and certification • Trade root identification and appropriate transport system	Mass vaccination • Developing capacity of NVI • Concentration to highly livestock producing pastoral areas
Surveillance • Passive and active • outbreak investigation • Serological survey	Progressive control pass way • Stage 1 (2009 – 2012) • Stage 2 (2013 – 2015) • Stage 3 (2016 – 2019) • stage 4 (2020)
Disease control by strategic vaccination • Ring vaccination around outbreak • Export animal • Dairy farms • 10Km radius around quarantine	

Conclusions

- FMDV is endemic in different regions of Ethiopia (A, O, SAT 1 and SAT 2) were confirmed
- SAT 1 serotype circulate virus was similar to Nigeria, 1976 and poorly matched with reference vaccine strains
- The distribution of SAT 2 Serotypes has been changed from border lowland pastoral areas of South and west of Ethiopia to the central highland part of the country
- It is possible to reduce risks of FMD through PCP by developing control program on major risk areas that can access to country for livestock export market

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EUFMD

Sero-surveillance in the PCP of FMD - Experiences from West Eurasia -

Carsten Pötzsch[1],
N.Bulut[2], T.Aliyeva[1], S.Kharatyan[1],
Z.Rukhadze[1], K.Sumption[1]

[1] EuFMD/FAO
[2] SAP Institute Ankara, Turkey

EUFMD

Conclusions

Sero surveys

- have been successfully used in Turkey and the Trans Caucasus for many years
- have improved FMD control and the knowledge about FMD epidemiology
- need to be combined with additional tools (clinical/virological/molecular surveillance, good descriptions of the situation, economic analyses)
- should have robust & risk based designs

EUFMD

PCP requirements → sero surveys to:

Stage 1 - estimate level of virus circulation in cattle (NSP Ab survey)
- identify high-risk populations

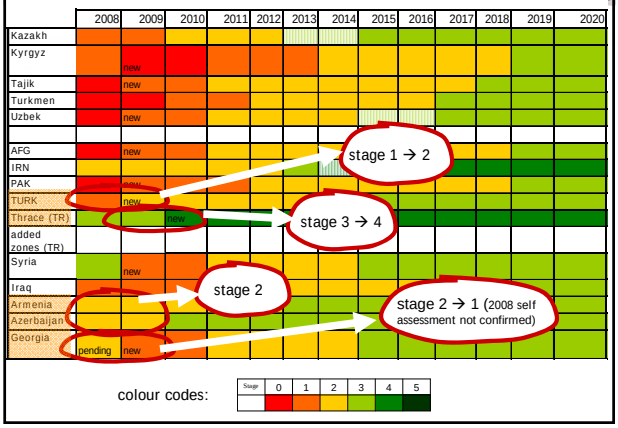
Stage 2 - monitor the efficacy of the preventive measures (mainly vacc.) in high-risk populations and the background FMD situation in large and small ruminants (NSP & SP Ab surveys)
- follow-up epidemiological sign. sero results/FMD events

Stage 3 - demonstrate that FMD exposure is limited in space & time
- support outbreak investigations

Stage 4 prove FMD freedom with vaccination

Stage 5 prove FMD freedom without vaccination

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FMD situation



- Turkey: - Thrace free with vaccination since 2010
  - Anatolia endemic for A (Ira 05) & O (PanAsia 2)
- TCC no outbreaks reported to OIE since 2002
  FMD is a highly political disease
- FMD mass vaccination of large ruminants, small ruminants: partly and irregularly



Vaccination coverage (%), spring 2010; average

	Turkey	Trace	Azerbaijan	Armenia	Georgia
LR	75-100	≈100	≈100	≈100	15
SR	60	50-100	32	0	0

EUFMD

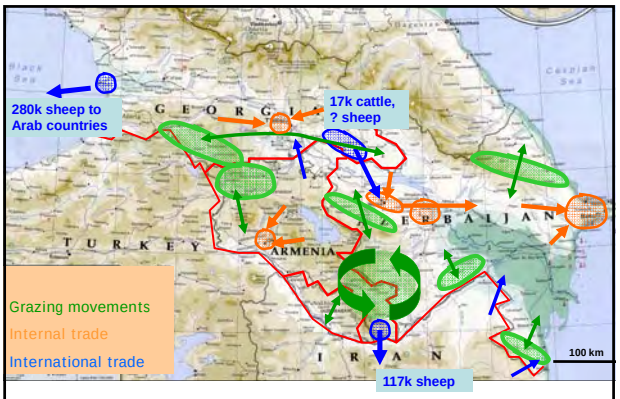
Risk of FMD introduction and spread

- migration to seasonal pastures
- open borders with neighbouring FMD infected countries
- increasing national and international trade of live ruminants

= uncontrolled ruminant movements

- TCC: no early detection of newly introduced FMDV





Ruminant movements in the TCCs

EUFMD

Sero surveys 2000-2010
for NSP and SP-Ab



Survey design: for estimating NSP-Ab distribution
= Risk based surveillance, two-stage sampling

Thrace/Turkey:
Since 2000: annual cattle surveys
Infrequent SR surveys

Anatolia/Turkey:
2007: market and slaughterhouse survey (4-12 & 4-36 mo.)
2008-10: annual cattle and one SR survey
→ For Thrace/Anatolia surveys: LR: 4-24 mo., SR: 4-18 mo.

Trans Caucasus:
2005 & 2006: in high risk cattle pop. (4-18 mo.)
2008/9: national cattle surveys (4-18 mo.)
2009: cattle and SR surveys (follow-up of 2008/09 results; 4-12 mo.)



EUFMD

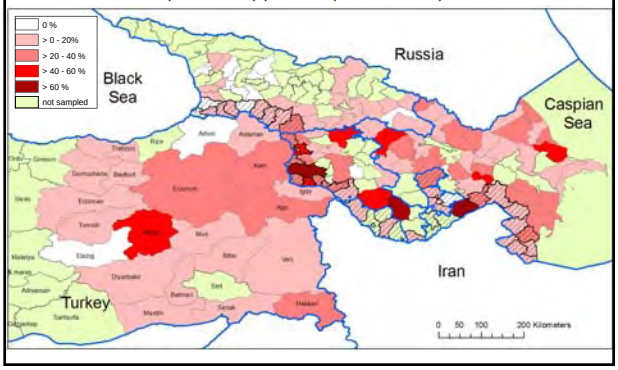
Sero surveys 2000-2010: NSP-Ab results (%)
national averages, apparent prevalence

	Species	Turkey/Trace	Turkey/Anatolia	TCC
2000-04	LR	1-2		
	SR	0.1-1		
2005	LR	0.3		3-15
2006	LR	1.3		10-58
2007	LR	0.8	27 & 52 *	
2008	LR	<0.1	8	11-31
2009	LR	0.1	10	3-11
	SR	<0.1		9-20
2010	LR		12	
	SR		17	

* market & slaughterhouse survey

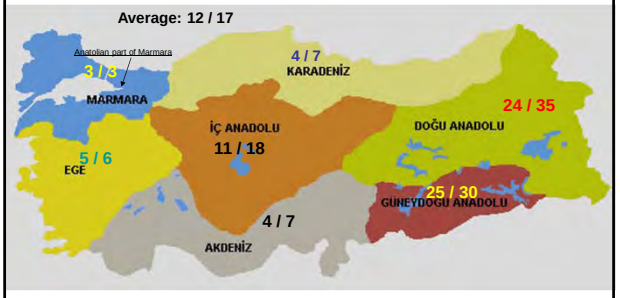
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NSP antibody results: TCC & E.Anatolia, 2008/09
(cattle, apparent prevalence)



EUFMD

NSP antibody results: Turkey, 2010
(LR / SR in %, apparent prevalence)



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Lessons learnt: Value of sero surveys

- Key element in PCP (assessment of FMD virus circulation) and understanding FMD epidemiology (esp. where the disease is underreported - E.Anatolia, TCC)
- Results lead to adjusted / improved FMD control
- Countries want to progress (competition between them)
- Increasing national capacities in:
  - Epidemiology (survey design, reporting, risk assessments, data analysis)
  - Field work (logistics, sampling)
  - Diagnostics (testing in national laboratories)

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Lessons learnt: Limitations of sero surveys



Survey design
- expected prevalence based on retrospective data/info

Conclusions:
- causality, time from exposure to test results (sampling age, time to availability)
- Comparability between surveys difficult
- Complex patterns, dynamic systems, socio economics
- Data analysis often insufficient

Execution: s.surveys are large, resource intensive undertakings in the field and lab (difficult to manage, many uncertainties)

Diagnostics:
- test sensitivity (variation for exposed populations)
- NSP induction of vaccines (lack of data)

EUFMD

… therefore



for sero surveillance in the PCP we need:
- Clear objectives – harmonisation (or standards ?)
- Robust designs and robust analyses

Increasing use of the following as moving from stages 1 to 3:
- good descriptions of the disease control & production systems
- virological/molecular & clinical surveillance, SP-Ab serology, good vaccine characterisation
- Outbreak investigations
- (socio-)economic assessments (e.g. market chain analysis)

FMD Week 2010

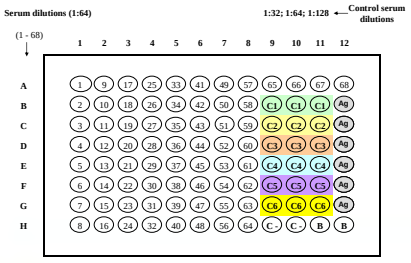
QUANTITATIVE SINGLE SERUM DILUTION COMPETITIVE BLOCKING lpELISA FOR THE ASSESSMENT OF HERD IMMUNITY AND EXPECTED PROTECTION AGAINST FMDV IN VACCINATED CATTLE

Nora Mattion
CEVAN, ICT Milstein, CONICET, Argentina

Aims

- ✓ Validation of a single serum-dilution liquid phase ELISA (**slpELISA**) for serological evaluation of herd immunity against FMDV.
- ✓ Replacement of the conventional serial dilution lpELISA for the assessment of the protection status of cattle in epidemiological studies and for monitoring the effectiveness of vaccination campaigns.

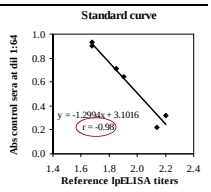
Single serum dilution lpELISA. Microplate layout



Validation of control sera and standard curves for the titration of antibodies by slpELISA.

A24/Cruzeiro

Inv. dilution	Absorbance 32	64	128	r	Calculated lpELISA titer	Reference lpELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	0.028	0.321	0.702	1.00	2.14	2.20
C2	0.251	0.714	1.104	1.00	1.83	1.85
C3	0.447	0.899	1.140	0.98	1.72	1.68
C4	0.020	0.220	0.769	0.97	2.12	2.14
C5	0.188	0.646	0.986	1.00	1.90	1.90
C6	0.461	0.931	1.269	1.00	1.69	1.68



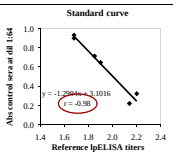
Validation of each plate/strain

- ✓ Standard curves: A straight line should be obtained by linear regression analysis (r ≥0.90) in the titer range of 1.40 to 2.40 (dilution 1:64)
- ✓ Control sera: Calculated lpELISA titers should differ from reference titers in not more than ±0.200
- ✓ Antigen control (100% reactivity). Avg. OD: >0.750 and <1.950, not differing in more than 0.300 from each other
- ✓ Negative control titer : ≤1.40
- ✓ Blank OD: <0.300

Validation of control sera and standard curves for the titration of antibodies by slpELISA.

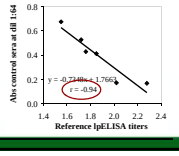
A24/Cruzeiro

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A/Arg/01

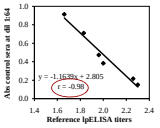
Inv. dilution	Absorbance 32	64	128	r	Calculated lp ELISA titer	Reference lp ELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	-0.003	0.167	0.523	0.98	2.13	2.28
C2	0.110	0.429	0.658	1.00	1.93	1.76
C3	0.331	0.676	0.818	0.97	1.69	1.55
C4	0.007	0.171	0.450	0.99	2.22	2.02
C5	0.087	0.413	0.681	1.00	1.92	1.85
C6	0.173	0.527	0.737	0.99	1.84	1.72



Validation of control sera and standard curves for the titration of antibodies by slpELISA.

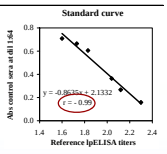
O1/Campos

Inv. dilution	Absorbance 32	64	128	r	Calculated lp ELISA titer	Reference lp ELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	0.030	0.218	0.574	0.98	2.18	2.26
C2	0.155	0.475	0.786	1.00	1.94	1.96
C3	0.399	0.711	0.958	1.00	1.72	1.83
C4	0.019	0.152	0.435	0.98	2.39	2.30
C5	0.100	0.384	0.634	1.00	2.07	2.00
C6	0.603	0.915	1.003	0.95	1.46	1.66

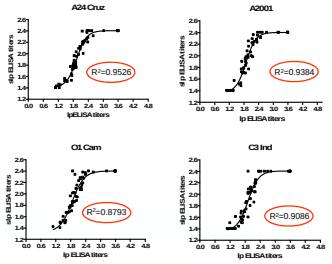


C3/Indaial

Inv. dilution	Absorbance 32	64	128	r	Calculated lp ELISA titer	Reference lp ELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	0.046	0.158	0.426	1.00	2.26	2.30
C2	0.068	0.267	0.572	0.99	2.04	2.12
C3	0.341	0.665	0.832	0.98	1.67	1.73
C4	0.105	0.364	0.617	1.00	1.97	2.04
C5	0.277	0.606	0.770	0.98	1.74	1.83
C6	0.453	0.709	0.818	0.97	1.54	1.60



Assay validation. Correlation between antibody titers of 60 cattle sera determined by end point dilution lpELISA and slpELISA



Assay validation and assessment of EPP

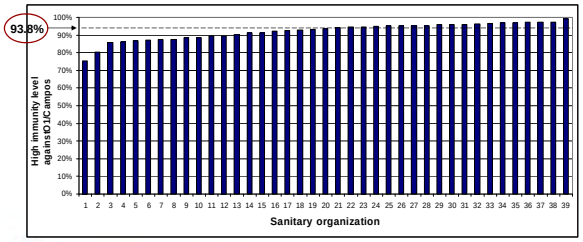
- Antibody titers of 60 cattle sera titrated by slpELISA and lpELISA, showed an acceptable correlation (R^2 >0.87) for viral strains A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial
- Titers of paired samples analyzed by both methods were not significantly different (p>0.05)
- Intra-laboratory repeatability (intermediate precision) of slpELISA: CV < 11%, in the titer range 1.40–2.40 for A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial
- Therefore, the Expected percentage protection (EPP) may be calculated through the validated curves of lpELISA titers vs. PPG, using the slpELISA titers

Distribution of cattle with high immunity level (EPP ≥75%), by age category and FMDV serotype in Buenos Aires Province in the year 2004

Age category	N° of animals	High immunity level A/Arg/01 n	EPP	95% CI	O1/Campos n	EPP	95% CI
< 1 year	13 832	8 230	59.5	[58.1-60.1]	8 686	62.8	[61.4-64.2]
1-2 years	4 118	3 616	87.8	[86.6-89.0]	3 786	91.9	[90.1-93.0]
> 2 years	2 792	2 668	95.6	[94.7-96.4]	2 704	96.8	[96.1-97.6]

Data from 39 Sanitary Units - The proportion of animals with high immunity level was significantly higher (P < 0.05, 95% CI) for O1/Campos than for A/Arg/01 strain, for the three age categories

Distribution of cattle (1-2 years) with high immunity levels for FMDV O1/Campos, among 39 Sanitary Units participating in the study.



CONCLUSIONS

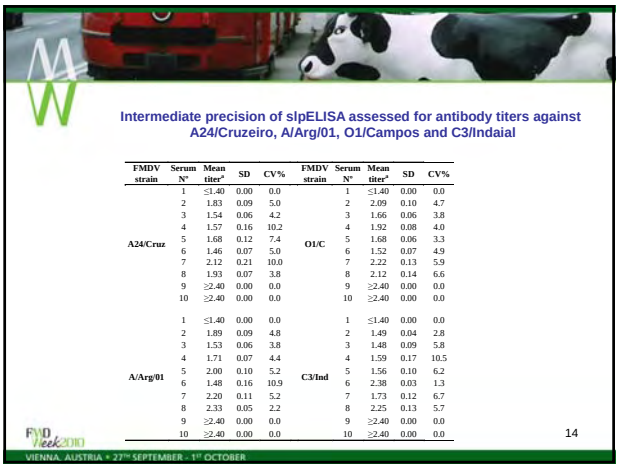
- ✓ slpELISA was successfully validated to replace the conventional serial dilution ELISA for the assessment of the protection status of cattle in epidemiological studies, in the titer range of 1.40 to 2.40.
- ✓ The method is a reproducible and practical epidemiological tool suitable for monitoring the effectiveness of vaccination campaigns of a large number of animals in extended geographical regions, at a considerable lower cost and speed
- ✓ The method may be also used for the assessment of seroconversion of naive animals during early stages of infection



Blanca Robiolo, Cristina Seki, José La Torre, Nora Mattion

Sergio Duffy, Emilio Leon

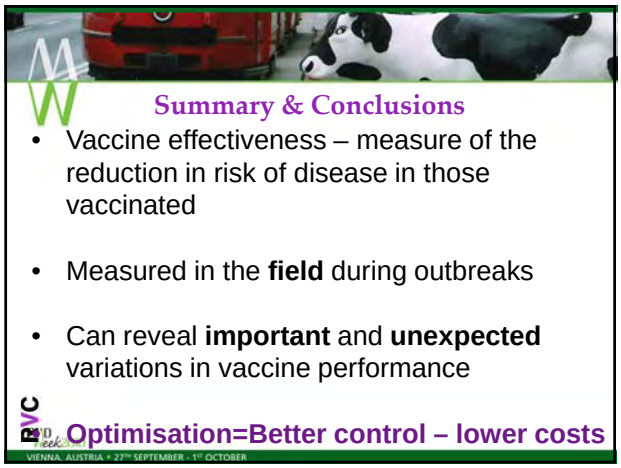
Argentine Inter-Institutional FMD R&D Network (RIIDFA)

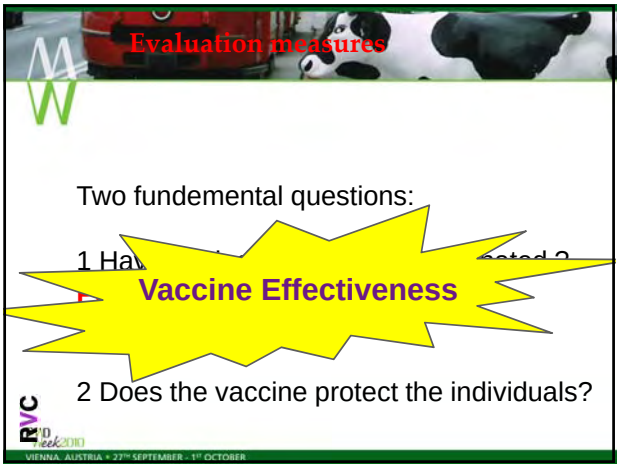


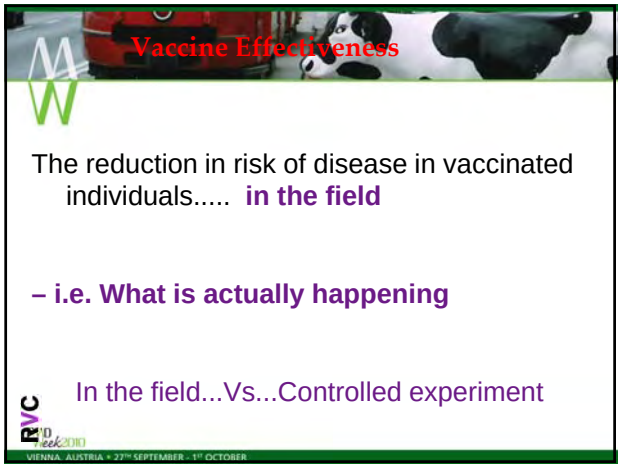
Intermediate precision of slpELISA assessed for antibody titers against A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial

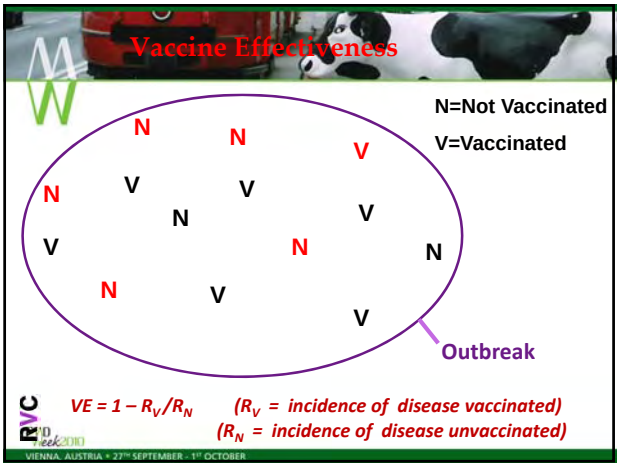
FMDV strain	Serum N°	Mean titer[a]	SD	CV%	FMDV strain	Serum N°	Mean titer[a]	SD	CV%
A24/Cruz	1	≤1.40	0.00	0.0	O1/C	1	≤1.40	0.00	0.0
	2	1.83	0.09	5.0		2	2.09	0.10	4.7
	3	1.54	0.06	4.2		3	1.66	0.06	3.8
	4	1.57	0.16	10.2		4	1.92	0.08	4.0
	5	1.68	0.12	7.4		5	1.68	0.06	3.3
	6	1.46	0.07	5.0		6	1.52	0.07	4.9
	7	2.12	0.21	10.0		7	2.22	0.13	5.9
	8	1.93	0.07	3.8		8	2.12	0.14	6.6
	9	≥2.40	0.00	0.0		9	≥2.40	0.00	0.0
	10	≥2.40	0.00	0.0		10	≥2.40	0.00	0.0
A/Arg/01	1	≤1.40	0.00	0.0	C3/Ind	1	≤1.40	0.00	0.0
	2	1.89	0.09	4.8		2	1.49	0.04	2.8
	3	1.53	0.06	3.8		3	1.48	0.09	5.8
	4	1.71	0.07	4.4		4	1.59	0.17	10.5
	5	2.00	0.10	5.2		5	1.56	0.10	6.2
	6	1.48	0.16	10.9		6	2.38	0.03	1.3
	7	2.20	0.11	5.2		7	1.73	0.12	6.7
	8	2.33	0.05	2.2		8	2.25	0.13	5.7
	9	≥2.40	0.00	0.0		9	≥2.40	0.00	0.0
	10	≥2.40	0.00	0.0		10	≥2.40	0.00	0.0

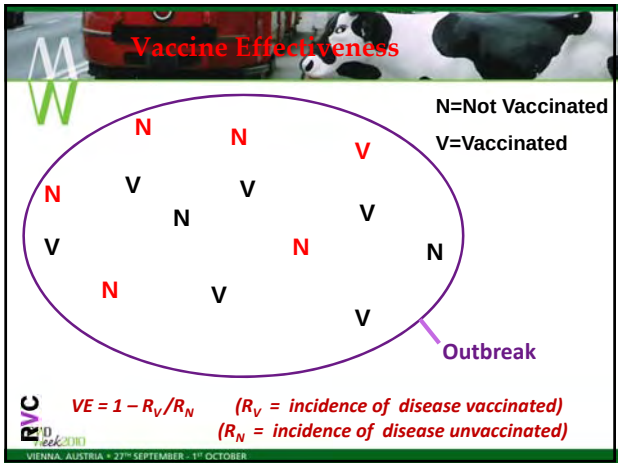

FMD Week2010
eufmd
Can evaluation methods used for human vaccination programmes be used to improve the control of foot and mouth disease (FMD)?
Theo Knight-Jones & Paul Fine
Royal Veterinary College,
London School of Hygiene and Tropical Medicine
RVC
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


Summary & Conclusions
• Vaccine effectiveness – measure of the reduction in risk of disease in those vaccinated
• Measured in the **field** during outbreaks
• Can reveal **important** and **unexpected** variations in vaccine performance
Optimisation=Better control – lower costs
RVC
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


Evaluation measures
Two fundemental questions:
1 Ha
Vaccine Effectiveness
2 Does the vaccine protect the individuals?
RVC
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Vaccine Effectiveness
The reduction in risk of disease in vaccinated individuals..... in the field
– i.e. What is actually happening
In the field...Vs...Controlled experiment
RVC
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Vaccine Effectiveness
N=Not Vaccinated
V=Vaccinated
Outbreak
VE = 1 – RV/RN (RV = incidence of disease vaccinated)
(RN = incidence of disease unvaccinated)
RVC
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Vaccine Effectiveness
N=Not Vaccinated
V=Vaccinated
Outbreak
VE = 1 – RV/RN (RV = incidence of disease vaccinated)
(RN = incidence of disease unvaccinated)
RVC
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Vaccine Effectiveness

Vaccine factors + Host factors + Environment factors + Agent factors

Value of vaccine effectiveness

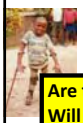


Smallpox

Variations in vaccine effectiveness

– Led to req

Many different FMD vaccines, do they all provide sufficient protection?



Polio

Little progress in north India 2000-2009

Vaccine found to be less effective than elsewhere

Are there areas where current vaccine strategy is not working? Will other vaccine schedules work better?



TB

Vaccine found less effective in tropics

– Focus on early detection and treatment

Should we concentrate on other control measures?

And much, much more...



Seasonal influenza

- When the vaccine is poorly matched – does it still protect?

Does the vaccine protect against a genetically distinct strain? How do r-values/serology relate to protection in the field?



Haemophilus influenzae type b

-Three doses found to be as effective as four – big saving (assessed using vaccine effectiveness & serology)

Which vaccine schedule is most cost-effective?

And even more.....!



Pertussis

-Canada –Should new (acellular) vaccine be used?

-Economic analysis done considering vaccine effectiveness, cost, adverse reactions, etc...

Which vaccine is most cost-effective?



Mumps

UK & USA - Effectiveness 96% in 2 year old but only 66% at age 11-12 years .....is this important?

Are there important groups lacking protection?

Current FMD measures

- Challenge studies
- Sero-surveys – "Coverage" – Complex!
- r-values

Current FMD measures

- Challenge studies

-small numbers, artificial conditions

- Sero-surveys – "Coverage"

Proportion Vaccinated + Proportion that sero-convert + Wild virus sero-conversion

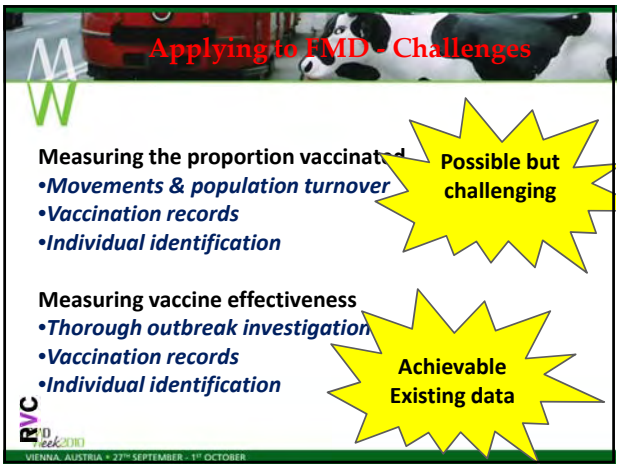
– does not consider vaccine history

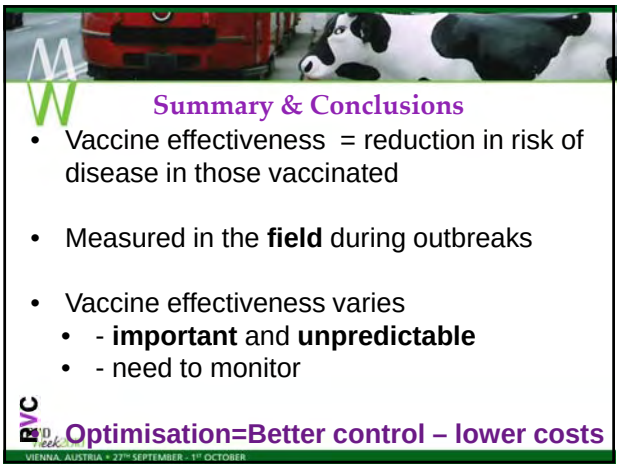
-are they protected?

- r-values

-But are they protected?

-How does serology relate to field protection?


Applying to FMD - Challenges
Measuring the proportion vaccinated
•Movements & population turnover
•Vaccination records
•Individual identification
Possible but challenging
Measuring vaccine effectiveness
•Thorough outbreak investigation
•Vaccination records
•Individual identification
Achievable Existing data


Summary & Conclusions
• Vaccine effectiveness = reduction in risk of disease in those vaccinated
• Measured in the field during outbreaks
• Vaccine effectiveness varies
• - important and unpredictable
• - need to monitor
Optimisation=Better control – lower costs

1

Foot-and-Mouth Disease Virus Genotype Definitions and Nomenclature

Nick J. Knowles, Jemma Wadsworth, Jef M. Hammond and Donald P. King

Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK.

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2

Introduction

- Foot-and-mouth disease viruses (FMDV) belonging to each of the serotypes have been classified into a number of topotypes (VP1 genotypes which occupy distinct geographical niches).
- Currently, these number 11 for type O, 3 for type A, 3 for type C, 1 for type Asia 1, 9 for type SAT 1, 14 for type SAT 2 and 5 for type SAT 3.
- There are also a number of older viruses which are distinct or difficult to place within this framework.
- Within topotypes viruses are sometimes placed in named clusters, called genotypes, lineages or strains, which do not necessarily have consistent defining criteria.

3

Material & Methods

- Phylogenetic trees were generated using the Neighbor-joining (N-J) method with bootstrap re-sampling (MEGA 4.0).
- Bayesian evolutionary analysis was performed using the BEAST software package. The Bayesian Markov Chain Monte Carlo (MCMC) method, using virus isolation times, was used to generate dated trees.
- VP1 sequences of the prototype viruses were used in the construction of both trees.

4

Results

- In general the tree topology was similar using the two methodologies, however, the Bayesian trees were more informative concerning the evolution and dating of the viruses.

5

FMDV O

•EURO-SA (Europe-South America)
•ISA-1 (Indonesia 1)
•ISA-2 (Indonesia 2)

•ME-SA (Middle East-South Asia)
•SEA (Southeast Asia)
•EA-1 (East Africa 1)
•EA-2 (East Africa 2)
•EA-3 (East Africa 3)
•EA-4 (East Africa 4)
•WA (West Africa)

•CATHAY (Far East)

•AFRICA?

FMDV A

•AFRICA
  •G-I
  •G-II
  •G-III
  •G-IV
  •G-V
  •G-VI
  •G-VII
•ASIA
•EURO-SA

FMDV C

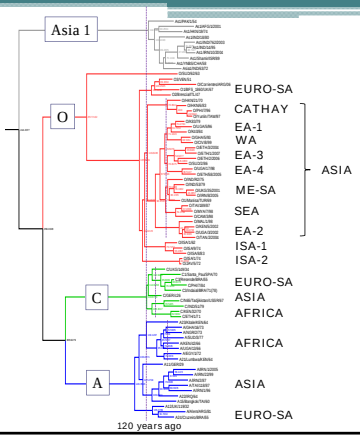
•AFRICA
•ASIA
•EURO-SA

FMDV Asia 1

•ASIA

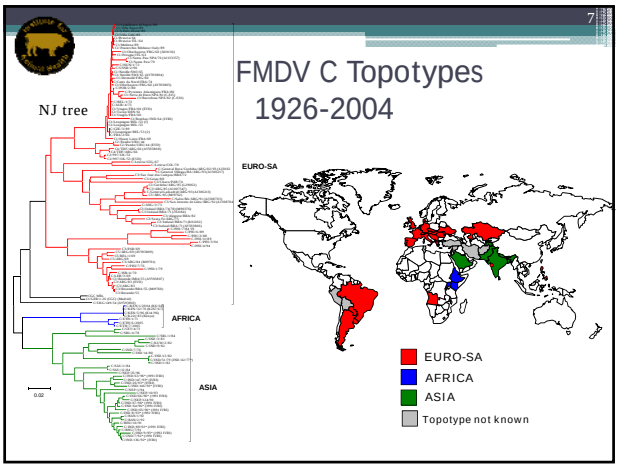
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Maximum clade credibility tree

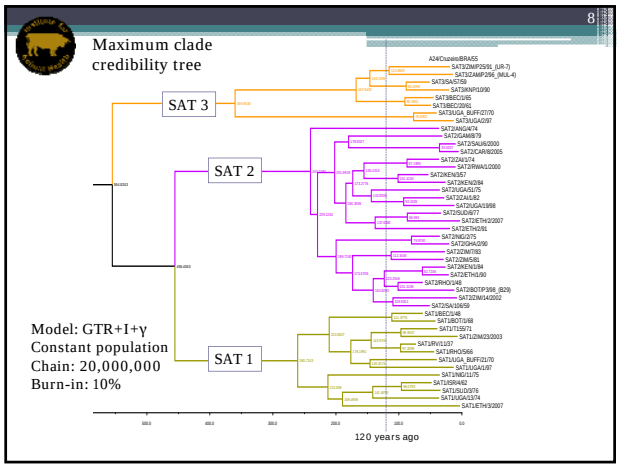


Model: GTR+I+γ
Constant population
Chain: 20,000,000
Burn-in: 10%

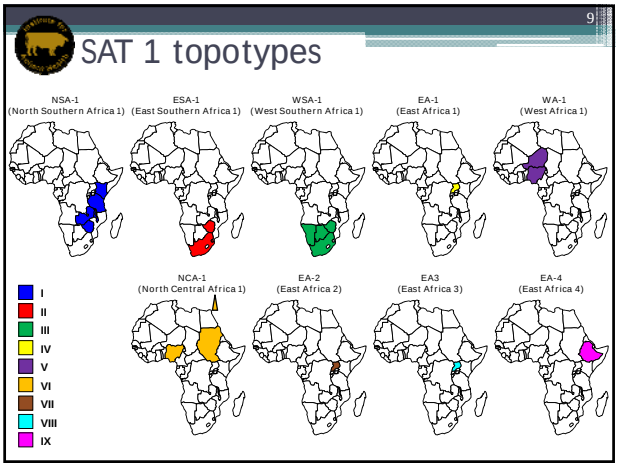
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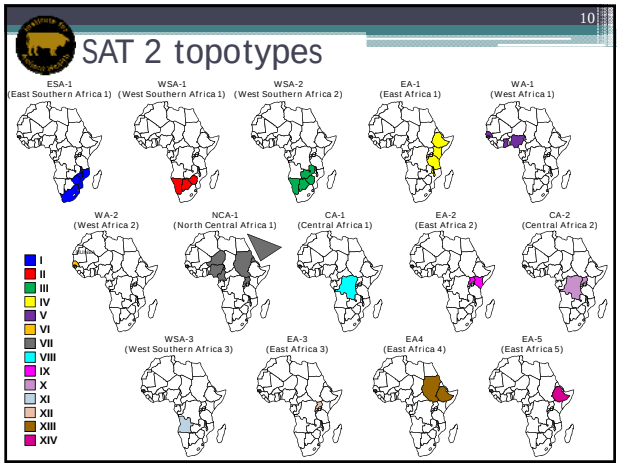
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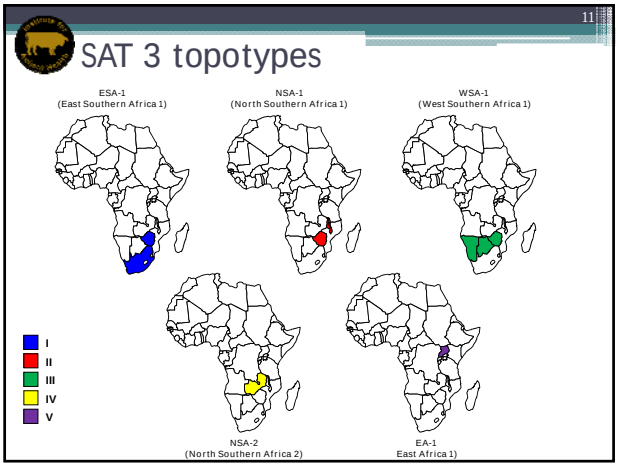
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10



11



12

Bayesian analyses

- Within-topotype diversity only dates back less than 120 years
- This suggests that almost all type O, A and C viruses were introduced into Africa following the African Rinderpest Pandemic of the 1890's.
  - Type O: two introductions, probably from Asia, i) the ancestor of EA-1/EA-3/EA-4/WA and ii) the ancestor of EA-2.
  - Type A: one introduction from Europe or Asia.
  - Type C: one introduction from Europe or Asia (possibly in the 1940's) and one from South America circa 1973.

13

Summary

- The topotypes of FMDV O, A and C have been assigned geographically-based names (e.g. O WEST AFRICA), while those belonging to the SAT types have been given arbitrary numbers (in Roman numeral format).
- We suggest that the former system is more informative and should be adopted for the SAT topotypes.
- Using both Bayesian and distance methods to construct trees provides a better basis for the definition of genetic groupings below the level of serotype.

14

Questions

- How should we define FMDV topotypes?
- How should we define FMDV strains or named lineages?

15

Acknowledgements

- Funding from Defra, UK

defra Department for Environment Food and Rural Affairs

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FULL-GENOME SEQUENCE ANALYSIS OF FOOT-AND-MOUTH DISEASE VIRUSES IN WESTERN EURASIA

Müge Firat Sarac,
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Faizah Abdul Hamid,
Begoña Valdazo-González,
Jemma Wadsworth,
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Institute for Animal Health, Pirbright, United Kingdom

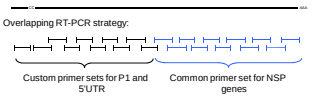
Summary and conclusions

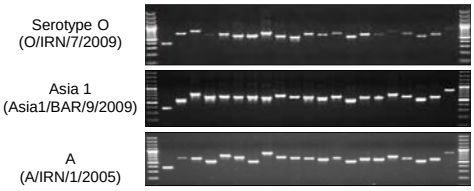
- This presentation outlines results from a 1 year collaborative project funded by FOA (EuFMD): *Application of Tools for High-Resolution FMDV Molecular Epidemiology in Western EurAsia*
- Complete genome sequencing protocols developed for FMDV serotype O, A and Asia circulating in the region
- Applying these approaches to field outbreaks can improve our understanding of the processes by which FMDV is maintained year-on-year in the Middle East. Specifically:

[1] Improve resolution by which outbreaks can be resolved
[2] Investigate the mechanisms by which FMDV evolves and generates diversity
[3] Estimate disease prevalence where there are gaps in epidemiological knowledge

Protocol development

- Strategy:





- Simple protocols
- Common amplification conditions for all PCRs
- PCR primers also used for sequencing

FMD viruses sequenced

- Characteristics of 22 fully assembled genome FMDV sequences
- 13 serotype O, 5 serotype 5 and 4 serotype Asia 1 (including Shamir [ISR/3/89])

Isolate name	Host species	Origin	Sequence length		Seq avg coverage
			nt	aa	
O/BAR/1/2008	Cattle	OS	8189	2336	3.45
O/IRN/8/2005	Not recorded	CC	8188	2336	3.18
O/IRN/7/2009	Not recorded	CC	8191	2336	2.97
O/IRN/9/2007	Cattle	OS	8190	2336	3.1
O/IRN/23/2006	Cattle	OS	8188	2336	3.92
O/IRN/34/2006	Cattle	OS	8181	2336	3.5
O/IRN/12/2006	Cattle	OS	8193	2336	3.73
O/TUR/2/2009	Cattle	CC	8190	2336	4.69
O/TUR/18/2007	Cattle	OS	8193	2336	3.19
O/TUR/30/2007	Cattle	OS	8190	2336	3.23
O/TUR/30/2008	Cattle	OS	8188	2336	3.02
O/AFG/34/2007	Cattle	CC	8191	2336	3.83
O/UKG/5470/2001	-	I	8193	2336	n.d.
A/BAR/6/2008	Cattle	OS	8198	2336	2.79
A/IRQ/22/2009	Cattle	OS	8203	2336	2.76
A/IRN/1/2005	Cattle	CC	8202	2336	2.95
A/IOR/4/2006	Cattle	OS	8196	2336	2.94
A/TUR/2/2008	Cattle	OS	8198	2336	3.53
ASIA1/ISR/3/89	Cattle	CC	8187	2336	3.09
ASIA1/BAR/9/2009	Cattle	CC	8135	2336	4.55
ASIA1/PAK/26/2009	Buffalo	OS	8182	2336	3.47
ASIA1/PAK/29/2009	Buffalo	CC	8183	2336	3.93

OS = Original suspension
CC = Cell culture
I = Inoculum

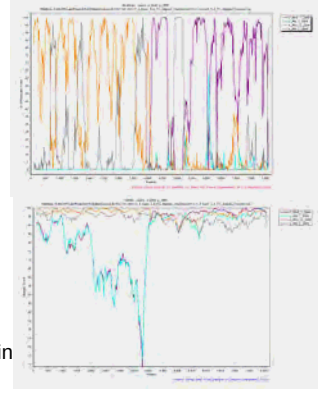
Uses of full genome data:

- Improve resolution by which outbreaks can be resolved
- Investigate the mechanisms by which FMDV evolves and generates genetic and antigenic diversity
- Estimate FMD prevalence where there are gaps in epidemiological knowledge?



Insights into mechanisms generating genetic diversity

- SimPlot and BootScan analysis
- 3' end of genome (NSP region) has closer relationship to O viruses in Middle East than A/IRN/1/2005
- Two separate events?
- Indicative of recombination between A/BAR6/08 and a circulating serotype O virus in Bahrain?



Insights into mechanisms generating antigenic diversity

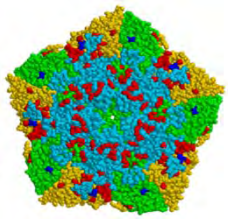
•Asia isolate received from Pakistan

•Poor match of PAK/29/2009 to Asia 1/Shamir (ISR/3/89)
- in vitro VNT r1 value = 0.14

•Circulation of this lineage currently restricted

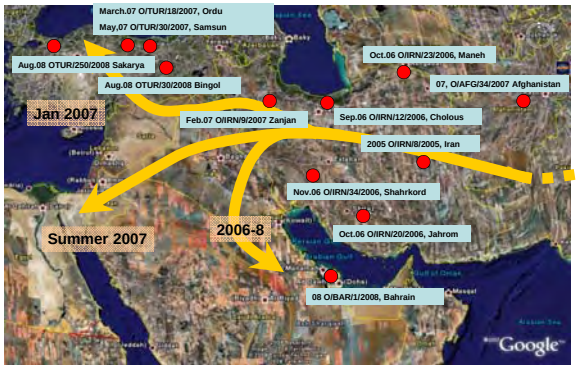
•Natural immunity may be low in the Middle East

•Surface exposed residues that underpin this phenotype?

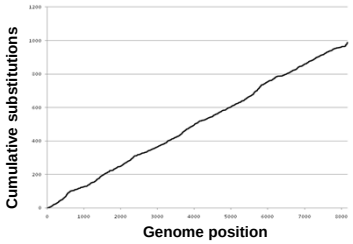


Cyan = VP1 Yellow = VP2 Green = VP3

Serotype O (PanAsia-2) outbreaks



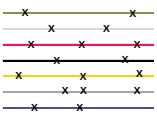
Distribution of nt substitution sites



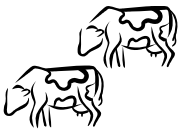
- 12 full genomes (~ 5 year period)
- substitutions are observed at 989 sites
- distributed across the genome
- UK outbreak (197 substitutions/7 months) from 23 viruses

How might these data be used?

- Sequences recovered over same temporal scale
- HOW MANY INFECTED ANIMALS/FARMS have generated this diversity?
  - assuming single introduction



- Coalescent analysis of sequences
- **Calibrated** using available data:
  - Experimental infection studies
  - UK 2001 and UK 2007 outbreaks
  - Serosurveys from endemic countries?



- Could this approach be used to monitor FMD control programmes?

Long term goals of project:

- An improved understanding of the manner by which FMDV is maintained year-on-year in The Middle East

- Determination of evolutionary rates and mechanisms by which FMDV evolves within an endemic situation

- Indication of the degree of undisclosed FMDV infection in endemic regions such as the Middle East

We now have the tools – we need to discuss how they will be used

–Critical input from field researchers and epidemiologists

Acknowledgements

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

EPIZONE

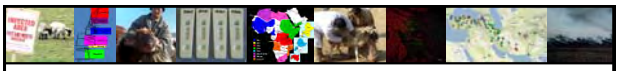
defra

SE2938

BBSRC bioscience for the future



- Further details (protocols etc…), please contact:
  - donald.king@bbsrc.ac.uk



Inter- and intra-herd sequence variability of foot-and-mouth disease viruses recovered during the 2007 UK outbreak

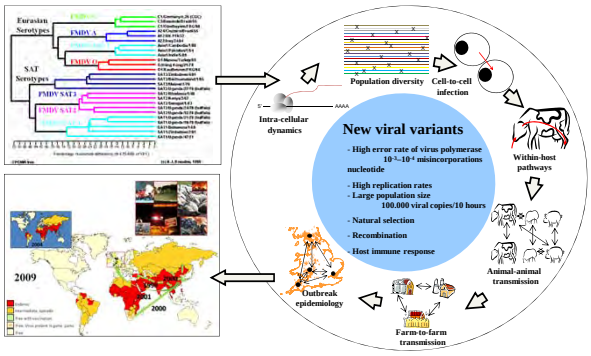
Begoña Valdazo-Gonzalez, Nick J. Knowles, Jemma Wadsworth, Donald P. King
Molecular Characterisation and Diagnostics Group
Institute for Animal Health
United Kingdom

BBSRC bioscience for the future | defra | Institute for Animal Health

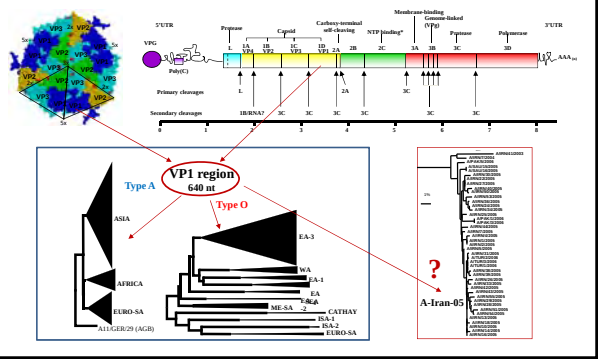
Summary and Conclusion

- **Aim**
  - 2007 UK outbreak: Inter- & intra- herd FMDV variability
- **M&M**
  - 44 field samples, 39 animals, 8 IP
  - FG sequencing strategy: *Cottam et al*, 2008
  - Statistical parsimony methods (TCS)
- **Results**
  - 50 nt changes along the genome
  - Intra-herd clustering
  - Probably chain of transmission events
  - Different degree of inter- and intra-herd variability
- **Discussion and conclusions**
  - Further knowledge epidemiological dynamics of FMDV
  - Different evolutionary processes
    - ✓ Acute vs chronically infected animals

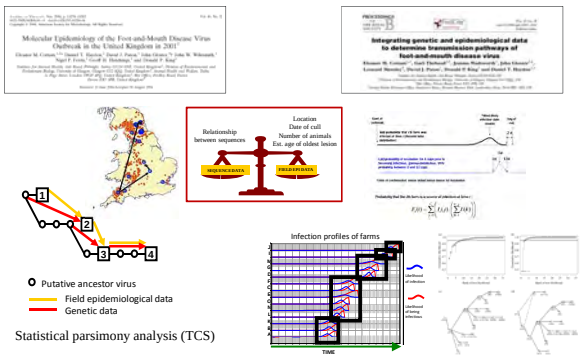
FMDV evolution and diversity



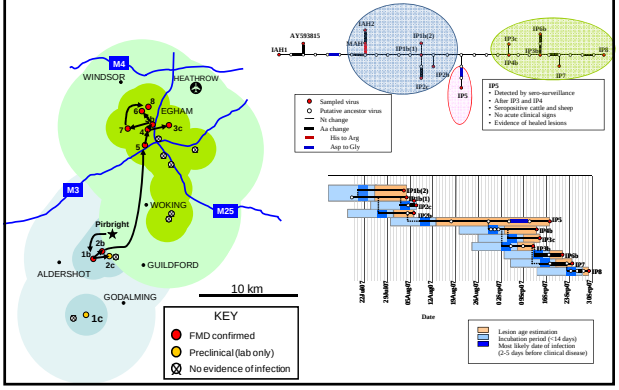
FMDV molecular characterization



Background: 2001 UK outbreak



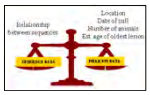
Background: 2007 UK outbreak



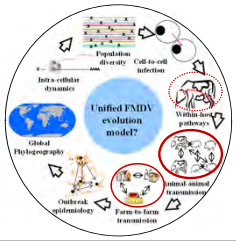
Objectives:

- Detailed investigation of the inter- and intra- herd consensus sequence variability during the 2007 UK outbreak

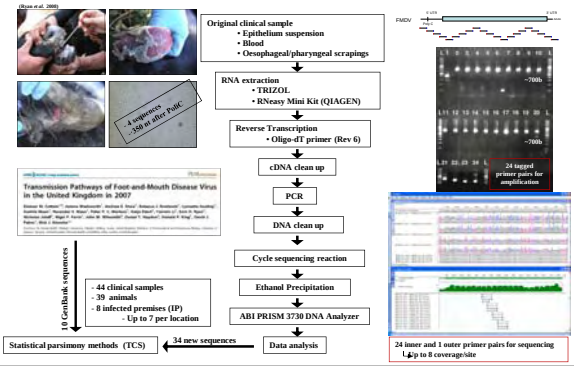
1. To develop application tools for fine-scale molecular FMDV epidemiology
   - ❖ Analytical models to integrate molecular and field epidemiology data
     - ✓ Estimation of undisclosed FMD circulation in endemic regions
     - ✓ Give clues about how virus persistence is maintained and could be blocked



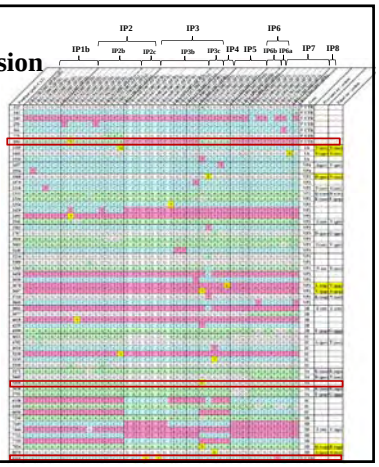
2. To increase the knowledge about FMDV evolution
   - ✓ Evolution rates
   - ✓ Sites and importance of recombination
   - ✓ Identification of ordered structures
   - ✓ Contribution of quasi-species to evolution



Material & Methods



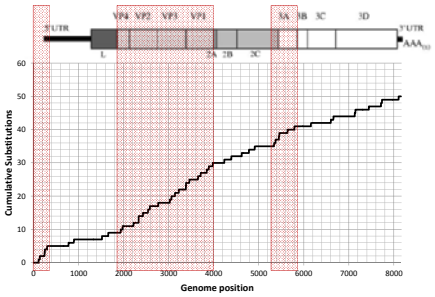
Results & Discussion



- ➢ Full genome
  - ❖ 50 substitutions
    - †62 considering ambiguities
      - ✓Exclusive: 12
        - 12 sites, 9 isolates
          - ↳ 6, 4 esophageal/pharyngeal scrapings
      - ✓Not exclusive: 4
        - 3 sites, 4 isolates
          - * Blood, IP-3
- ➢Polyprotein
  - ❖ 42 substitutions
    - †54 considering ambiguities
      - ✓ 7 out of 12 non synonimous
        - ↳ 5, 4 esophageal/pharyngeal scrapings
    - ➢25 synonymous
    - ➢17 non synonymous

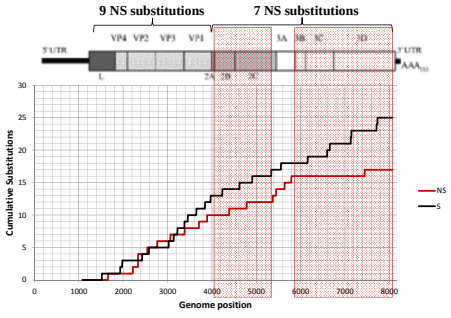
Results & Discussion

- ➢ Full genome



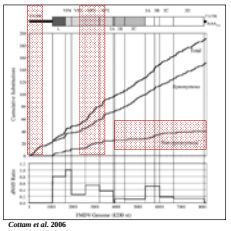
Results & Discussion

- ➢ Poliprotein

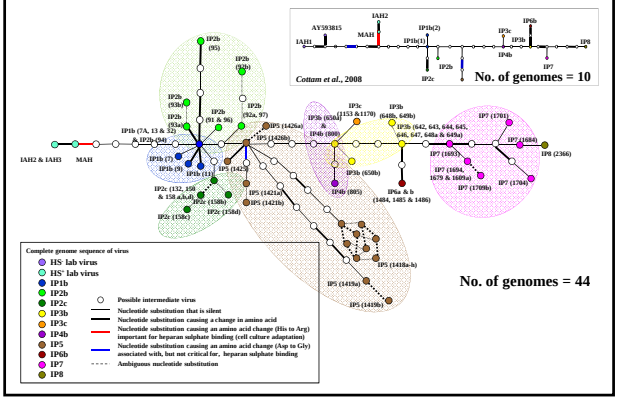


Results & Discussion

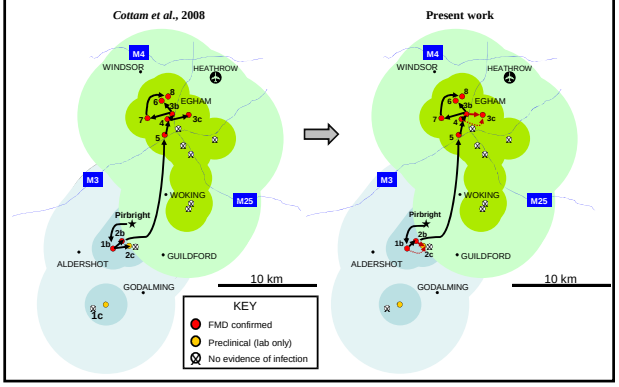
- ➢ 2001 UK outbreak (*Cottam et al.*, 2006)
  - ❖ 23 full genome sequences
  - ❖ 191 nt substitutions
  - ❖ 7 months
    - ✓ Nt substitution rate: 2.26 X 10^{-5}/site/day
- ➢ Future work
  - ❖ Mathematical analysis
    - ✓ dN/dS
    - ✓ Recombination
  - ❖ Analytical models



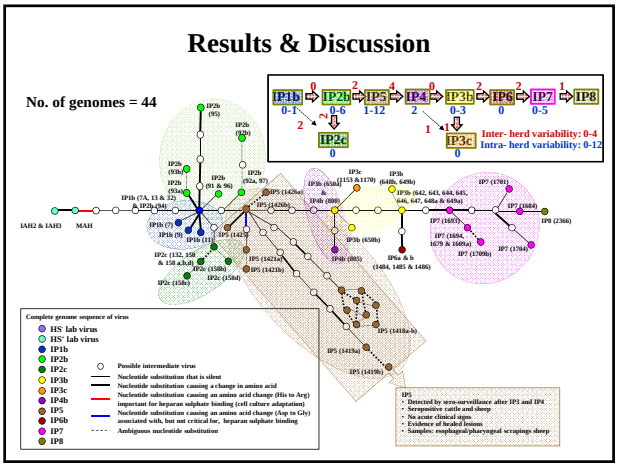
Results & Discussion



Results and Discussion



Results & Discussion



Acknowledgements

- Institute for Animal Health
  - Staff involved in the data and specimen collection from the 2007 UK outbreak
    - Nigel Ferris
    - Geoff Hutchings
- Department for Environment, Food and Rural Affairs (SE2938)
- Biotechnology and Biological Sciences Research Council

Can next generation sequencing

be used to unravel

fine scale

FMDV population dynamics?

Caroline Wright
Institute for Animal Health, Pirbright, UK
30.09.10

Summary and conclusions

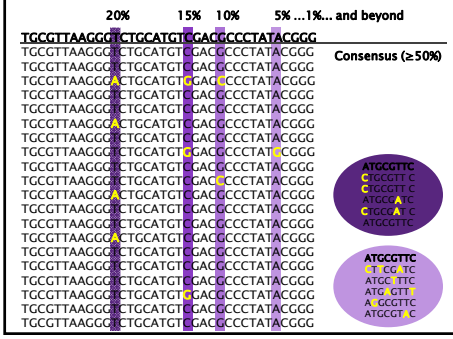
- First time next generation sequencing (NGS) has been used to investigate within-host FMDV population diversity
- NGS demonstrates a 'step-change' improvement in the depth of detection of FMDV population diversity present within-host
- Intermediate stages in the evolution of a cell culture to host adapted virus revealed for the first time
- Such data leads to an improved understanding of FMDV evolution and the more accurate reconstruction of transmission events

FMDV and the 'virus swarm'

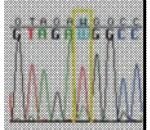
- Small genome ~ 8500 nt long
- Rapid replication rate
- Large population sizes
- High error rate of viral polymerase – *virus swarm*

The consensus and beyond

Full genome sequencing has proven to be a powerful tool for epidemiological tracing of FMD during outbreaks (UK '07)



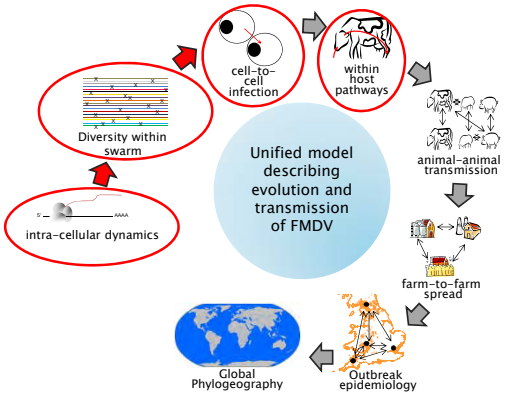
Limitations to date



- Consensus (Sanger) sequences average the sequence at any one nucleotide position
- Limited resolution achieved by cloning approaches

Problem distinguishing the genuine mutations from experiment introduced error

Why look beyond the consensus?

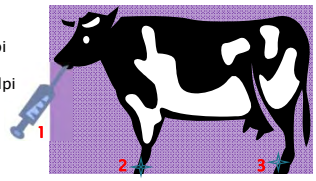


Pilot study utilising NGS

1 O_1BFS1860 (inoculum)

2 Front left foot lesion (FLF) – 2dpi

3 Back right foot lesion (BRF) – 2dpi







Sample preparation for sequencing

IAH

RNA extraction

RT

2x long PCR (overlapping fragments) **1ug** of product required

Optimisation

1 2

CCCC AAAA

L-fragment

Transportation to Glasgow

Glasgow

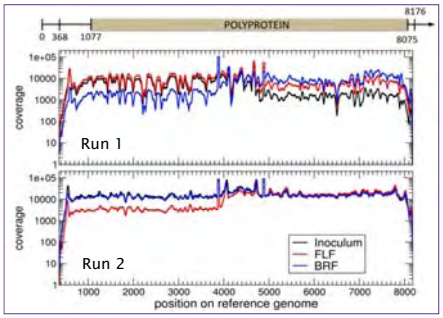
Sample preparation

Illumina sequencing platform (2x runs)



- High fidelity RT&PCR enzymes used
- Reproducibility study carried out from PCR step

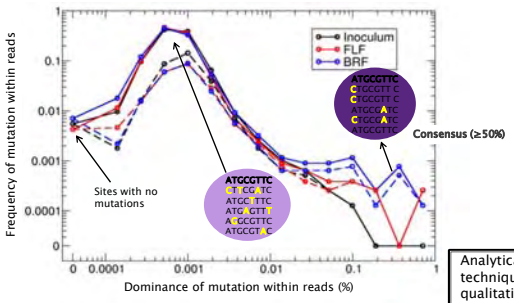
Greater resolution



Only the best quality reads (50nt) used

Raw data	Data used
• 7,190,884	⋙ 2,828,554 A (run 1)
• 10,116,147	⋙ 8,969,902 B (run 2)

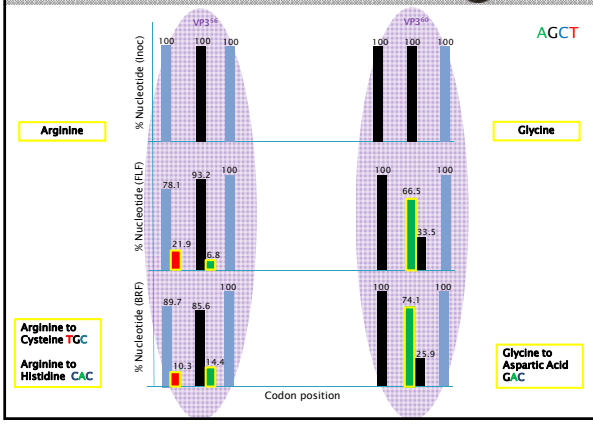
Estimates of site specific mutation (SSM)



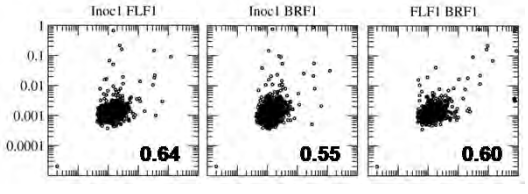
Validated STOP codons within ORF used to infer upper limit of virus intra-cellular mutation rate (μ=7.8 x 10^{-4})

Analytical techniques used to qualitatively validate sites and quantitatively assess repeatability of SSM between runs

Sites of biological significance (run1)



Genome wide correlation

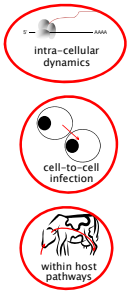


Correlation of mutation frequencies between samples for ALL qualitatively validated mutations receiving coverage above 100x in run 1

Conclusions of pilot study

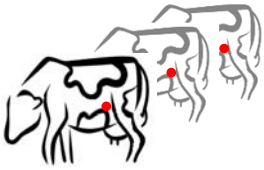
NGS demonstrates a 'step-change' improvement in the depth of detection of FMDV population diversity present within-host

- The majority of mutations are low dominance and most likely represent progeny from replication **within** cells prior to onset of any selective pressures (STOP codons within ORF)
- We hypothesize the higher dominance mutations have been selected over multiple replication rounds **between** cells
- Linkage between foot samples of higher dominance mutations (<50%) associated with heparan sulphate binding reveals intermediate stages in the evolution of a cell culture to host adapted virus



Future work

1. How does the viral swarm vary over time within a naturally infected host?



2. How does the viral swarm vary between different locations within a naturally infected host?



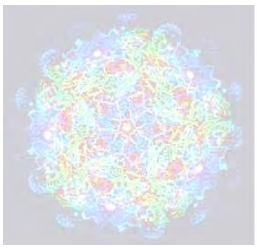
Acknowledgements

Supervisors:

Don King (IAH)
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Dan Haydon (Glasgow)

Marco Morelli (Glasgow)
Nick Knowles (IAH)
Nick Juleff (IAH)

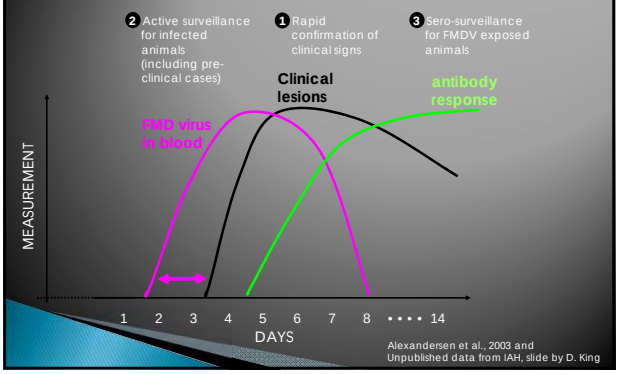
Pawel Herzyk (Glasgow)
Julie Galbraith (Glasgow)



The changing landscape of diagnostics

Wilna Vosloo, David Paton,
Emiliana Brocchi, Kris de Clercq,
Don King, Samia Metwali

Diagnostic window



Parameterization of the duration of infectious stages

- Review of serotype O experimental infections and set of regression models used
  - Latent phase: 3.1–4.8 days
  - Sub-clinical phase: 2–2.3 days
  - Incubation: 5.5–6.6 days
  - Infectious period: 3.3–5.7 days

Mardones et al, 2010

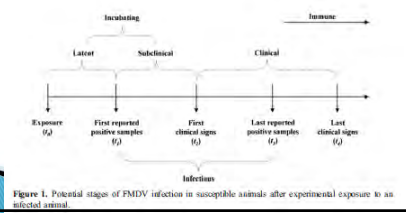


Figure 1. Potential stages of FMDV infection in susceptible animals after experimental exposure to an infected animal.

Parameterization of the duration of infectious stages

- Factors that impacted on the duration of phases:
  - Species – duration of infectiousness is longer in small ruminants than cattle
    - Latent–subclinical and incubation periods longer for small ruminants than cattle
  - Longer incubation times when exposed to infected animals from different species
    - Duration of latency and sub-clinical period longer in pigs exposed to ruminants
    - Shorter time to clinical disease in small ruminants exposed to pigs
  - Specific virus strain

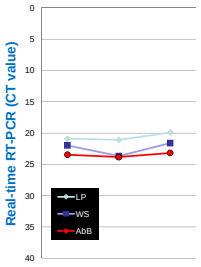
POC devices

The future of POC devices

- Policies needed for notifiable diseases
- Control over sales and distribution
- Used by competent persons
- Fit for purpose
- Rules on notification for pos and neg results
- Rules on submission of samples to labs
- Record keeping (species, age, epidemiological info, etc.)
- Validation in different countries/regions

Lateral flow devices

- Detection of FMD antigen
  - One device for 6 serotypes
  - Separate device for SAT-2
    - Ferris et al, 2009; 2010
- Retrieval from LFD for further characterisation
  - Genomic material found to be stable over 1 month
  - Potential for retrieval of larger regions to sequence



Data provided by D. King and B. Bankowski

Lateral flow devices

- Detection of FMD antibodies
  - Abs to structural proteins
  - Abs to NSPs
- Detection of antibodies to FMD NSPs
  - Device made with peptide within 3B-2
  - Limited validation: Sp 100%, Se 95%
  - Sp comparison to Ceditest and UBI ELISA: 98.6% and 96.6%

Yang et al., 2010

- IgA detection in saliva or nasal swabs?

Enzyme immunoassay membrane tests

- Comparable to a 'dry' ELISA for ag detection
  - BD Directigen™ Flu A+B
- Test is performed on membrane
- Test time ~15 minutes
- Suitable for clinical samples
- Negative results to be confirmed



Progress with the Enigma Diagnostics FL (Porton Down)

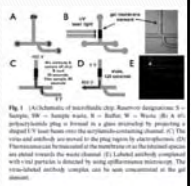
- Enigma have licence to use TaqMan® PCR technology
  - basis for routine molecular assays (FMDV, ASFV, BTV, BVDV, CSFV etc...)
- 2009-2010: Evaluation of machine's capability at IAH
  - wet assay with MagNA PURE reagents
  - equivalent performance to routine rRT-PCR assays used in the WRL
- Results in < 60 minutes
- Future project to develop a dry-down consumable for FMDV?



(D. King & M. Madi, IAH)

Rapid microchip based electrophoretic immunoassays

- Conc and detection on microfluidic chip with integratred nanoporous membrane
- Method relies on separation of labelled ab from ab/ag complex and detection via laser-induced fluorescence
- Assay for swine flu where virus is concentrated and separated
  - More sensitive
  - Reduced assay complexity and time
  - Could include secondary abs
  - 6 minute reaction time
  - 50ul sample volume



Reichmuth et al, 2008

Infrared thermography (IRT)

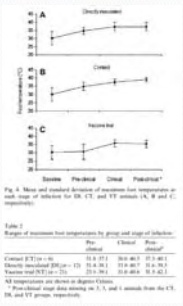
- IRT could assist in selecting infected animals for further testing
  - Decrease # of lab tests
  - Decrease # of animals handled and restrained
  - Images can be sent remotely for decisions
  - Used in combination with POC assays



Rainwater-Lovett et al, 2009

Infrared thermography (IRT)

- IRT detected increased foot temps in infected cattle
- IRT was less successful in detecting vaccinated and infected animals in the pre-clinical phase with lower temp increases
- Difficulties in determining the baseline that impacts on Se and Sp
- Limitations



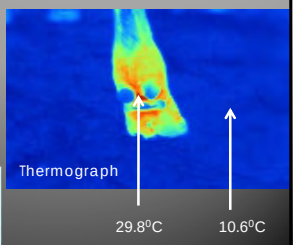
Rainwater-Lovett et al, 2009

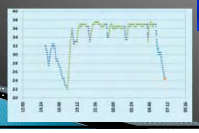
Infrared thermography (IRT)

- Measuring the temperatures of normal cattle feet with a temperature recording button and an infra red digital camera



Snapshot from digital camera:





Infrared thermography (IRT)

- Foot temperatures are greatly affected by ambient temperature and activity (e.g. lying down on straw)
- In temperate climates, thermal image derived hoof temperatures can only be used to indicate an inflammatory condition such as FMD by reference to the other feet of an animal and its herd-mates and not on the basis of a simple comparison to a threshold for normality

Poster by J. Gloster et al., IAH

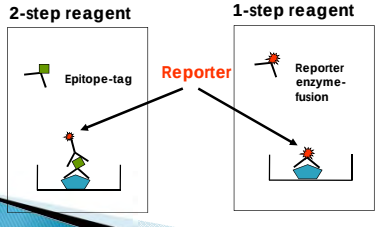
Progress/developments of 'old' ideas

'Novel' reagents for NSP ELISA

- Recombinant reagents expressed in *E. coli* (3ABC and rec abs)
  - Stable immortalised reagents, safe, cheap, consistent, well characterised, unlimited quantities, eliminate need for animals
  - Rec ab can be mutated (affinity maturation, altered specificity, reporter genes)

One step cELISA for DIVA

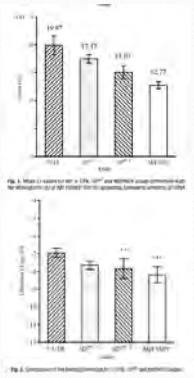
- Alkaline phosphatase (AP) reporter gene genetically fused to the scFv to generate a new FM27-AP construct
- cELISA worked well with limited number of sera, further validation is needed



Muller et al., 2010
Provided by H. Heine, AAHL

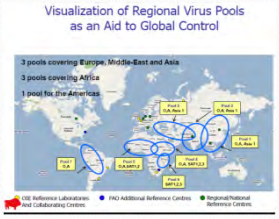
Multiplex rt-RT PCR

- Improved assays to ensure pan serotype PCR picks up all variants
- Multiplex assay with 6 3D and 2 3C targets (Tam et al., 2009)
- More sequences needed in databases



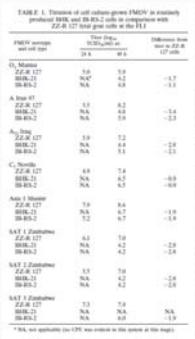
Multiplex rt-RT PCR

- Serotyping assays still needed
- Lower sens with assays covering all topotypes
- Region specific assays most promising



New cell lines

- Primary cells for virus isolation – BTY most sensitive
  - Laborious and expensive
  - Batch variation
- Cell lines preferable in some labs
  - Usual lines BHK, IBRS2
- Fetal goat tongue cell line (ZZ-R 127)
- LF-BK (bovine kidney) and MVPK (porcine kidney) cell lines showed promising performance when compared to 2^{O} LK and IBRS-2. Further evaluation needed (PIADC-FADDL)



Brehm et al, 2009

FMDV genotyping microarray

- Microarrays represent large quantities of sequence data
- Large regions of the genome can be sequence characterized using a 'low' density, multiplex array
- 8 individual samples can be tested on a single microarray slide
- Doesn't require specific primers for amplification – prior knowledge of sequence unnecessary

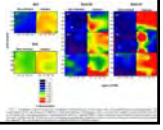
PIADC- FADDL
Roger Barrette et al., unpublished

Novel tests – the future?

Allosteric biosensors

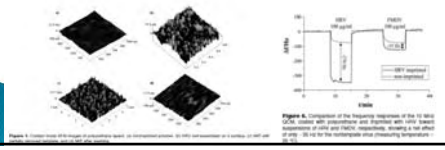
- B-galactosidase allosteric biosensor
  - Decreased enzymatic activity when foreign peptides are inserted
  - Reactivated/increased activity in the presence of Mabs or sera from infected pigs
  - Reaction conditions to perform DIVA
- Advantages of biosensors as diagnostic tools
  - Homogenous nature of assay
  - Short reaction time
  - Potential for automation
  - No need for species specific reagents
  - Potential for POC devices

Sanchez-Aparicio et al. 2009



Molecular imprinting techniques on quartz crystal microbalance

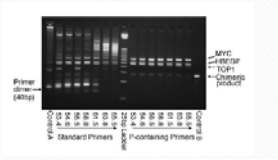
- Designed a functionalised material using MIP that interacts noncovalently with the analyte – an artificial antibody to the virus that recognises the whole virus particle
- Used MIP on HRV and found it could distinguish HRV serotypes and FMD



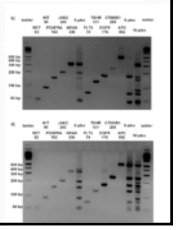
Jenik et al, 2009

Artificial genetic systems

- Artificially expanded genetic information system (AEGIS)
  - bind to each other and not to natural DNA by different hydrogen-bond patterns – 6 letter PCR
- Self-avoiding molecular-recognition system (SAMRS)
  - binds to natural DNA, but not to other members of the same SAMRS species



(Yang et al, 2010)



(Hoshika et al, 2010)

Using 'novel' markers of infection

Use of IgA to detect infection and carrier status in ruminants

- Vaccinated and infected and naïve infected cattle
  - Only carriers, whether vaccinated or not, tested positive for IgA
  - Saliva gave more consistent results than probang and nasal fluids
  - IgA in saliva correlated with persistence of virus or viral RNA in OP fluids

Parida et al, 2006

Parida, IAH, presentation

Mucosal immune responses

- Responses post vaccination
  - Vaccinated pigs had a low IgA response correlated to ag dose (> dose = > response)
- Reponses post challenge:
  - IgA response depended on immune status
  - Lasted longer in non-vaccinated and lower ag dose

Eble et al, 2007

- Vaccinated and infected pigs
  - Vaccinated pigs were negative and specific IgA could only be detected PI

Pacheco et al, 2010

Quality, validation, harmonisation

Quality control and validation

- OIE *ad-hoc* group on "Principles and methods of diagnostic test validation", OIE Paris 20-22 January 2010
  - OIE manual now has only one chapter on test validation that includes principles and methods for test validation and 7 annexes as best practice documents
    - Antibody detection tests (Ab)
    - Antigen detection tests (Ag)
    - Nucleic acid detection tests (NAD)
    - Statistical approaches to test validation including modern Non-Gold Standard methods (NGS), such as Bayesian stats
    - Method comparison or equivalence testing
    - Measurements of uncertainty
    - International evaluation of NSP tests (reference panel)

Info provided by Axel Colling, AAHL

Need for in-country validation

- USA (APHIS) when using the Prionics ELISA
  - Nonspecific reactivity on sera of small ruminants acutely infected with parapox virus (Orf)
  - Low specificity on U.S. cattle population (94%)

New MAb based kits for standardisation and harmonisation of results

- Ready to use ELISA kits for serotype specific ab detection (O, A and Asia-1)

  Brocchi et al, poster
- Ag detection kits for O, A, C and Asia-1

  Grazioli et al, presentation

Fitness for purpose

- Tests needed for different PCP stages
  - Free regions – early warning system
  - Stage 1 – determine level of virus circulation
    - Serological assays and especially NSP
    - Typing of circulating viruses
  - Stages 2 – 4: control measures with improvement of labs
    - All the above assays
    - RT-PCR
    - Further characterisation

The future role of laboratories

- Confirmation of the index case in QA environment
- POC devices may become more prevalent
  - Labs role to confirm negative/inconclusive results
  - Developing and validating devices and making recommendations
- Surveillance – high throughput (post outbreak and vaccine monitoring)
- Characterisation of disease agents
- Responsible for reagent stockpiles
- Responsible for validation, determining uncertainty of measurement and precision

Conclusions

- Lab needs to adjust its role
- POC devises need improvement, further validation and policies on use
- Ensure fitness for purpose
- High throughput needed in PCP stages
- More sensitive assays to detect infection in 'latent phase'

RESCUE OF HIGHLY PATHOGENIC FOOT-AND-MOUTH DISEASE VIRUSES FROM PRESERVED VIRAL RNA SAMPLES COLLECTED IN PAKISTAN AND AFGHANISTAN

Graham J. Belsham[1], Syed M. Jamal [1,2], Kirsten Tjørnehøj[1] and Anette Bøtner[1]*

[1]National Veterinary Institute, Technical University of Denmark, Lindholm, 4771 Kalvehave, Denmark;
[2]National Veterinary Laboratory, Park Road, 45500, Islamabad, Pakistan.

DTU Vet
National Veterinary Institute

Summary & Conclusions

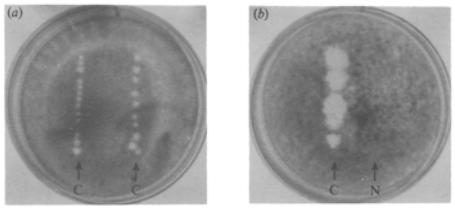
- FMD viruses of three different serotypes and multiple lineages have been rescued from preserved RNA samples derived from clinical samples collected in Pakistan and Afghanistan.
- Two of the rescued viruses were inoculated into bull calves and caused acute clinical disease in each case which spread rapidly to in-contact animals. Thus the rescued viruses were highly pathogenic.
- The availability of the rescued viruses enabled serotyping by antigen ELISA and facilitated genome sequencing.

Conclusions

- The procedure should improve the characterization of FMDVs circulating in countries where the disease is endemic and thus enhance disease control globally.

FMDV RNA is infectious

Microinjection of RNA

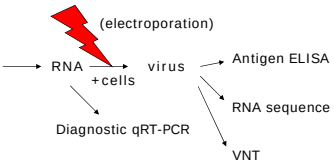


(Belsham & Bostock, 1988)

Concept of FMDV rescue system from RNA

- Clinical samples (e.g. from Pakistan, Uganda)
- Inactivation of virus but preservation of RNA
- Safe transportation
- RNA isolation (+ qRT-PCR for virus diagnosis)
- Rescue of virus
- Characterization of virus (serotype, sequence etc. - for vaccine selection)





Rescue of O-UKG/34/2001 virus from RNA isolated from bovine epithelium

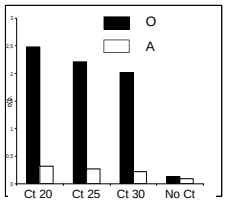
A

RNA Dilution	Ct - values	
	Qiagen	MagNa Pure
Undiluted	10.7	12.6
1:10	14.0	16.7
1:100	17.7	20.8
1:1000	21.6	24.4

B

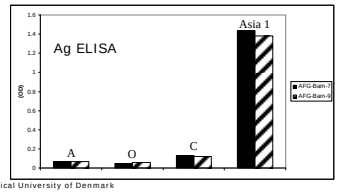
Sample	1. passage (BHK/BTY)		2. passage (BTY)	
	Day 1	Day 2	Day 1	Day 2
Ct 20	?	++	++	+++
Ct 25	?	neg	neg	+++
Ct 30	?	neg	neg	++

C



Rescue of Asia 1 viruses

Samples	qRT-PCR		Virus isolation (CPE)	
			1. passage	2. passage
	5'-UTR	3D	BHK	BTY
AFG-Bam-7/2009	19.4	16.1	neg	+++
AFG-Bam-9/2009	19.3	16.4	neg	+++
AFG-Bam-10	21.8	19.9	neg	Neg
PAK-1872	26.6	20.2	neg	Neg
PAK-1873	21.4	19.3	neg	Neg
PAK-1874	24.2	18.2	neg	Neg
PAK-1875	27.6	19.3	neg	Neg
PAK-1876	21.8	19.8	neg	Neg
PAK-1877	29.0	21.1	neg	Neg
O UKG/34/2001	20		neg	+++
Neg contol	-	-	neg	Neg



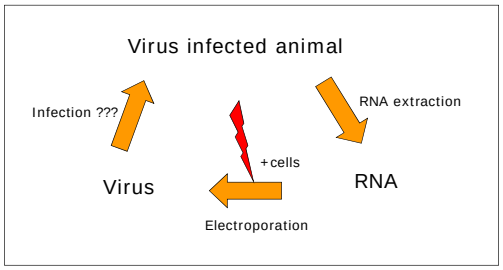
Rescue of multiple serotypes and strains

Virus strain and lineage (from VP1 sequencing)	qPCR (Ct) (5'UTR)	Virus rescued	Serotype O Ag ELISA (OD)	Serotype A Ag ELISA (OD)
Pak A-IRN05^{Bar-08}	21.8	-		
Pak O PanAsia-III	20.2	-		
Pak A IRN05^{AFG-07}	13.8	-		
Pak A IRN05^{AFG-07}	21.0	-		
Pak O PanAsia-II	17.9	-		
Pak A IRN05^{AFG-07}	19.2	-		
Pak O PanAsia-III	19.0	+	1.56	0.09
Pak A IRN05^{AFG-07}	18.7	-		
AFG A IRN05$^{AFG-07/BAK-08}$	18.2	-		
AFG A IRN05^{AFG-07}	13.7	+	0.15	1.53
AFG A IRN05^{AFG-07}	14.4	+	0.03	0.31*
AFG A IRN05^{AFG-07}	15.1	-		
AFG A IRN05^{AFG-09}	16.3	+	0.21	1.75

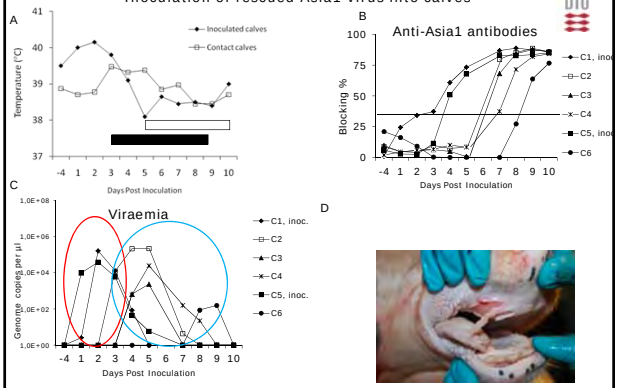
Sequence analysis

- Near complete genome sequences determined from the rescued viruses
- Complete VP1 sequences determined from both the input RNA and from the 6 rescued viruses (12 x 880 nt) - 1 nt discrepancy.

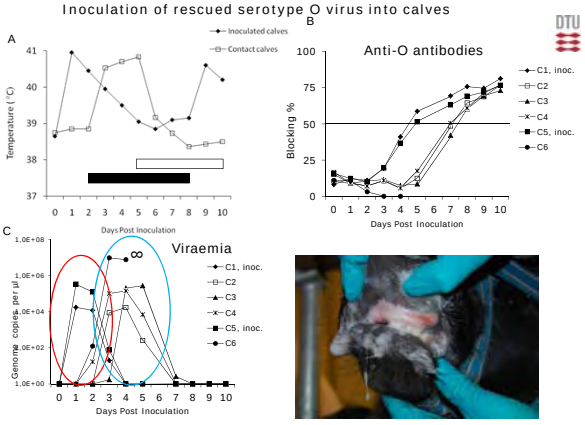
Completing the circle...



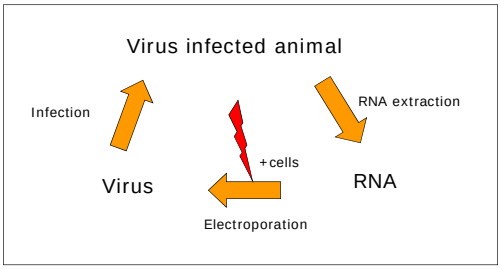
Inoculation of rescued Asia1 virus into calves



Inoculation of rescued serotype O virus into calves



The circle is completed





Development of a FMD diagnostic multiple bead immunoassay

Sandra Blaise-Boisseau, Monique Guy, Anthony Relmy, Kamila Gorna, Margot Carocci, Stéphan Zientara and Labib Bakkali-Kassimi

anses

VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER

Objectives

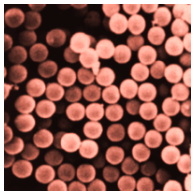
Development of a multiplex immunoassay for FMD diagnosis by using the Luminex liquid array technology

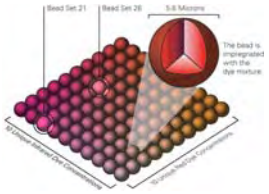
✓Simultaneous detection of antibodies against vesicular disease viruses and FMDV structural and non structural proteins

✓Serological discrimination between FMDV-infected and vaccinated animals in a single reaction

anses 2

Principles of the luminex technology (1/2)





Polystyrene microspheres

- **Ø 5,6 µm**
- **each bead is impregnated with a unique infrared/ red dye ratio**

anses 3

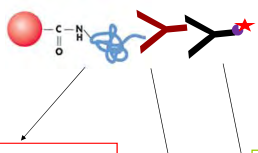
Principles of the luminex technology (2/2)



Bead ID and reporter quantity determined by laser detector

anses 4

FMD diagnostic multiple bead immunoassay (1/2)



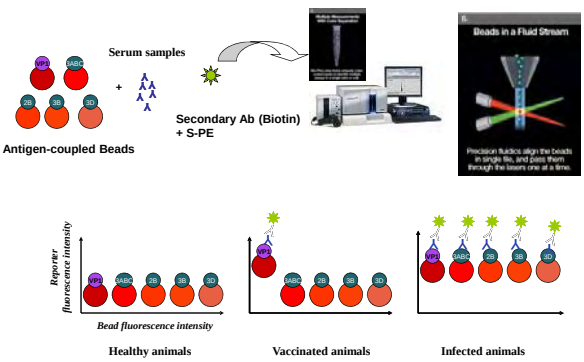
Capture molecule:
FMD SP : VP1 (of all serotypes)
FMD NSP: 3ABC, 3A, 3B,3D and peptide 2B
VSV SP
SVDV SP

Analyte:
Serum samples

Reporter molecule:
Secondary anti-species antibody, biotin labeled. + Streptavidine-Phyco-Erythrine (S-PE)

anses 5

FMD diagnostic multiple bead immunoassay (2/2)



anses 6

OPTIMIZATION OF ANTIGEN COUPLING CONDITIONS

anses 7

ANTIGEN COUPLING (1/3): Protein preparation, two different protocols

For convenience, preliminary experiments were performed using capsid protein 1D (VP1, 30kDa) from EMCV (EncephaloMyoCarditisVirus)

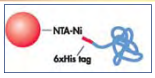
- ✓**1D protein produced in *E.coli***
- ✓**Double His tagged (NH2 and COOH extremities)**
- ✓**Protein insoluble, accumulated in *E.coli as* inclusion bodies**
- ✓**2 Purification methods**

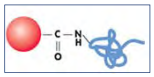
Solubilization with Urea 8M → **Buffer exchange column (PBS pH 7.4)** → **1D(U)**

Solubilization with GuHCl 6M (guanidine hydrochloride) → **Dialysis** → **Ultrafiltration and exchange buffer (PBS pH 7.4)** → **1D (Gu)**

anses 8

ANTIGEN COUPLING (2/3): Two different bead sets were compared





Liquichip Ni-NTA beads

- ✓Universal directed immobilization of 6xHis-tagged proteins
- ✓Ni-NTA-6His-Tag interaction
- ✓1µg/ coupling reaction (2.5 x 10^5 beads)
- ✓1250 to 2500 beads/assay
- ✓Protease resistant, High binding capacity

!!!Restrictions on use:

Not compatible with buffer components disturbing the Ni-NTA-6His-Tag interaction (e.g., Imidazole)

Bioplex carboxyl beads

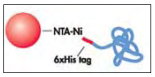
- ✓ Immobilization of 6–150 kD proteins
- ✓ carbodiimide reaction (covalent)
- ✓ 5-10µg/ coupling reaction (1.25 x 10^6 beads)
- ✓2500 to 5000 beads/assay

!!!Restrictions on use:

The protein sample must be free of sodium azide, BSA, glycine, Tris or amine-containing additives and must be suspended in PBS, pH 7.4.

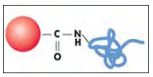
anses 9

ANTIGEN COUPLING (3/3)



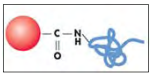
Ni-NTA<>1D (U)

Ni-NTA<>1D (Gu)



COOH<>1D (U)

COOH<>1D (Gu)



COOH<>AntiHisAb + 1D(U)

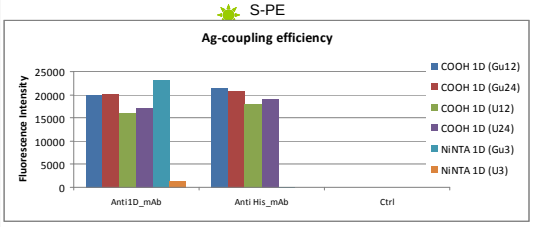
COOH<>AntiHisAb + 1D(Gu)

anses 10

Results (1/3):

Protein coupling efficiency

S-PE



% Beads Aggregats
27
30
19
17
39
14

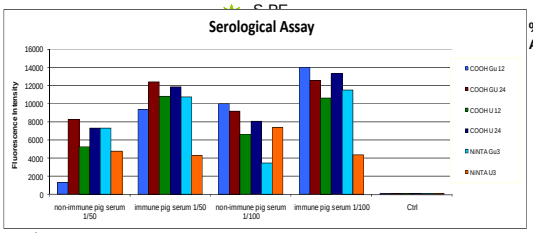
Comparable results except with NiNTA-1D (Urea).12 µg of protein (Urea or GuHCl solubilization) per coupling reaction are sufficient (use of larger quantity doesn't improve the results).Use of protein solubilized with GuHCl results in more beads aggregates.

anses 11

Results (2/3):

Serological assay using 1D-coupled beads

S-PE



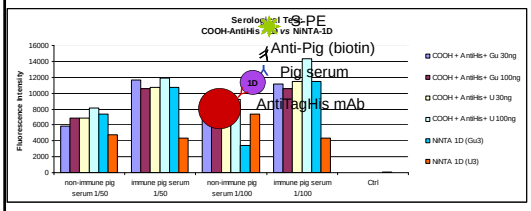
% Beads Aggregats
29
45
16
21
39
15

The best results are obtained for the serological assay using COOH-1D(Gu12) but the assay need to be improved to reduce background.

anses 12

Results (3/3):

Serological assay using AntiHis-mAb-coupled carboxyl beads
(comparison with 1D coupled NiNTA beads)



% Beads Aggregats
34
37
20
19
39
15

AntiHis-mAb-coupled COOH beads require an additional incubation step with the protein of interest and don't lead to better results than 1D coupled NiNTA beads. According to these results there's no benefit to use such coupled beads.

anses

Conclusions

- The Luminex technology permits multiplexed antibody detection from a single sample ➔ time and cost saving.
- Development of the test needs optimization of antigen production, purification and coupling
- According to these preliminary results, optimal antigen coupling conditions are as follows:

✓ Protein (12µg/30kDa, GuHCl solubilized) + Bioplex COOH beads
Or
✓ Protein (3µg/30kDa, GuHCl solubilized) + Qiagen NiNTA beads

anses
14

PERSPECTIVES…

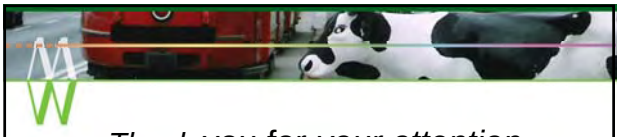
✓ Improving GuHCl solubilization protocol to increase yields of purified recombinant proteins (*currently in progress* for the structural protein VP1 of FMDV)

✓ Producing proteins of interest (***in progress)*** :

- FMD SP : VP1 (of all serotypes)
- FMD NSP: 3ABC, 3A, 3B,3D and peptide 2B
- VSV SP
- SVDV SP

✓Developing/optimizing simplex serological immunoassay for each antigen coupled beads set (***in progress)*** and then developing multiplex assays

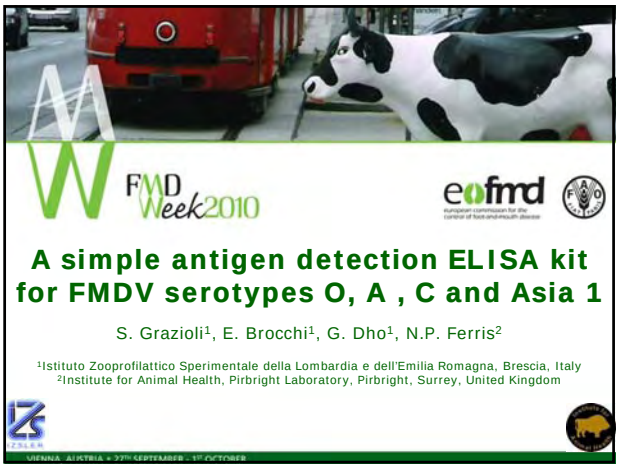
anses
15



Thank you for *your attention…*

"The research leading to these results have received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 226556. "

anses
16



A simple antigen detection ELISA kit for FMDV serotypes O, A , C and Asia 1

S. Grazioli[1], E. Brocchi[1], G. Dho[1], N.P. Ferris[2]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy
[2]Institute for Animal Health, Pirbright Laboratory, Pirbright, Surrey, United Kingdom

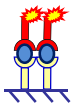
Why ready-to-use kits for MAbs-based FMDV antigen detection?

- ✓ User friendly
- ✓ Stability
- ✓ Limited assay steps
- ✓ Need for endemic countries (PCP)
- ✓ Emergencies, Reagents Bank ?
- ✓ Coverage of FMDV antigenic diversity



FMDV antigen detection kit – design sandwich ELISA – Monoclonal Antibodies

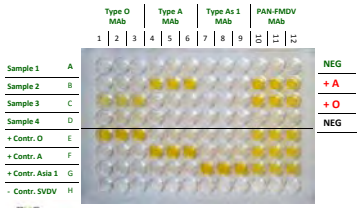
Catching MAb	Unique conjugated MAb pan-FMDV	FMDV specificity
type O	1F10	O
type A	1F10	A
type Asia 1	1F10	Asia 1
Type C	1F10	C
pan-FMDV	1F10	FMDV



DETECTOR MAb PO-conjugated

FMDV Antigen (positive sample)

CATCHING MAb (coated)



- ✓ **Stable at 5°C**
- ✓ **Customised layout**
- ✓ **FMDV control antigens already trapped onto the plate by relevant MAbs**
- ✓ **Stabilized liquid-phase reagents (conjugates, buffers, chromogen)**

Selection of appropriate catching MAbs / 1



Work flow / 1st step

- ➢ **For each of four FMDV serotypes, analysis of available panels of type-specific MAbs (30 → ≈150) with a restricted spectrum of FMDV isolates**
- ➢ **First selection of more restricted MAbs panels (5-10 MAbs) with broad intra-type reactivity for each of four FMDV serotype**

Example for type A:
150 MAbs generated against six different type A strains evaluated against 20 FMDV isolates of type A

Selection of appropriate catching MAbs / 2

Work flow/2nd step

- ➢ Analysis of pre-selected MAbs extended to a wider spectrum of FMDV isolates representative of the wide antigenic and molecular diversity within each serotype
- ➢ Type-specificity cross-checked, including SATs
- ➢ Final selection of 1-2 MAbs with the broadest intra-type reactivity
- ➢ Studies in progress to further check synergy of pooling two MAbs

FMDV Serotype	N. isolates tested	Period covered		Final selection of catching Monoclonal Antibodies						
Type A	130	1943 2010	MAbs	4D12	5F6	1C11				
			N. isolates reactive	122	112	113				
Type O	108	1950 2010	MAbs	3B11	A8	7E1	3C8			
			N. isolates reactive	105	105	105	81			
Type Asia 1	53	1954 2009	MAbs	3D8	1F10	4G6	3C6	5E10		
			N. isolates reactive	53	51	48	43	35		
Type C	33	'60s 2005	MAbs	3E9	2B1	5C4	4D7	2E5	3E5	4C4
			N. isolates reactive	32	32	32	31	30	27	27

Evaluation of diagnostic performances of the ready-to-use kit

SAMPLES → EPITHELIUM SUSPENSIONS POSITIVE BY VI / PCR

n. 136 → type 0
n. 103 → type A
n. 30 → type Asia 1
n. 29 → type C

n. 9 → type SAT 1
n. 8 → type SAT 2
n. 3 → type SAT 3
n. 4 → SVDV

TOTAL 298 Epith. Susp.

representative of different:

animal species
regions
time periods
genotype / topotype
antigenic diversity

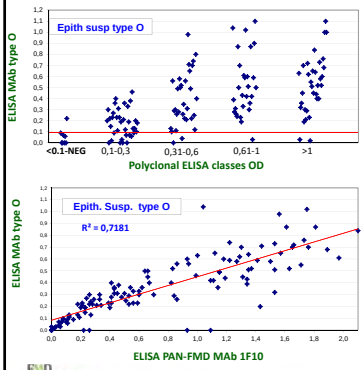
n. 14 → NVD

n. 119 FMDV isolates grown in cell cultures

Ready-to-use kit compared with Polyclonal ELISA performed at the time of sample receipt

Results of FMDV Ag detection kit

N. 136 epithelium / organ suspensions pos for type O



Type O Epith. Susp.		Type O MAb ELISA +	-	Total
ELISA polyclonal	+	115	14	129
	-	1	6	7
Total		116	20	136

CONCORDANCE 121/136 = 89%

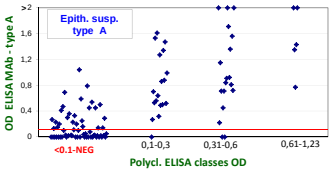
14 missed by MAb ELISA type O
- ✓ 2 from ECU 2010 (pos pan-FMD)
- ✓ 12 also neg pan-FMD ELISA (antigen deteriorated ?)

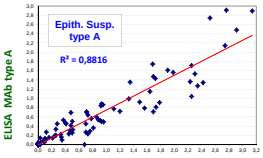
TEST	N.pos/N.tested	Se*
Polyclonal ELISA	129/136	95%
Type O Mab ELISA	116/136	85%
PAN-FMD ELISA	118/136	87%

Se* re. to VI/PCR

Results of FMDV Ag detection kit

N. 103 epithelium / organ suspensions pos for type A





Type A Epith. Susp.		Type A Mab ELISA +	-	Total
ELISA polyclonal	+	38	3	41
	-	29	33	62
Total		67	36	103

CONCORDANCE 72/103 = 69%

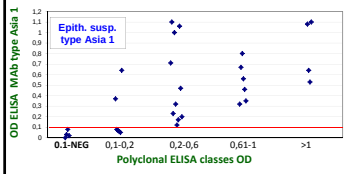
3 missed by MAb ELISA type A
- ✓ 2 borderline (positive after culture amplification)
- ✓ 1 KEN 8/2008 non detected

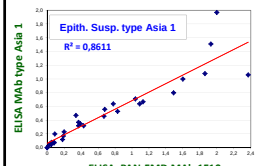
TEST	N.pos/N.tested	Se*
Polyclonal ELISA	41/103	40%
Type A Mab ELISA	67/103	65%
PAN-FMD ELISA	67/103	65%

Se* re. to VI/PCR

Results of FMDV Ag detection kit

N. 30 epithelium suspensions pos. for type Asia 1





Type Asia 1 Epith. Susp.		Type Asia 1 MAb ELISA +	-	Total
ELISA polyclonal	+	22	4	26
	-	0	4	4
Total		22	8	30

CONCORDANCE 26/30 = 87%

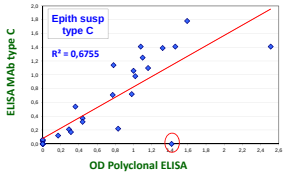
4 missed by MAb ELISA type Asia 1
- ✓ all borderline in both assays (with OD values for polyclonal ELISA ranging from 0.1 to 0.15)

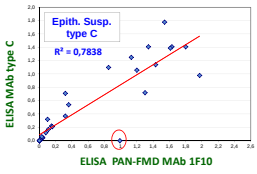
TEST	N.pos/N.tested	Se*
Polyclonal ELISA	26/30	86%
Type Asia 1 MAb ELISA	22/30	73%
PAN-FMD ELISA	21/30	70%

Se* re. to VI/PCR

Results of FMDV Ag detection kit

N. 29 epithelium suspensions pos. for type C





Type C Epith. Susp.		Type C MAb ELISA +	-	Total
ELISA polyclonal	+	19	1	20
	-	0	9	9
Total		19	10	29

TEST	N.pos/N.tested	Se*
Polyclonal ELISA	20/29	69%
Type C MAb ELISA	19/29	66%
PAN-FMD ELISA	20/29	69%

Se* re. to VI/PCR

Summary of diagnostic performances

Sensitivity

FMDV types	N.	Type-specific MAbs-based ELISAs POS	Sens.	Polyclonal ELISA POS	Sens.	Pan-FMD ELISA based on MAb 1F10 POS	Sens.
O	136	116	85%	129	95%	118	87%
A	103	67	65%	41	40%	67	65%
Asia	30	22	76%	26	90%	21	72%
C	29	19	66%	20	69%	20	69%
TOTAL Epithelium suspensions	298	224	75%	216	72%	226	76%

Specificity

- ✓ Epithelium suspensions Positive for O, A, Asia 1 and C cross-checked in heterologous tests:
  Low and inconsistent cross-reaction of type O isolates (culture grown) in MAb-based type A ELISA
- ✓ 24 SATs and SVDV + 14 Negative epith. suspensions:
  ALL NEGATIVE

Conclusions

- ✓ A simple, rapid and stable kit based on ELISA and monoclonal antibodies was developed for antigen detection and typing of four FMDV serotypes (O, A, Asia 1 and C)
- ✓ Maximum simplification was achieved thanks to use of a unique pan-FMD detector MAb combined with type-specific catching MAbs
- ✓ Diagnostic performances are similar or better (for type A) than those of the more complex polyclonal ELISA
- ✓ Perspectives of availability of end-products suited for world distribution are realistic

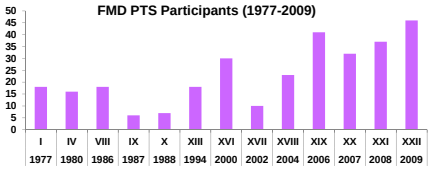
Overview of the FAO/WRL/CRL Phase XXII Studies (Proficiency Test Scheme 2009) for FMD/SVD

Yanmin Li

WRL/CRL for FMD
IAH, Pirbright Laboratory

Summary and Conclusion

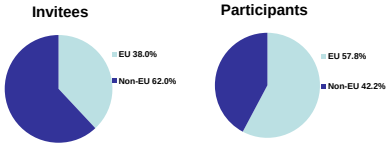
- ❑45 labs participated in PTS 2009. In general, most labs showed a good level of consistency for all tests
- ❑81%, 46%, 80% and 100% of labs meet the criteria preset for all tests from panel 1, panel 2, panel 3 and panel 4, respectively.
- ❑ 4 labs have received the panels they requested but have not reported their results
- ❑ 5 labs have not tested all samples in a panel.



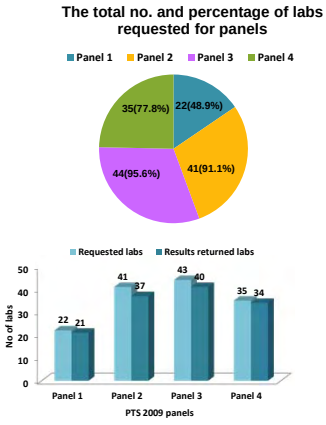
PTS 2009 - Phase XXII

- **A combined proficiency studies for FMD and SVD**
- **An advisory board was constituted: Dr Kris De Clercq (Belgium), Dr Aldo Dekker (Netherland), Dr Emiliana Brocchi (Italy) and Dr Bernd Hass(Germany) are members of the board.**
- **Four panels**
- **All samples in each panel were repeatedly tested 10 times to establish the status of the samples to set up the criteria to assess the performance**
- **Tests not specified for each panel. Each sample from each panel for each lab was uniquely coded and labelled.**

Phase XXII – Participants



Phase XXII (country/lab)	EU	Non-EU	Total
No. if invitees	26/27	42/44	68/71
No. of responses	25/26	18/19	43/45
No. of non-responses	1/1	24/25	25/26
Participants	25/26	18/19	43/45



Phase XXII-PTS 2009 Panel 1

Panel 1: Infectious material from 2 cases of suspected vesicular disease for virus detection.

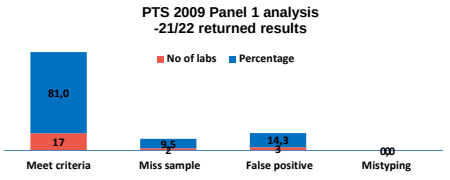
Case 1a 4 epithelial suspensions samples from cattle in a herd affected with a vesicular condition-2x O UKG 685/2007+2 x Neg.

Case 1b
2 epithelial (SVDV + Neg) and 2 faecal suspension (SVDV+Neg) samples from pigs in a herd affected with a vesicular condition.

Interpretation required – which samples are positive with what viruses in which tests and overall which cases are positive with which virus?

Panel 1 results-VI and Ag-ELISA /PCR

sample	1	2	3	4
	1a			
case	P1a-1	P1a-2	P1a-3	P1a-4
Lab Code	O UKG 685/2007	O UKG 685/2007	Neg	Neg
1	O	O	neg	neg
3	O	O	neg	neg
21				
22	O	O	neg	neg
23	O	O	neg	neg
24	O	O	neg	neg
38				
31	O	O	neg	neg



Phase XXII-PTS 2009 panel 2

Panel 2: Non-infectious material* from cattle (case a)or pigs (case b) with each originating from a different case of a herd with a vesicular condition, for virus genome/antigen detection.

Interpretation required – which samples are positive with what viruses in which tests and overall interpretation for each sample?

Case	P2a-1	P2a-2	P2a-3	P2a-4	P2a-5	P2a-6	P2b-1	P2b-2	P2b-3	P2b-4
	1	2	3	4	5	6	7	8	9	10
Sample	O IRN 34/2007	A IRN 37/2007	Asia 1 HKN 3/2005	SAT 2 BOT 2/2007	Neg	Neg	SVDV ITL 9/2008	SVDV ITL 9/2008	Neg	Neg

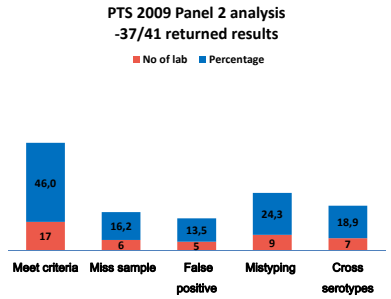
***Samples were inactivated using Binary ethyleneimine and inocuity tested by two passages in primary bovine thyroid cells with negative results.**

Ag-ELISA - Panel 2 FMD

Laboratory	O IRN 34/2007	A IRN 37/2007	Asia 1 HKN 3/2005	SAT 2 BOT 2/2007	Negative	Negative
1	O	A	Asia 1	SAT 2	neg	neg
3	O	A	Asia 1	SAT 2	neg	neg
4	O	A	Asia 1	SAT 2	neg	neg
5	FMDV pos	FMDV pos	FMDV pos	FMDV pos	neg	FMDV incon
6	SVDV neg	SVDV neg	SVDV neg	SVDV neg	SVDV neg	SVDV neg
7	O	A	Asia 1	SAT 2	neg	neg
8	O	A/Asia 1	Asia 1	SAT 2	neg	neg
9	O	FMDV pos	Asia 1	SAT 2	neg	neg
11	O	SAT 1/A cross	SAT 1/SAT 2 cross	SAT 2	neg	neg
12	FMDV pos	FMDV pos	FMDV pos	FMDV pos	neg	neg
13	O	O/A/X/SVDV	Asia 1	FMDV pos	neg	neg
14						
15	O	A	Asia 1	SAT 2	neg	neg
16	O/SAT 3	A/SAT 3/Asia 1	SAT 3/Asia 1	SAT 2/SAT 3	neg	neg
17						
47	O	A/C	Asia 1	SAT 2	neg	neg

RT-PCR data - Panel 2 FMD

lab Code	sample	1	2	3	4	5	6
	case	P2a-1	P2a-2	P2a-3	P2a-4	P2a-5	P2a-6
		O IRN 34/2007	A IRN 37/2007	Asia 1 HKN 3/2005	SAT 2 BOT 2/2007	Negative	Negative
1	FMDV 5'UTR	23.8	18.8	21.4	30.3	neg	neg
	FMDV 3D	23.9	19.3	22.1	22.8	neg	neg
	SVDV	neg	neg	neg	neg	neg	neg
	VESV	neg	neg	neg	neg	neg	neg
3	FMDV	POS	POS	POS	POS	neg	neg
	SVDV	neg	Neg/contam	neg	neg	neg	neg
5	FMDV 3D	19.3	16	17.7	21.1	neg	neg
	FMDV 3D	POS	POS	POS	neg	neg	neg
	FMDV 3D	n.t.?	n.t.?	n.t.?	inconcl.	neg	inconcl.
	SVDV 3D	neg	neg	neg	neg	neg	neg
6	SVDV 5'UTR	neg	Neg	neg	neg	neg	neg
7	FMDV 3D	17	14.4	16.5	19.5	neg	neg
	FMDV 5'UTR	25.1	18.5	20	30.9	neg	neg
	FMDV 3D	POS	POS	POS	NEG	neg	neg
8	FMDV	POS	POS	POS	POS	neg	neg



Phase XXII-PTS 2009 Panel 3

Panel 3: non-infectious material *for FMD serology

<u>Case 3a</u>
4 bovine sera from a suspected FMDV infection case in UK. The cattle showed no vaccination history.

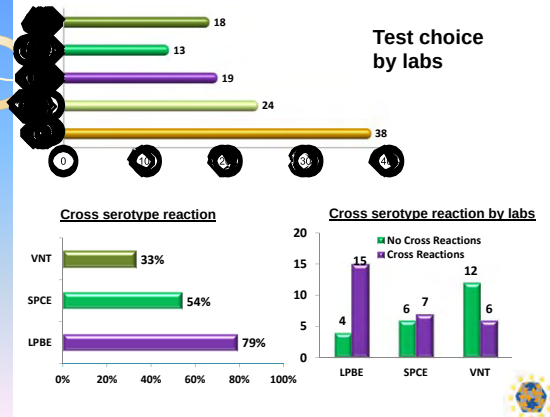
<u>Case 3b</u>
4 bovine sera from a suspected FMDV infection case in Africa. The cattle were vaccinated against FMDV O1 Manisa and SAT 2 Eritrea.

Interpretation required – which samples are positive for which virus/serotype in which tests; overall interpretation for each sample and which cases are infected with which virus?

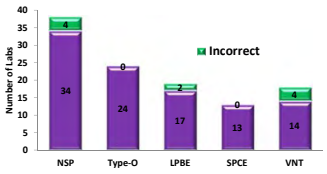
Samples were all tested 10 x by NSP ELISA, Type O ELISA, SPCE, LPBE and VNT.

Case	Sample			
	Code		Vaccinated	Infected
3a	3a-1	1	NBS	
	3a-2	2	NBS	
	3a-3	3	NBS	
	3a-4	4	NBS	
3b	3b-1	5	O1 Manisa	O1 Manisa
	3b-2	6	O1 Manisa	NA
	3b-3	7	Sat2 Eritrea	Sat2 Eritrea
	3b-4	8	Sat2 Eritrea	NA

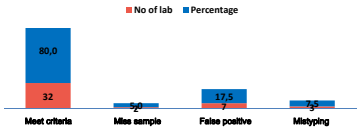
***Sera were inocuity tested by two passages in primary bovine thyroid cells with negative results and then inactivated using Binary ethyleneimine to kill any live virus not detected by inocuity testing.**



Result analysis by test



PTS 2009 Panel 3 analysis -40/43 returned results



Phase XXII-PTS 2009 panel 4

Panel 4: non-infectious material[2] for SVD serology

8 sera from pigs from a suspected SVDV historical infection case.

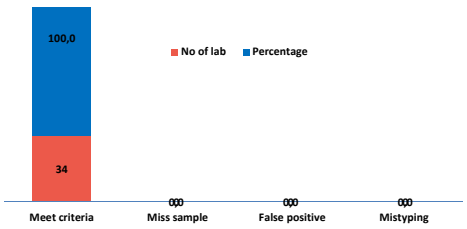
Interpretation required – which samples are positive in which tests and an overall interpretation for each sample?

All samples were tested 10x by 5B7 Mac ELISA, c70 ELISA, Isotype ELISA, PrioCHECK SVD ELISA and VNT

***Sera were inocuity tested by two passages in primary bovine thyroid cells with negative results and then inactivated using Binary ethyleneimine to kill any live virus not detected by inocuity testing.**

Sample	Origin
1	Invitrogen Negative Pig serum
2	SO11 7dpi Dil 1/2 in NPS
3	Sigma Negative Pig Serum
4	PAA Negative Pig Serum
5	VOS2 22dpi Dil 1/6 in NPS
6	SL98 6dpi Dil 1/8 in NPS
7	Farnborough Abbatoir Negative Pig Serum
8	SL82 9dpi Dil 1/6 in NPS

PTS 2009 Panel 4 analysis -34/35 returned results



Recommendation from PTS 2009

Panel 1

- ❑4 labs to improve the sensitivity of cell cultures/cross contamination
- ❑3 labs to improve the sensitivity of PCR
- ❑1 lab to improve the specificity of SVD PCR

Panel 2

- ❑11 labs to improve the Ag ELISA (10 x Sp+ 1x Sp&Sn)
- ❑6 labs to improve the PCR (2x Sn+ 4x Sp)
- ❑1 lab to establish FMDV SAT typing ELISA
- ❑1 lab to establish the FMD tests (ELISA and/or PCR)
- ❑3 lab to establish the SVD Ag ELISA and SVD PCR, respectively.
- ❑2 labs to establish the SVD tests (ELISA and/or PCR)

Recommendation from PTS 2009

Panel 3

- ❑5 labs to improve the Sp and/or Sn of the test (1 x VNT &LPBE Sp; 1 x LPBE Sp; 2x VNT Sn&Sp; 1x NSP ELISA Sp)
- ❑6 labs to establish the non-type O SP tests
- ❑7 labs to establish the SAT tests.
- ❑2 labs to establish the tests to detect the antibody against the structural protein (SP) of FMDV

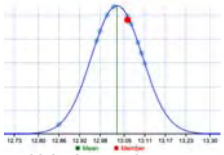
Phase XXIII-PTS 2010

- **Panel 1: infectious material from 2 cases of suspected vesicular disease for virus detection- 6 samples in total.**
- **Panel 2: non-infectious material from cattle or pig with each originating from a different case of a herd with a vesicular condition- 7 samples in total.**
- **Panel 3: non-infectious material for FMD serology: 6 bovine sera from FMD endemic country Pakistan for importation screening. The cattle were vaccinated against both FMDV vaccines A_{22} Iraq and Asia1 Shamir.**
- **Panel 4: non-infectious material for SVD serology- 6 samples in total.**
- **56 participants with 34 labs having returned back their results**

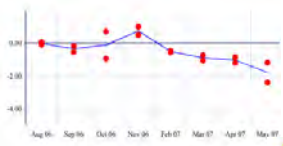
Future work – WCRLFMD interlab



The PTS organiser prepares the samples and creates a testing round on-line at the web portal. The system automatically informs members per e-mail and provides relevant information about the sample type, distribution and closing date. Once the documents ready, the participants receive their samples, test them and enter their results on-line.
After there are enough verified results are available to make statistical sense, members may immediately see their comparative stats and performance indicators. When the testing round closes, members receive their final performances per e-mail
Analysis results are archived and presented to members graphically via distribution curves, method comparisons and chronological Z-value plots. The system delivers immediate turnaround.



Interlab distribution graph. Mean in green, logged in users see their own value in red



Acknowledgements

Everyone for participating the studies

Phil Keel, Ginette Wilsden, Pip Hamblin, Nigel Ferris, Geoff Hutchings, Don King, Valerie Mioulet, Miki Madi, Bartek Bankowski, Sheila Wilsden, Elisabeth Wilson,

Paul-Michael Agapow,

David Paton and Jef Hammond

WRL/CRL, IAH, Pirbright Laboratory

Defra, EC and FAO

THANK YOU

Institute for Animal Health

USE OF PRIOCHECK® FMDV-NS ELISA AND PRIOCHECK® FMDV TYPE O ELISA IN UGANDA.

Tjørnehøj, K., Elsohaby, I., Ayebazibwe, C., Mwiine, F.N., Stenfeldt, C., Sangula, A.K., Balinda, S.N., Alexandersen, S.

Department of Virology, Lindholm
National Veterinary Institute
Technical University of Denmark



DTU Veterinærinstituttet

Conclusions:

- **Non-purified vaccines used in e.g. Uganda elicits antibodies measurable in the PrioCHECK® FMDV-NS.**
- **The usage of the PrioCHECK® FMDV TYPE O ELISA for screening for antibodies against FMDV cannot be recommended in populations where the SAT-serotypes of FMDV are prevalent.**

Uganda:
- Chris Ayebazibwe
- Frank Mwiine
- Sheila Balinda
- Abraham Sangula.



- Rose Ademun Okurut
- Vincent Muwanika
- Charles Masembe

- Martin Esau



Denmark:
- Ibrahim Elsohaby
- Carolina Stenfeldt

- Soren Alexandersen
- Graham Belsham
- Hans Siegismund
- Anette Bøtner

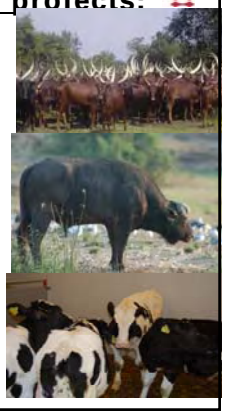
- Jane Borch
- Jani Christiansen
- Jonna V. Jensen





Based on data from two projects:

- **Field data:**
  - Livestock Wildlife Diseases in East Africa Project (LWDEA) funded by the Danish International Development Agency (DANIDA).
- **Experimental study:**
  - National veterinary institute, Technical university of Denmark.



Objective:

- **Examine the use of PRIOCHECK® FMDV-NS ELISA AND PRIOCHECK® FMDV TYPE O ELISA for screening Ugandan field sera for antibodies against FMDV.**
- **Hypothesis: possible to identify FMDV-infected animals in an endemic area in an easy and convenient way using two commercially available antibody ELISAs.**

Antibodies against NSP	Antibodies against SP	Expected conclusions:
-	-	No exposure
-	+	Vaccination
+	+	Infection
+	-	Depending on kinetics of antibodies and sesitivities of tests

Field work:

- **Samples:**
  - **Cattle:**
    - 2006
    - Kasese District
    - Post-outbreak sampling (serotype O)
    - Acute, subacute or healing lesions
    - The majority of herds vaccinated with non-purified trivalent vaccine (O, SAT 1, SAT2) >7 months before sampling
  - **Buffalo:**
    - 2001-2008
    - Random sampling
    - 4 Ugandan national parks
    - No vaccinations

Cattle in Kasese District

Screening for antibodies against FMDV after 2006 outbreak.

Mwiine et al., 2010, TBED.

Herd	No. of samples tested	PRIOCHECK® FMDV-NS ELISA % positive	PRIOCHECK® FMDV TYPE O ELISA % positive
Ks2	6	83	100
Ks3	8	88	88
Ks4	27	81	93
Ks5	21	71	67
Ks6	16	44	94
Ks7	4	100	100
Ks8	14	93	79
Ks9	13	100	100
Ks10	9	100	100
Ks11	19	84	95
Ks12	30	**63**	**7**
Ks13	2	50	50
Ks15	3	33	100

Herd Ks12:

Serotype-specificity of antibodies against FMDV.

PRIOCHECK® FMDV-NS ELISA	PRIOCHECK® FMDV TYPE O ELISA	Serotype-specific ELISAs for antibodies against FMDV serotypes. No. pos./no. tested			
No. pos/no. tested	No. pos/no. tested	O	SAT 1	SAT 2	SAT 3
17/20	2/20	0/19	3/16	15/15	11/13

Serotype-specific antibody titres in herd Ks12.

PRIOCHECK® FMDV-NS ELISA	PRIOCHECK® FMDV TYPE O ELISA	Antibody titre for serotype: O	SAT 1	SAT 2	SAT 3
+	-	nd	-	40	20
+	-	-	-	20	10
+	-	-	10	10	nd
+	-	-	-	40	10
+	-	-	-	10	40
+	-	-	-	20	20
+	-	-	-	20	-
+	-	-	640	20	10
+	-	-	inc.	40	10
-	-	-	20	10	20
+	-	-	-	nd	nd
-	+	-	-	10	80
-	-	-	-	20	nd
+	-	-	-	20	inc.
+	-	-	inc.	80	160
+	-	-	-	40	160

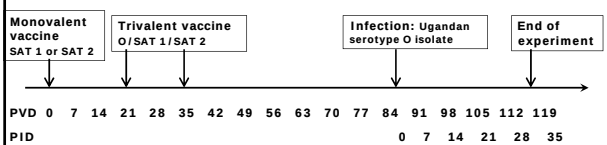
Screening for antibodies against FMDV in Ugandan buffalos.

Ayebazibwe et al., in preparation.

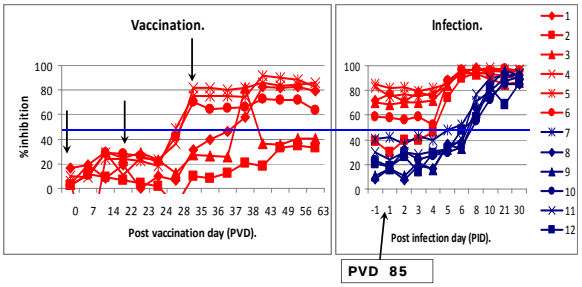
National park	No. of samples tested	% positive in PRIOCHECK® FMDV-NS ELISA	% positive in PRIOCHECK® FMDV TYPE O ELISA
MFNP	53	96	19
LMNP	18	94	6
KVNP	39	92	14
QENP	108	92	27

Experimental study:

- **Vaccination:**
  - 6 steers in 2 groups
  - 1*monovalent (SAT 1 or SAT 2) + 2*trivalent (O/SAT 1/SAT 2)
- **Infection:**
  - 6 vaccinated steers + 6 age-matched naive steers
  - Ugandan serotype O isolate



Antibodies against FMDV measured with the PRIOCHECK® FMDV-NS ELISA:



Conclusions:

- **Non-purified vaccines used in e.g. Uganda elicits antibodies measurable in the PrioCHECK® FMDV-NS.**
- **The usage of the PrioCHECK® FMDV TYPE O ELISA for screening for antibodies against FMDV cannot be recommended in populations where the SAT-serotypes of FMDV are prevalent.**

Evaluation of Lateral Flow Device 'Svanova' for Detection of FMD Virus During Course of Infection



Samia Metwally, DVM, PhD

Foreign Animal Disease Diagnostic Laboratory (FADDL)
USDA, APHIS, VS, NVSL
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Safeguarding Animal Health

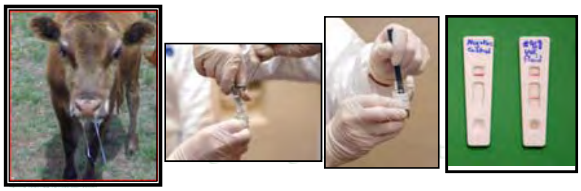
Diagnosis of FMD on Farm

- The use of 'Point of care' or 'Lateral Flow Device, LFD' diagnostic test would have the advantage of providing support to foreign animal disease diagnosticians while conducting a field investigation.
- Early identification of positive farms for immediate response should FMD occur in the U.S.
- Increase FMD awareness and improve epidemiological information.

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Svanova ® LFD

- Svanova LFD has shown overall 84% sensitivity and 99% specificity in detection of FMDV in archived vesicular tissues from field submissions (Ferris et al., 2009 & 2010).
- To enhance performance, two LFDs were produced to use in tandem to cover the seven serotypes.
- Results can be obtained in less than 30 min.



Overarching Goals

Aims:

- Determine the performance of Svanova® LFD on fresh clinical samples representing the major FMDV serotypes and subtypes,
- Determine window of detection during the course of infection in bovine and swine,
- Determine the diagnostic sensitivity during FMD epidemics,
- Determine diagnostic specificity in the US animal population.

Deliverables:

- To define the overall performance characteristics and application:
  - When to use in the field,
  - Test limitation, recommendation and guidance,
  - National Veterinary Stockpile acquisition.

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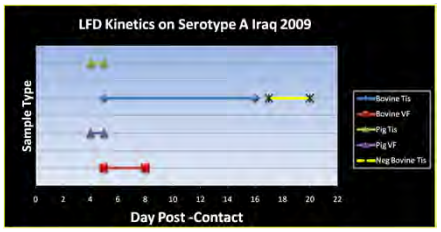
Kinetics of Detection on Serotype O Israel 2008



- One inoculated cattle by IDL and 4 contacts
- One inoculated pig by ID in heel bulb and 4 contacts
- Sequential samples were collected until lesions were healed

- **Bovine:** clinical signs developed in contact animals as early as 1 dpc and lesions healed within 8 days. One bovine died from myocarditis at 6 dpc. LFD positive on vesicular fluid and tissues 0-5 day-old lesions (1-8 dpc)
- **Pigs:** mild clinical signs that healed 6 dpc. Three of 4 pigs died acutely from myocarditis 2-3 dpc. LFD positive on vesicular fluid and tissues on 0 (2-4 dpc) and 0-3 (5-6 dpc) day-old lesions, respectively

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- Vesicular lesions lingered in bovine but healed quickly in pigs
- **Bovine:** LFD positive on 0-2 day-old vesicular fluid (5-8 dpc), 1-11 day-old vesicular tissues (5-16 dpc), and negative on 15 day-old foot scab (20 dpc)
- **Swine:** LFD positive on 2-3 day-old vesicular fluid and foot epithelium (4-5 dpc)

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Kinetics of Detection on serotype A24 Cruzeiro











- Domestic swine: LFD positive on 0- 9 day-old (1-10 dpc) vesicular tissues
- Feral swine: LFD positive on 0- 10 day-old vesicular tissues (3-12 dpc) and 0-4 day old vesicular fluid (4-6 dpc). Vesicular tissues were negative on 14-19 dpc (11-15 day-old) lesions

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Overall LFD Analytical Sensitivity in Bovine and Swine

Serotype	Bovine			Swine		
	No. Tested	No. Positive	% Sensitivity	No, Tested	No, Positive	% Sensitivity
O	24	22	92	15	14	93
A	19	18	94.7	16	16	100
Total	**43**	**40**	**93**	**31**	**30**	**98.9**

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LFD Limit of Detection Against AgELISA & rRT-PCR

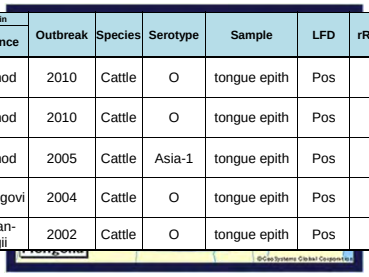
- A/Iraq/2009: Vesicular fluid of bovine tongue

Dilution	AgELISA	LFD	rRT-PCR
$10^{-3.3}$	Positive	Positive	Positive
$10^{-4.3}$	Positive	Weak Positive	Positive
$10^{-5.3}$	Negative	Negative	Positive

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Clinical Samples from Outbreaks 2002-2010 in Mongolia

Origin Province	Outbreak	Species	Serotype	Sample	LFD	rRT-PCR
Dornod	2010	Cattle	O	tongue epith	Pos	Pos
Dornod	2010	Cattle	O	tongue epith	Pos	Pos
Dornod	2005	Cattle	Asia-1	tongue epith	Pos	Pos
Dornogovi	2004	Cattle	O	tongue epith	Pos	Pos
Bayan-Ulgii	2002	Cattle	O	tongue epith	Pos	Pos



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Diagnostic Sensitivity - FMD Outbreak in Mongolia 2010







Origin Province	Species	Sample	LFD	rRT-PCR
Sukhbaatar	Cattle	tongue epith	Pos	Pos
Sukhbaatar	Cattle	tongue epith	Pos	Pos
Sukhbaatar	Sheep	tongue epith	Pos	Pos
Sukhbaatar	Cattle	tongue epith	Pos	Pos
Sukhbaatar	Gazelle	tongue epith	Pos	Pos
Sukhbaatar	Gazelle	Saliva, swab	Negative	Negative
Dornod	Cattle	tongue epith	Pos	Pos
Dornod	Cattle	tongue epith	Pos	Pos
Dornod	Camel	tongue epith	Pos	Pos
Sukhbaatar	Cattle	tongue epith	Pos	Pos
Sukhbaatar	Cattle	tongue epith	Pos	Pos
Sukhbaatar	Camel	tongue epith	Pos	Pos
Sukhbaatar	Sheep	Saliva, swab	Negative	Negative

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Conclusions

- LFD correctly identified positive samples up to 11 day-old vesicular tissues from bovine and 10 day-old vesicular tissues from swine
- Preliminary analytical sensitivity was 93% for bovine and 98.9% for swine
- LFD limit of detection was comparable to AgELISA but lower than rRT-PCR
- Diagnostic sensitivity was 100% on tongue epithelium (N= 16) that were collected from bovine, gazelle and camel in outbreak in Mongolia 2010. Limited number of swabs and saliva from sheep and gazelle tested negative
- A training video on the use of LFD was prepared

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In the Pipeline

- Continue to determine diagnostic sensitivity– 2010 outbreak in Pakistan
- Testing additional serotypes to fine-tune the window of detection per species
- Determine diagnostic specificity on US animal population
- Determine LFD on oral swabs from small ruminants while the vesicular lesions are not apparent as well as swabs of oral lesion in bovine and swine

Safeguarding Animal Health

Acknowledgments

- **FADDL:**
  - Erin Mulhern
  - Andy Fabian
  - Emily O'Hearn
  - Dr. Fawzi Mohamed
  - Dr. Mike McIntosh
  - Dr. Bill White
- **Jeff Babcock and animal care taker staff at PIADC**
- **State Central Vet Lab, Ulaanbaatar, Mongolia**
  - Dr. Sodnomdarjaa
  - Dr. Dashko Bold
- **Dept of Homeland Security- PIADC**
  - Dr. Bruce Harper
  - Dr. David Brake
  - Dr John Neilan

Safeguarding Animal Health

Thank you for your Attention



National Agro Bio Defense Facility (NBAF), Manhattan, KS

June 2018

Safeguarding Animal Health

DIAGNOSTIC PERFORMANCE OF TWO COMMERCIALLY AVAILABLE CELL VIABILITY ASSAYS IN FOOT-AND-MOUTH DISEASE RESEARCH

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Dr. David Lefebre[1]
Dr. Johan Neyts[2]
Dr. Kris De Clercq[1]

[1] *Unit of Vesicular and Exotic Diseases, Department of Virology, CODA-CERVA-VAR, Groeselenberg 99, 1180 Ukkel, Belgium*
[2] *Department of Microbiology and Immunology, Rega Institute, KULeuven, Minderbroedersstraat 10, 3000 Leuven, Belgium*

Short Summary

- Comparison Microscopy vs. Viability assay
- Compounds & Redox reactions
  - Principles
  - Protocols
  - Assay characteristics
- Assays with Cell TiterBlue ®
  - Virus Neutralisation Test
  - Antiviral screeninng assay
- Final Conclusion

Conclusions

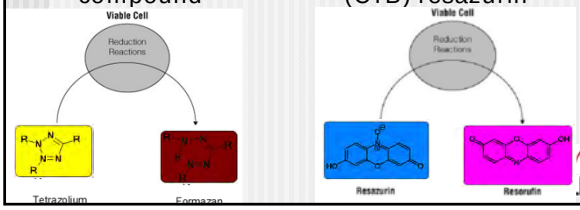
- All compared cell viability assays are suitable and fit for purpose
  MTS (absorbance), CTB_{fluo} (fluorescence), CTB_{abs} (absorbance).
- CPE can be determined by a cell viability assay and colourimetric reading
- Colourimetric reading has advantages with respect to high throughput screening

Microscopy vs. Viability assay

- Microscopy
  - Variability
    - Interpretation
    - Tiring reading
  - Data generation:
    + Qualitative
    - Difficult manual data entry (PC)
- Cell Viability assay
  - Variability
    + Objective interpretation
    + Automated reading
  - Data generation:
    + Quantitative
    + Automated storage (PC)

Redox Reaction

- Indirect Cell Viability Assay
- Viable cells reduce dye to substrate
- Linear range until 5h incubation time
- MTS tetrazolium compound
- CellTitre Blue® (CTB) resazurin



Testing principles

- Old medium is exchanged for new medium containing the viability dye
- Incubation time (1h30)
- Read/evaluate Colour production

MTS	CTB_{fluo}	CTB_{abs}
Absorbance 490 nm	Fluorescence Ex: 560 nm Em:590 nm	Absorbance 570 nm - 600 nm



Basic Protocol



Incubation (1h)



Incubation (48 h)

Microscopic Reading

&

Cell viability assay

- Virus and serum OR cells and antiviral compound
- Addition of cells OR virus
- 2 days incubation before reading & cell viability assay

Assay characteristics

Summary of test characteristics of three cell viability assays to determine CPE.

Assay	MTS	95% CI	CTB_{fluo}	95% CI	CTB_{abs}	95% CI
N° of wells	576	-	576	-	672	-
C/O[a] (%)	75.0	-	75.0	-	70.0	-
Z'-factor	0.755	0.599 - 0.912	0.853	0.811 - 0.895	0.854	0.768 - 0.941
ACC[b]	0.988	0.963 - 0.996	0.984	0.962 - 0.993	0.982	0.962 - 0.991
Precision	1.000	0.989 - 1.000	0.977	0.952 - 0.989	0.989	0.970 - 0.996
κ[c]	0.974	0.952 - 0.986	0.969	0.942 - 0.984	0.964	0.937 - 0.980
Sensitivity:	96.8	93.5 - 98.4	99.3	97.5 - 99.8	97.8	96.7 - 98.9
Specificity:	100	98.9 - 100	97.5	94.9 - 98.8	98.7	96.7 - 99.5

a: C/O= cut-off point; b: ACC= accuracy; c: κ= Cohen's Kappa index.

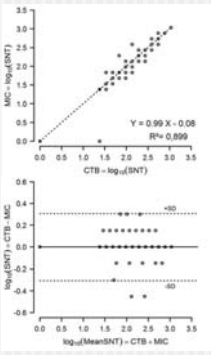
Assay characteristics

	MTS_{abs}	CTB_{fluo}	CTB_{abs}
ACC	0.988	0.984	0.982
Presicion	1.000	0.977	0.989
Sensitivity	96.8	99.3	97.8
Specificity	100.0	97.5	98.7

- Characteristics of all viability asays are comparable
- CTB_{abs} handling advantages and is the most cost effective assay
- CTB_{abs} futher developed and validated for the VNT and antiviral screening assay

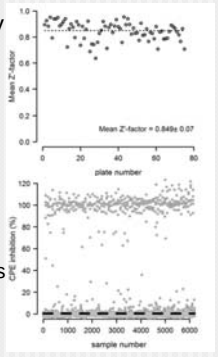
Virus Neutralisation test with CTB® Abs

- Diagnostic sensitivity & specificity
  - 84 infected and vaccinated and infected cattle
  - 190 naïve cattle
  - sensitivity: 98.9% (95% CI: 93.5% - 99.8%)
  - specificity: 100% (95% CI: 98.1% - 100%)
  - R^2 : 0.899
  - Log_{10}(SNT) difference < 0.3



Anti viral screening assay with CTB® Abs

- Z'-Factor for repeatability
  Z'-factors >0.5 : GOOD
  - Z'-factor range: 0.64 to 0.95
  - Mean Z'-factor : 0.85 (95% CI: 0.83- 0.86%)
  - Positive controls and cell controls ≥ 90 % colouring
  - Negative controls and virus controls ≤ 3 % colouring



Conclusions

- All compared cell viability assays are suitable and fit for purpose
  MTS (absorbance), CTB_{fluo} (fluorescence), CTB_{abs} (absorbance).
- CPE can be determined by a cell viability assay and colourimetric reading
- Colourimetric reading has advantages with respect to high throughput screening

Thanks for your attention



Compounds

- MTS (Promega, Leiden, The Netherlands)
  CellTiter 96® AQueous One Solution Cell Proliferation Assay
  (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
- CTB (Promega)
  CellTiter-Blue® Cell Viability Assay
  Resazurin

COMPARISON OF TWO ONE-STEP REAL-TIME RT-PCR ASSAYS FOR FMDV DIAGNOSIS

[1]Valérie Mioulet, [2] Abdallah Traore, [1] Mikidache Madi, [1] Geoff Hutchings, [1] Nigel Ferris, [1] Don King

[1]Institute for Animal Health, Ash Road, Pirbright, Woking, Surrey GU 24 0NF, UK.
[2]Laboratoire Central Vétérinaire, Bamako, Mali

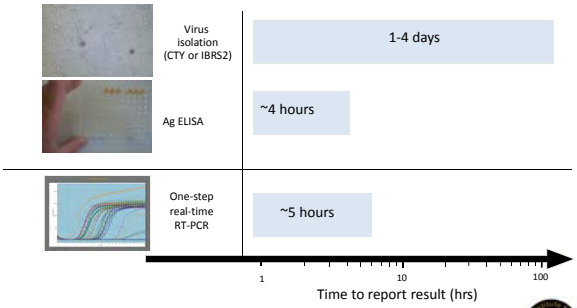
Summary of study

Objective:
Compare 2 real-time RT-PCR (rRT-PCR) assays currently in use over range of samples (2007-2010).

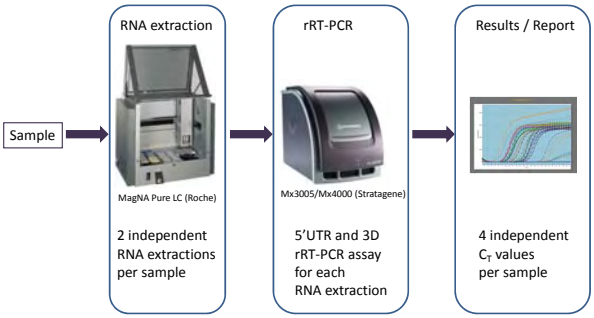
Results:
rRT-PCR more sensitive than traditional diagnostic methods.

3D rRT-PCR assay detects a greater number of isolates than 5'UTR rRT-PCR assay.

Laboratory assays for FMDV detection

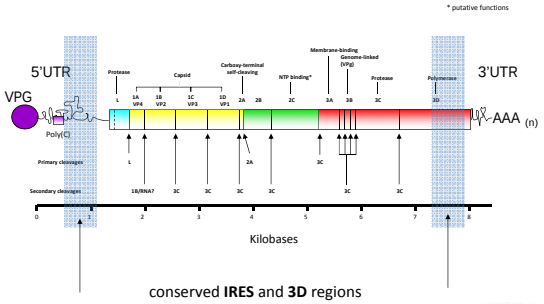


Sample preparation for rRT-PCR

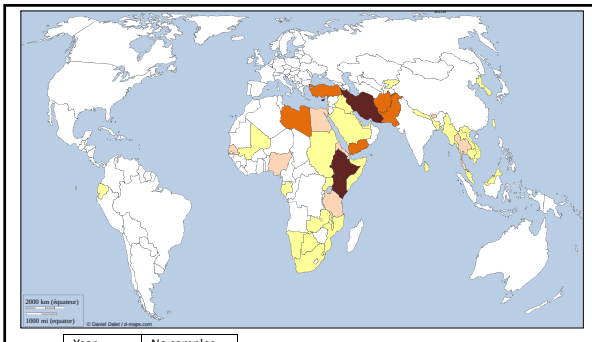


Foot-and-Mouth Disease Virus

Assay targets:



conserved **IRES** and **3D** regions
targets for pan-serotype reactive assays



Year	No samples
2007*	759
2008	330
2009	997
2010	850
Total	2936

samples<50
50<samples<100
100<samples<200
samples<200

52 countries

* samples from the UK 2007 outbreak were not included

Summary for submissions 2007 - 2010

	Sample No	%
No virus detected	1098	37.4
FMDV positive	1838	62.6
Total reported	2936	100

FMDV Positive = Samples positive by at least 1 test (VI, Ag ELISA or rRT-PCR)

Assay comparison

		rRT-PCR 3D and 5' UTR assays [1]		
		Genome detected	32 < Ct [2] < 50	Negative
Virus isolation	Virus detected	1211	16	3
	Negative	571	161	657

1. Both rRT-PCR assay are treated as independent diagnostic assays
2. Based on average Ct of each assay

Assay comparison

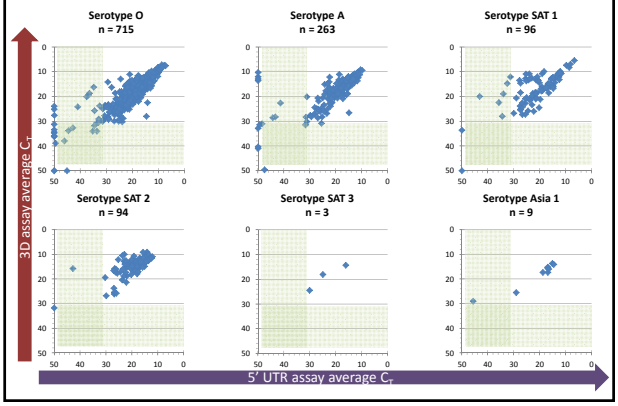
rRT-PCR 3D and 5' UTR assays [1]

WRL batch	Isolate name	Sample type	Serotype	Virus isolation
WRLFMD/2008/00016	KEN 4/2008	Epithelium	O	BTY1 48 hours
WRLFMD/2008/00006	PAK 74/2006	Swab	O	BTY3 24 hours
WRLFMD/2008/00009	ETH 18/2007	Probang	SAT 1	BTY1 48 hours
WRLFMD/2007/00018	AFG 37/2007	Fluid	O	BTY1 24 hours
WRLFMD/2009/00053	BAN 27/2009	Epithelium	O	BTY1 48 hours
WRLFMD/2009/00055	BHU 20/2009	Epithelium	O	BTY1 24 hours
WRLFMD/2009/00055	BHU 23/2009	Fluid	O	BTY1 48 hours
WRLFMD/2007/00005	IRN 7/2007	Epithelium	O	BTY1 48 hours
WRLFMD/2009/00051	KEN 65/2009	Probang	O	BTY1 48 hours
WRLFMD/2009/00051	KEN 66/2009	Probang	O	BTY1 48 hours
WRLFMD/2008/00006	PAK 66/2006	Swab	O	BTY1 48 hours
WRLFMD/2007/00019	PAK 15/2007	Epithelium	O	BTY1 48 hours
WRLFMD/2007/00019	PAK 32/2007	Epithelium	O	BTY1 48 hours
WRLFMD/2007/00019	PAK 34/2007	Epithelium	O	BTY1 48 hours
WRLFMD/2009/00003	IRQ 10/2009	Epithelium	A	BTY2 24 hours
WRLFMD/2008/00013	PAK 73/2007	Epithelium	A	RS1 48 hours
WRLFMD/2009/00036	PAK 17/2009	Epithelium	A	BTY1 48 hours
WRLFMD/2009/00036	PAK 18/2009	Epithelium	A	BTY1 48 hours
WRLFMD/2008/00009	ETH 19/2007	Probang	SAT 1	BTY1 48 hours

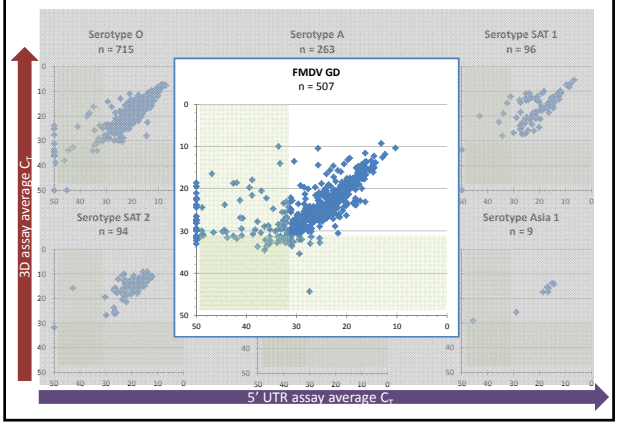
32 < Ct [2] < 50	Negative
16	3
161	657

Currently no sequence data for these isolates.

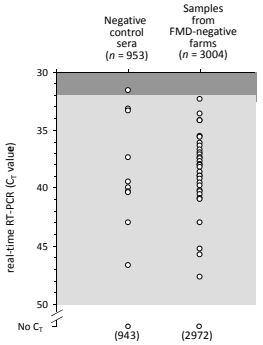
rRT-PCR assays comparison



rRT-PCR assays comparison



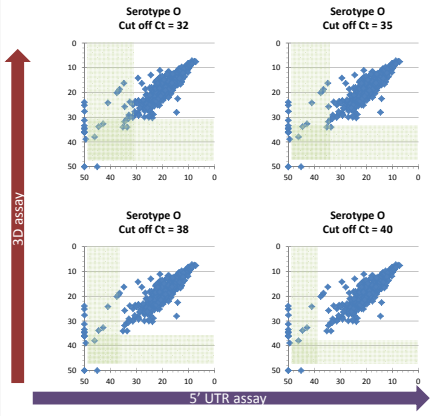
rRT-PCR Cut-off values: positive or negative?



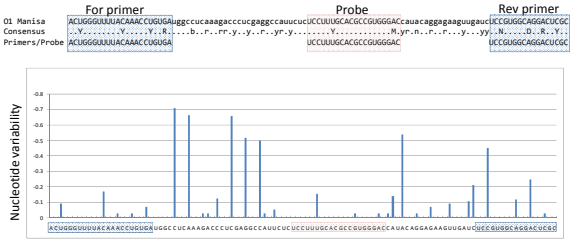
Absolute specificity	99.0 %	98.9 %
Specificity (using defined assay criteria)	99.9 %	100 %

(Shaw *et al.*, 2007)

rRT-PCR Cut-off values

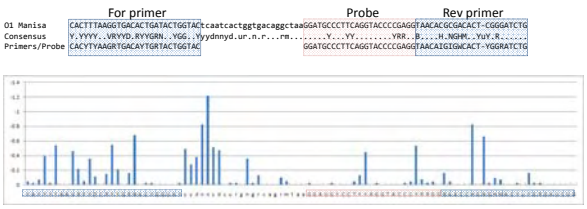


3D Assay: variations within PCR product



(based on alignment of 221 sequences)

5'UTR Assay: variations within PCR product



(based on alignment of 235 sequences)

Ongoing and future work

- Sequencing of rRT-PCR outliers ongoing
- Develop and improve new FMDV molecular assays?
- Serotyping rRT-PCR – currently limited, under development
- Are 2 rRT-PCR assays necessary as routine diagnostics (WRL *vs* NRL)?

Comparison of tests that detect Persistent FMDV Infection in Cattle

Jitendra K Biswal[1], Can Cokcaliskan[1&2], Antonello Di Nardo[1], Sahara Rai[1], Helen Ambrose[1], Philippa Grainger[1], Katja Ebert[1], Unal Parlak[2], Musa Alkan[2], Fuat Ozyoruk[2], *Geraldine Taylor*[1], *David J Paton*[1] & ***Satya Parida***[1]

[1]*Institute for Animal Health, Pirbright, UK*

[2] SAP FMD Institute, Ankara, Turkey

Carrier

1. Vaccinated animals may become infected with or without signs of disease
2. Continuing virus circulation or virus carriers must be identified in any vaccinated population
3. Recovery of FMD Viruses from oro-pharynx after 28 days of post infection is considered as carrier
4. Serosurveillance can be used to help substantiate freedom from infection in vaccinated population
5. Stamping out/Vacc/NSP-serosurvey may take 6 months to help substantiate freedom from infection
6. NSP serosurveillance detects infection in vaccinated herd, but not exclusively the FMD carriers
7. Alternative strategies are required to detect infection in vaccinated animal
8. Results indicate that IgA is a reliable indicator of FMDV replication in oro-pharynx.

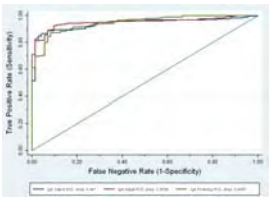
Validation of IgA test for FMDV O serotype and comparison of test efficacy with NSP test for detection of carrier

Animal Experiments	Clinically infected/ vaccinated challenged animals	Vaccinated carriers detected by VI+RT-PCR	Carriers detected by Cedi-NSP test	NSP seroconversion by Cedi test	Carriers detected by IgA test	Carriers concordantly detected by both Cedi and IgA test	Carriers detected by either Cedi or IgA test or both
UV	0/20	9	7	10	8	7	9
UY	0/20	3	3	7	3	3	3
VH	5/20	9	9	18	9	9	9
VD/VE	6/20	11	10	12	9	8	10
	11/80	**32 (100%)**	**29 (90.62%)**	**47 (146.87%)**	**29 (90.62%)**	**27 (84.37%)**	**31 (96.87%**

ROC Comparative Analysis

Test of Equality

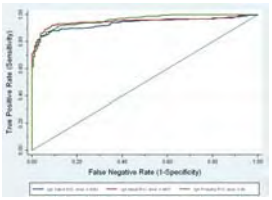
Vaccinated control



	ROC Area	SE	95% CI
IgA Saliva	0.941	0.0129	0.916 – 0.966
IgA Nasal	0.951	0.0112	0.929 – 0.973
IgA Probang	0.945	0.0120	0.923 – 0.969

$X^2 = 0.56$ $P=0.751$

Unvaccinated control



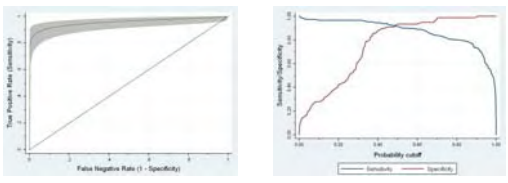
	ROC Area	SE	95% CI
IgA Saliva	0.936	0.0118	0.913 – 0.959
IgA Nasal	0.949	0.0110	0.927 – 0.970
IgA Probang	0.960	0.0080	0.944 – 0.976

$X^2 = 4.08$ $P=0.130$

IgA Nasal – Vaccinated Control

Assessing cut off by Maximum-likelihoods ROC model

Cut-off	Se	Sp	Correctly Classified	LR+	LR-
10 PP	92.31%	90.79%	91.78%	10.0220	0.0847
15 PP	90.21%	93.42%	91.32%	13.7119	0.1048
20 PP	88.81%	94.74%	90.41%	16.7413	0.1255
25 PP	83.92%	97.37%	88.58%	31.8880	0.1652
30 PP	81.12%	98.68%	87.21%	61.6504	0.1913
35 PP	**79.72%**	**98.68%**	**86.30%**	**60.5875**	**0.2055**
40 PP	78.32%	98.68%	85.39%	59.5245	0.2197
45 PP	73.43%	98.68%	82.19%	55.8042	0.2693
50 PP	69.93%	100%	80.37%	-	0.3007



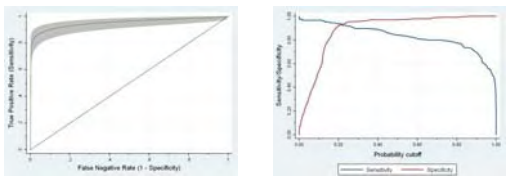
Index	Estimate	SE	95% CI
ROC Area	0.960	0.0124	0.936 – 0.985
$\delta(m)$	4.504	0.7520	3.030 – 5.978
d_e	2.678	0.2091	2.268 – 3.088
d_a	2.482	0.2055	2.079 – 2.885

Log-likelihood = -591.402 AIC = 1244.804

IgA Nasal – Unvaccinated Control

Assessing cut off by Maximum-likelihoods ROC model

Cut-off	Se	Sp	Correctly Classified	LR+	LR-
10 PP	90.91%	76.32%	85.84%	3.8384	0.1191
15 PP	88.11%	92.11%	89.50%	11.1608	0.1291
20 PP	86.71%	97.37%	90.41%	32.9510	0.1365
25 PP	80.42%	98.68%	86.76%	61.1189	0.1984
30 PP	79.02%	98.68%	85.84%	60.0560	0.2126
35 PP	**75.52%**	**98.68%**	**83.56%**	**57.3987**	**0.2480**
40 PP	70.63%	98.68%	80.37%	53.6784	0.2967
45 PP	61.54%	98.68%	74.43%	46.7693	0.3897
50 PP	53.15%	98.68%	68.95%	40.3916	0.4748

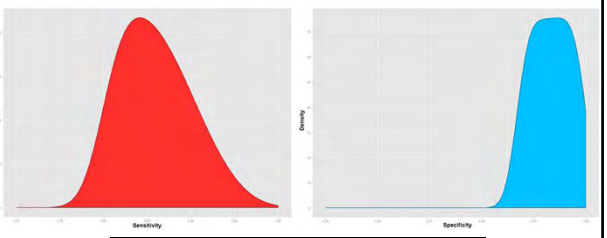


Index	Estimate	SE	95% CI
ROC Area	0.952	0.0132	0.926 – 0.978
$\delta(m)$	4.501	0.5772	3.370 – 5.633
d_e	2.565	0.1844	2.203 – 2.927
d_a	2.356	0.1887	1.986 – 2.726

Log-likelihood = -870.569 AIC = 1803.139

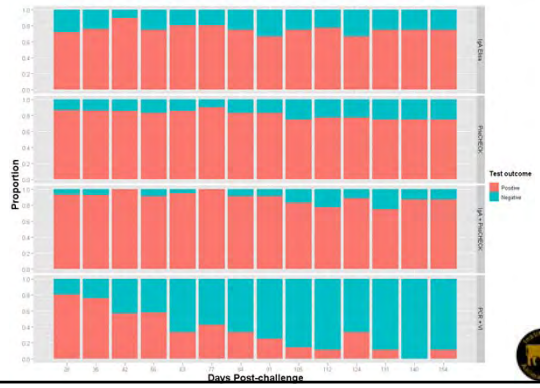
IgA Elisa Parameters Evaluation

Bayesian framework using probabilistic constrains



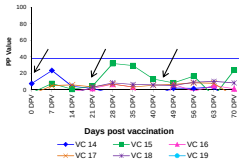
	Median	SD	95% BPI
Se	0.854	0.0396	0.801 – 0.944
Sp	0.993	0.0038	0.987 – 1
LR+	688.8	0.0399	65.83 – 2634
LR-	0.142	16770	0.056 – 0.201

Tests performance comparative analysis

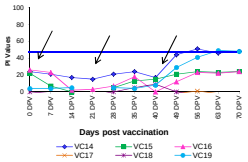


Comparison of serum and mucosal antibody response in multiply vaccinated cattle

Anti-FMDV IgA antibody



Anti-NSP antibody



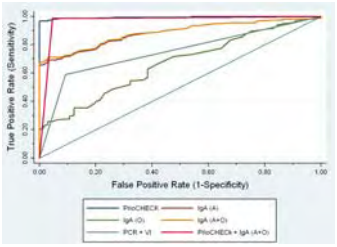
Analysis of cattle field sera from Turkey

		Total No/No Tested	% of Detection [95% CI]
PrioCHECK	+ve	157/226	69.47% [63.11% - 75.16%]
	-ve	69/226	30.53% [24.48% - 36.89%]
IgA (A)	+ve	99/228	43.42% [37.09% - 49.98%]
	-ve	129/228	56.58% [50.02% - 62.91%]
IgA (O)	+ve	32/229	13.97% [10.03% - 19.14%]
	-ve	197/229	86.03% [80.86% - 89.97%]
IgA (A + O)	+ve	102/230	44.53% [38.01% - 50.88%]
	-ve	128/230	55.65% [49.12% - 61.99%]
PrioCHECK + IgA (A+O)	+ve	162/232	69.83% [63.57% - 75.43%]
	-ve	70/232	30.17% [24.57% - 36.43%]
PCR + VI	+ve	101/232	43.53% [37.25% - 50.03%]
	-ve	131/232	56.47% [49.97% - 62.75%]
	Infected 161/232 [69.4%]		*Not-infected 71/232 [30.6%]*

		No/No TOT	% of Detection [95% CI]
PrioCHECK	+ve	155/161	96.88% [92.67% - 98.70%]
	-ve	69/71	96.97% [88.75% - 99.25%]
IgA (A)	+ve	98/161	62.03% [54.16% - 69.30%]
	-ve	69/71	98.57% [90.42% - 99.80%]
IgA (O)	+ve	31/161	19.62% [14.11% - 26.61%]
	-ve	70/71	98.59% [90.55% - 99.80%]
IgA (A + O)	+ve	101/161	63.52% [55.71% - 70.68%]
	-ve	70/71	98.59% [90.55% - 99.80%]
PrioCHECK + IgA (A+O)	+ve	158/161	98.14% [94.34% - 99.40%]
	-ve	67/71	94.37% [85.84% - 97.88%]
PCR + VI	+ve	95/161	59.01% [51.20% - 66.39%]
	-ve	65/71	91.55% [82.35% - 96.18%]
	Infected 161/232 [69.4%]		*Not-infected 71/232 [30.6%]*

IgA Elisa

ROC Comparative analysis (test of equality)



	ROC Area	SE	95% CI
PrioCHECK	0.994	0.0039	0.986 – 1
IgA (A)	0.883	0.0217	0.840 – 0.925
IgA (O)	0.664	0.0381	0.589 – 0.739
IgA (A+O)	0.886	0.0214	0.844 – 0.928
PrioCHECK + IgA (A+O)	0.970	0.0139	0.943 – 0.998
PCR + VI	0.749	0.0268	0.696 – 0.801

X^2 = 179.91 P=0.000

Conclusions

- NSP antibody tests could identify infection in vaccinated population
- NSP antibody tests detects past infection, but not exclusively carrier animals
- Salivary mucosal IgA detection test may be considered as a potential DIVA test for screening the persistently infected animals after application of vaccinate-to-live policy.
- Parallel NSP and IgA test may be useful to increase the sensitivity and specificity
- IgA test may be useful to use as a confirmatory test to NSP test to detect carrier

Acknowledgements

- Nigel Ferris
- Paul Barnett
- Sarah Cox

Funding

- Defra, UK
- Commonwealth Scholarship Commission, UK
- DISCONVAC, FP7
- EPIZONE





Characteristics of some of FMD outbreaks in Iran

N.Rasouli Beirami

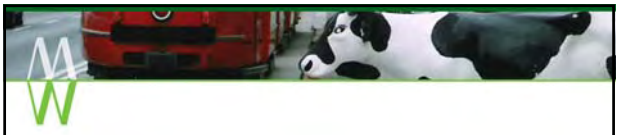
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER



Outbreaks in Jam County (Bushehr Province)

- Report of lambs and kids mortality in Jam County in the middle of Feb 2010
- Visiting the area in 27 and 28th of Feb. 2010
- At the end of January a nomadic sheep herd settled in the pastures around SarCheshme village.
- After few days death in the herd began, one of the signs being lameness,(Approx. 60 adults and lambs & kids deaths out of 650 animals)
- A friend and animal keeper from SarCheshme helped the nomads to deal with deaths
- After a few days the disease appeared in his herd in the village
- Later on most of the other herds in the village affected including cattles
- FMD vaccination on 28th Jan.

slide title (5/20)



summary

-In the last months of 2009 and first half of 2010 FMD outbreaks in Iran increased considerably.

- The most important reasons for the outbreaks are:
  - animal movement (Herds belonged to Nomadics, movement for fattening purposes, smuggling animals from neighbouring countries)
  - not observing biosecurity

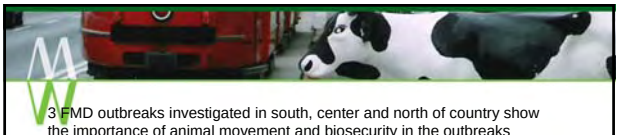
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Outbreaks in Jam County (Bushehr Province) Cont.

•About 15 Feb , a farmer from Cori Hayati village bought 2 goats from SarCheshme (60 kms far from each other)
•He added them to his herd at the outskirt of the village
•2 days later disease and mortality began in his herd
•At the day of visiting (27 feb) all the cattles had FMD lesions in the mouth and hooves, some fmd vesicles in the hooves of goats
•FMD vaccination on 2nd feb. cattle, 12th feb. for sheep & goat
•The rest of the village was free of the clinical disease
•In necropsy of 2 adult goats, entrotoxemia and pulpy kidney

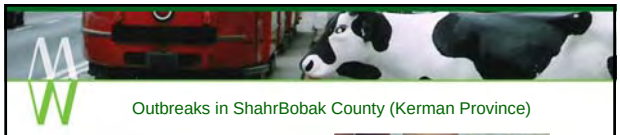
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3 FMD outbreaks investigated in south, center and north of country show the importance of animal movement and biosecurity in the outbreaks



slide title (5/20)



Outbreaks in ShahrBobak County (Kerman Province)

•Reports of kids and lambs mortality related to Fmd in the province,
•ShahrBobak the most affected county
•Visiting the area on 20 &21st of April 2010
•Investigating in 3 villages and 4 semi nomadic sites
•Only in 1 semi nomadic and 1 village (Chah-e-Shohadaye Gong) clinical signs of fmd observed
•Most were ppr





slide title (7/20)

FMD outbreak in Chah-e- Shohadaye Gong

.About 20 families lived in the village
.All had few cattles (Confined and feeding at home) and some sheep and goats feeding in 2 separate herds in the pastures around
•One herd of sheep and goats belonging to 5 family had lameness, the cattle belonged to them had lesions of fmd
•The other herd had no lameness, in the cattles no clinical fmd

Conclusion

1. Animal movement and not observing strict biosecurity are the main causes of FMD outbreaks
2. There must be strict epidemiological studies about the outbreaks
3. In the 3rd phase of EU-FMD project which recently signed a lot of epidemiological studies expected

Outbreaks in Sari County (Mazandaran Province)

•Repoirting FMD outbreaks after vaccination, condemning vaccine (similar reports from other provinces)
•Visiting ShirinBull village,
•A day after vaccination a lot of clinical disease and mortality appeared,
•the disease was present in the village before vaccination
•Maybe infected needles caused the intensity of the disease ?
•The same history in the next village (Toghdar)

•In West Azarbayjan and Lorestan provinces they stopped sheep and goat vaccination for the same reason.

Acknowledgments

Dr Ghesari for PPR pictures.



Thank you for your attention

FMDV-A/IRN/2005 endemics in WestEurasia: estimation of coascelent events in the population history

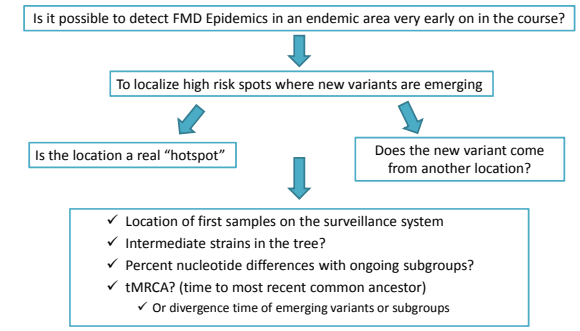
F. Özyörük, Ü.Parlak, M.Fırat Saraç
Foot-and-Mouth Disease Institute
Ankara, Turkey

Summary

- Bayesian coalescent approach offers a statistical model for estimating the TMRCs (time to most recent common ancestor) of subgroups.
- Epidemiological and evolutionary data were combined and the result indicated that the pandemic A/IRN/05 was existed one year before causing the reported epidemiological events in Iran and Turkey.
- Phylogenetic reconstruction of A/IRN/05 sequences revealed two major groups (ARD-07 and EZM-07) compatible with the previously denoted groups and multiple novel subgroups descendent of ARD-07.

Conclusion

- Time between major coalescent events is ~2 years in A/IRN/05 genotype.
- Multiplicity of emerging variants is an important constraint in tracing the origin.
- TMRCAs, when combined with phlyogenetic and geographical data, have potential to differentiate whether a new strain emerged in a surveillance system or imported.
- Time between divergence and detection could reflect the detection performance of a surveillance system.



Goals

- To identify coalescent events in the population history
- To estimate the time between divergence and detection of FMDV strains
- To exemplify the potential value of the method in FMD epidemiology

Facts about AIran05

•FMDV A/IRN/05 has been a dominant type A genotype since 2005 in WestEurasia

•First official report was from Qom-Iran in April 2005,

•Simultaneous spreading to Saudi Arabia, Pakistan and Turkey in December 2005.

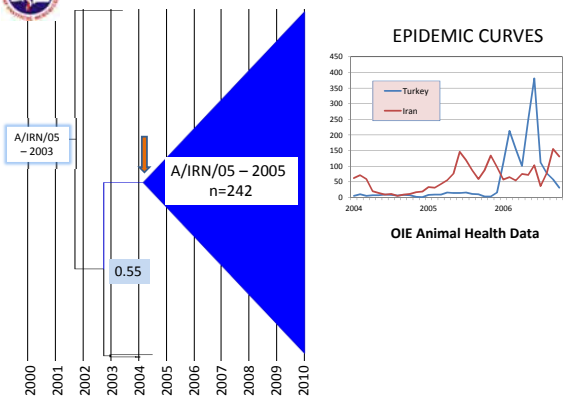
•Further spread of variants to Middle East and Libya in 2008-2009.

•Vaccine strains were changed twice. First A96 → A22, then A22 → homologous strain.

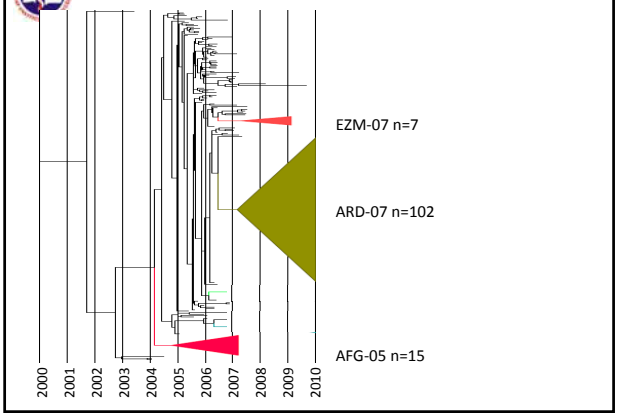
Material and Approach

- FMDV-VP1 sequences were produced at FMDI or downloaded from NCBI.
- 279 isolates having time-stamp with week segments (0,019= one week)
- Collection dates: between 1964.981-2010.058
- Countries: Turkey (215), Iran (41), Afghanistan (4), Pakistan (4), Iraq (4), India (4), S. Arabia (2), Bahrain(2), Syria (1), Jordan (2)
- Genotypes: A/IRN/05 (242), A22 (2), A99 (9), A96 (12), Others (14)
- Bayesian Markov Chain Monte Carlo approch and BEAST software was used to analyse molecular sequences.
- GTR substitution model, Relax molecular clock model and exponential growth tree prior were chosen.
- All analysis were performed on genogroups having posterior value > 0.9

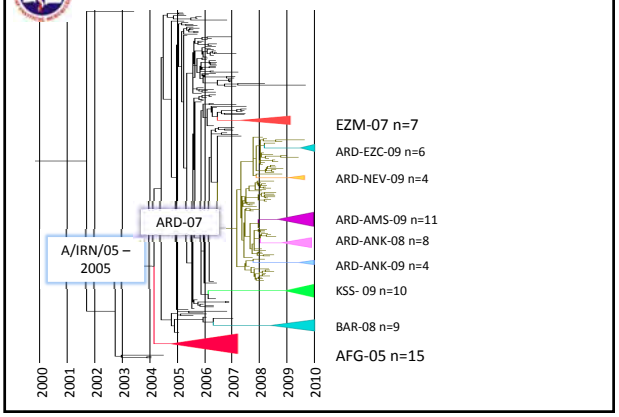
Coalescent Events in A/IRN/2005 pandemic lineage



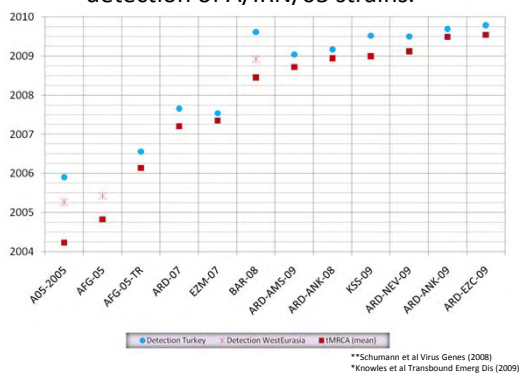
Coalescent Events in A/IRN/2005 pandemic lineage



Coalescent Events in A/IRN/2005 pandemic lineage



Estimated time between divergence and detection of A/IRN/05 strains.



**Schumann et al Virus Genes (2008)
*Knowles et al Transbound Emerg Dis (2009)

Tracing the origin of KSS-09

(estimated time between tMRCA and detection : 6.4 months)

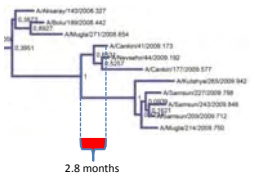


Tracing the origin of ARD-ANK-08

(estimated time between tMRCA and detection : 2.8 months)







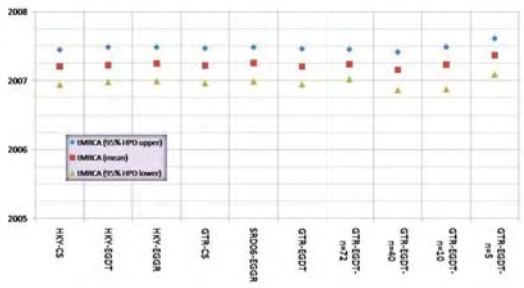
Acknowledgement

- Nick Knowles - IAH
- People who develop open access materials and tools such as;
  - FMD Molecular Epidemiology reports by WRLFMD
  - FMDBIOPORTAL by UC Davis
  - NCBI-Nucleotide by NIH
  - BEAST software
  - ClustalX software
  - WAHID by OIE
  - Google Earth
- People in FMD Institute
- Support

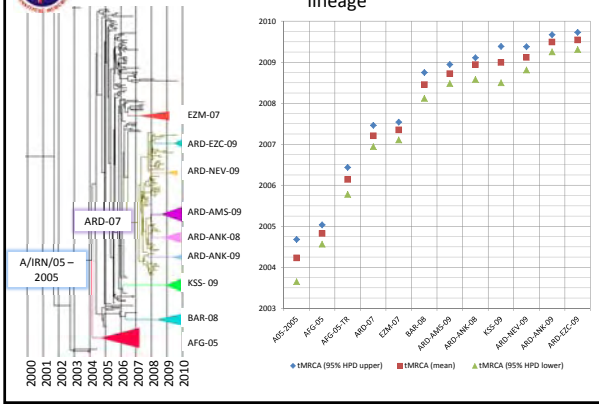
Sensitivity of the tmrca estimates (ARD-07)

Models (substitution-tree) n=102

Reducing the number of sequences backward in time



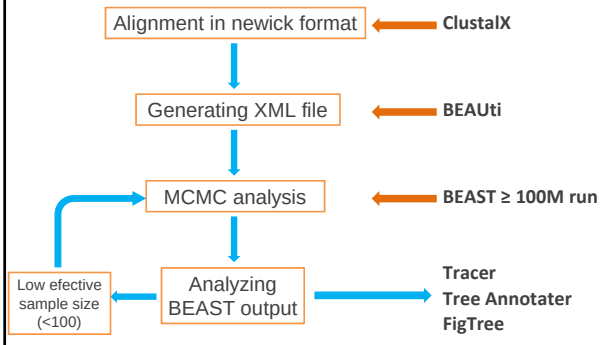
Estimates of Coalescent Events in A/IRN/2005 pandemic lineage



Model Combinations

Abbreviations	Substitution and site heterogeneity	Clock Model	Tree prior	Growth parameter
HKY-EGDT	Haseg awa-Kishino-Yano-γ invariant sites	Relaxed Uncorrelated lognormal dist.	Coalescent: Exponential growth	Doubling time
HKY-EGGR	Hasegawa-Kishino-Yano-γ invariant sites	Relaxed Uncorrelated lognormal dist.	Coalescent: Exponential growth	Growth rate
GTR-CS	General Time Reversible-γ invariant sites	Relaxed Uncorrelated lognormal dist.	Coalescent: Constant size	
GTR-EGDT	General Time Reversible-γ invariant sites	Relaxed Uncorrelated lognormal dist.	Coalescent: Exponential growth	Doubling time
SRD06-EGGR	Hasegawa-Kishino-Yano-γ invariant sites	Relaxed Uncorrelated lognormal dist.	Coalescent: Exponential growth	Growth rate
HKY-BSP	Hasegawa-Kishino-Yano-γ invariant sites	Relaxed Uncorrelated lognormal dist.	Coelescent: Bayesian skyline	

Experimental Flow of Bayesian MCMC analysis



FMD Week2010

MOLECULAR INVESTIGATION OF RECENT OUTBREAK OF THE TYPE O FMD IN IRAN

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[3]Iran Veterinary Organization

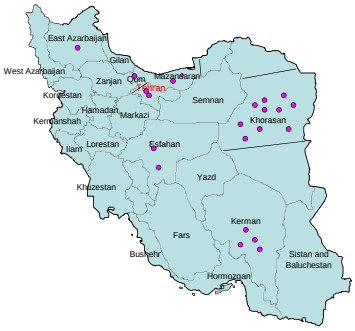
Summary and conclusion

Iran FMD outbreaks during late 2009:

affected cattle, sheep and goats, both commercial and small scale animal holdings, even recently vaccinated animals.

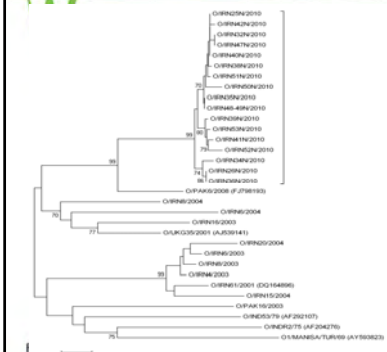
FMD serotype O viruses detected and phylogenetic studies with sequence information used to develop and apply serotype specific assays to achieve Rapid and Sensitive serotype identification method within a region.

FMD in Iran, 2009-2010



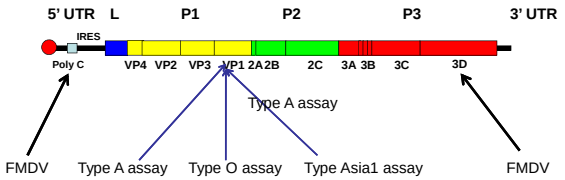
22 different epithelium samples, suspect FMD cases, collected from different geographical points of Iran

Tested at National Veterinary Institute, Lindholm FMDV was isolated from 18 of the 22, all identified as serotype O by antigen ELISA and from VP1 coding sequence (following RT-PCR)



Phylogenetic relationships among FMDV O isolates collected in Iran 2010(software MEGA 4.0,Model;Neighbor-joining). Phylogeny based on the nucleotide sequences of part of the VP1 coding sequence.

Strategy for serotype specific qRT-PCRs



Two step	3D assay	Ct (type O)	Ct (type A)	Ct (type Asia1)	
A TAI 4/2003	31.14	No Ct	No Ct	No Ct	
O UAE 2/2003	26.56	39.40	No Ct	No Ct	
A IRN 5/2003	27.43	No Ct	No Ct	No Ct	
SAT 2 KEN 2/2002	28.74	No Ct	No Ct	No Ct	←
Asia 1 HKN 5/2005	26.55	No Ct	No Ct	26.02	
A IRN 31/2005	32.53	No Ct	31.63	No Ct	
A IRN 51/2005	26.02	No Ct	21.99	No Ct	
O IRN 48/2006	27.08	23.96	No Ct	No Ct	
A TUR 7/2007	27.68	No Ct	No Ct	No Ct	
Asia 1 MYA 1/2005	21.04	No Ct	No Ct	26.37	
A IRN 37/2007	22.24	No Ct	20.44	No Ct	
SAT 2 BOT 2/2005	23.32	No Ct	No Ct	No Ct	←
O IRN 34/2007	26.66	27.05	No Ct	No Ct	
SAT2/UGA96	27.62	No Ct	No Ct	No Ct	←
C-Noville; (13/1-00) 1:7	20.28	No Ct	No Ct	No Ct	←
SAT 1 BOT V57	19.60	No Ct	No Ct	No Ct	←
SAT 2 ZIM 5/8 V72	20.02	No Ct	No Ct	No Ct	←
SAT 3 ZIM V83 4/81	24.96	No Ct	No Ct	No Ct	←
A-Iraq	21.91	No Ct	32.94	No Ct	
Asia 1-Shamir; (16/3-05)	30.98	No Ct	No Ct	26.67	
O/PAK	30.86	21.17	No Ct	No Ct	
Water (neg control)	No Ct	No Ct	No Ct	No Ct	

Testing serotype discrimination by real time RT-PCR assays

Only 3D assays detect type C and SAT strains

Some A strains not detected by A specifc assay but still detected by 3D assay

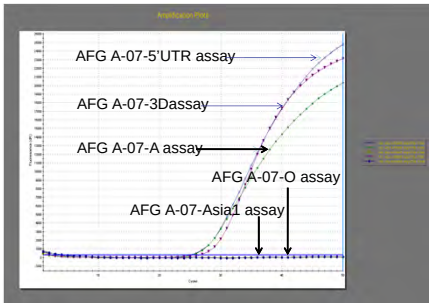
Two step assay	Ct 3D	Ct (type O)	Ct (type A)	Ct (type Asia1)	
O/IRN10/N2	21.88	35.73	No Ct	No Ct	
O/IRN10/N3	35.52	No Ct	No Ct	No Ct	←
O/IRN10/N4	25.50	34.70	No Ct	No Ct	
O/IRN10/N16	19.89	24.72	No Ct	No Ct	
O/IRN10/N18	28.46	31.35	No Ct	No Ct	
O/IRN10/N19	33.48	36.64	No Ct	No Ct	
O/IRN10/N20	No Ct	No Ct	No Ct	No Ct	
Neg control	No Ct	No Ct	No Ct	No Ct	
O/IRN10/N21	20.28	36.51	No Ct	No Ct	
O/IRN10/N22	25.90	32.32	No Ct	No Ct	
O/IRN10/N23	21.37	28.14	No Ct	No Ct	
O/IRN10/N25	19.27	22.82	No Ct	No Ct	
O/IRN10/N26	No Ct	No Ct	No Ct	No Ct	
O/IRN10/N28	19.10	22.70	No Ct	No Ct	
O/IRN10/N29	29.02	39.63	No Ct	No Ct	
O/IRN10/N30	30.25	32.57	No Ct	No Ct	
O/IRN10/N31	25.93	36.11	No Ct	No Ct	
Water	No Ct	No Ct	No Ct	No Ct	
Asia 1/AFG09/(AFG-BAM-7)	25.00	No Ct	No Ct	28.76	

One sample (high Ct in 3D assay) not detected by serotyping assay.

Serotyping assays on Iranian 2010 FMDV samples

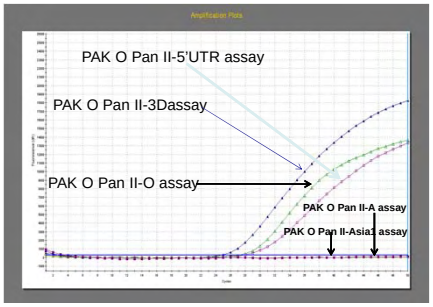
Amplification Plots

FMDV A viruses two step



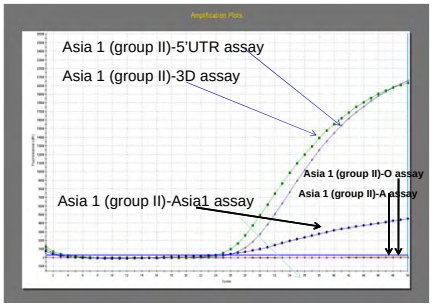
Amplification Plots

FMDV O viruses two step



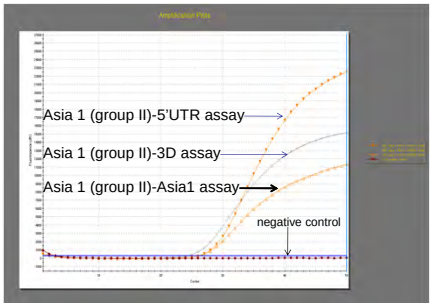
Amplification Plots

FMDV Asia1 viruses two step

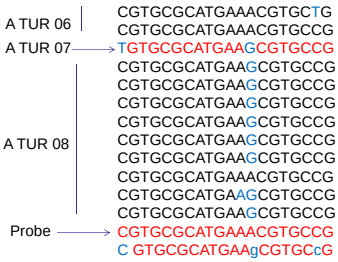


Amplification Plots

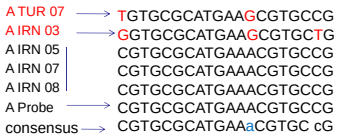
20100913nazem_55.mxp



Type A viruses alignment

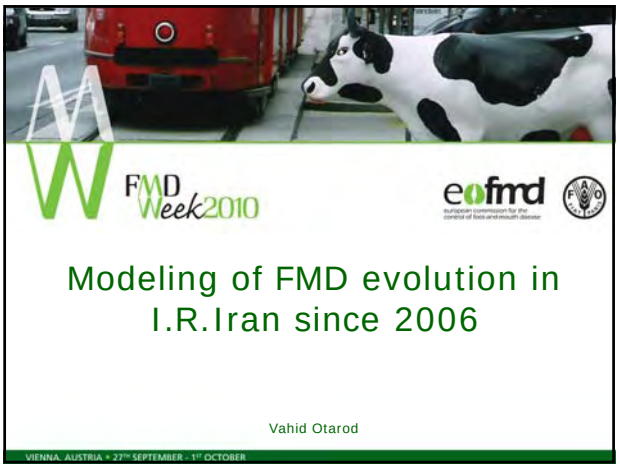


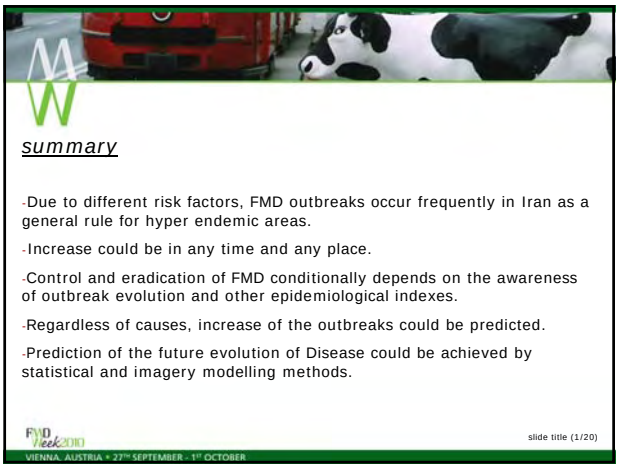
Type A viruses alignment

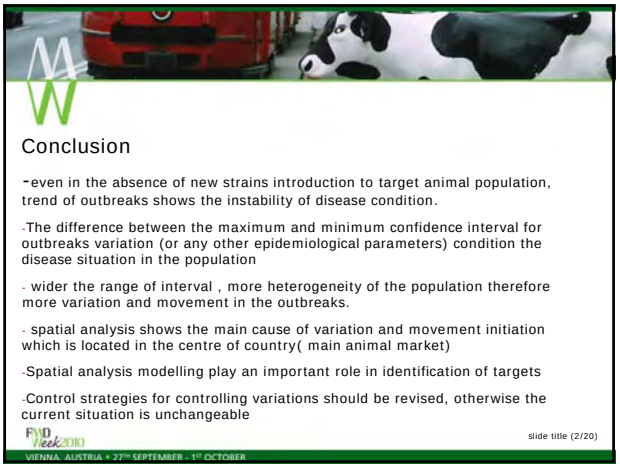


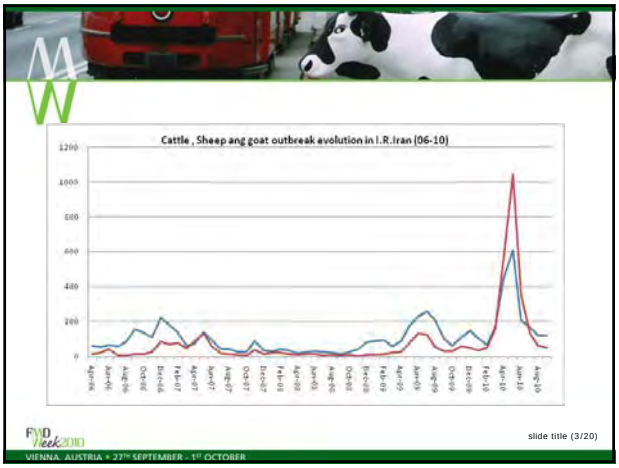
Acknowledgment

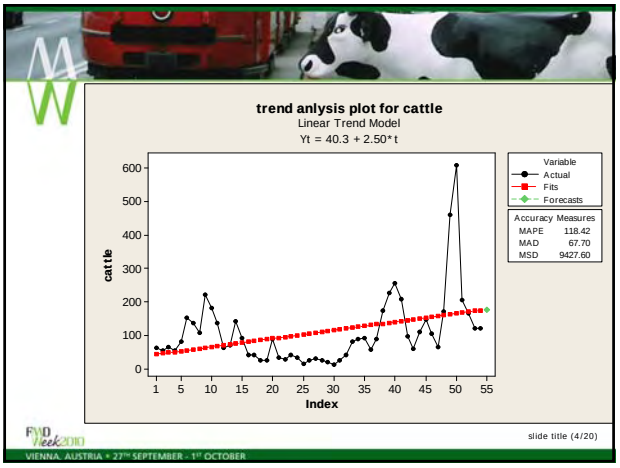
- IAH: D.P.King,N.J.Knowles and S.M.Reid
- Lindholm: P.Norman,T.Fredrechen
- EUFMD group: K.Sumption
- Iran veterinary Organization

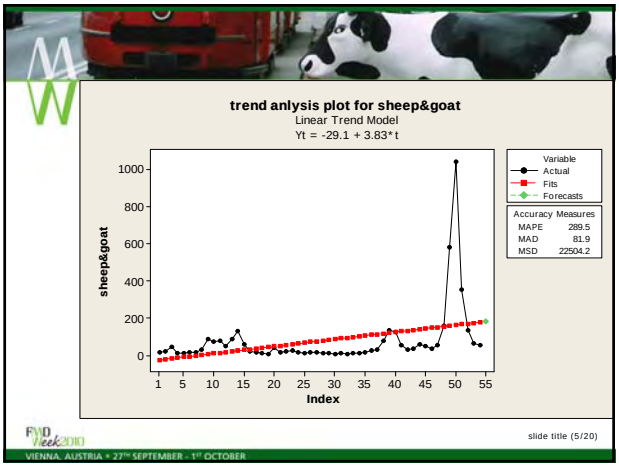

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eufmd
Modeling of FMD evolution in I.R.Iran since 2006
Vahid Otarod
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER

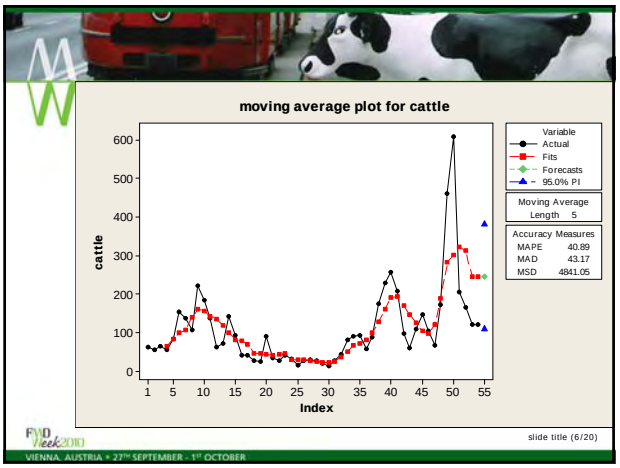

summary
-Due to different risk factors, FMD outbreaks occur frequently in Iran as a general rule for hyper endemic areas.
-Increase could be in any time and any place.
-Control and eradication of FMD conditionally depends on the awareness of outbreak evolution and other epidemiological indexes.
-Regardless of causes, increase of the outbreaks could be predicted.
-Prediction of the future evolution of Disease could be achieved by statistical and imagery modelling methods.
slide title (1/20)
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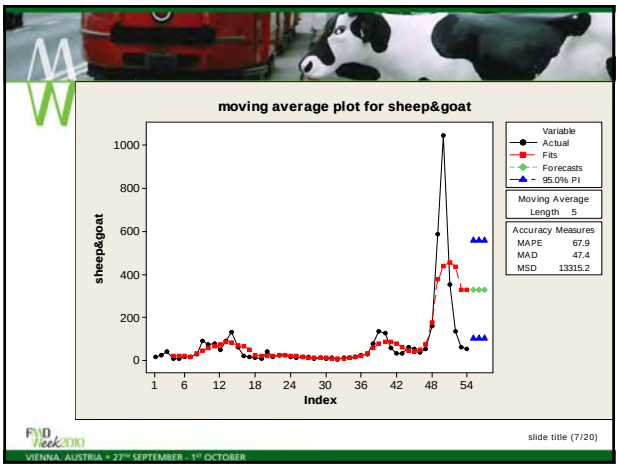

Conclusion
-even in the absence of new strains introduction to target animal population, trend of outbreaks shows the instability of disease condition.
-The difference between the maximum and minimum confidence interval for outbreaks variation (or any other epidemiological parameters) condition the disease situation in the population
- wider the range of interval , more heterogeneity of the population therefore more variation and movement in the outbreaks.
- spatial analysis shows the main cause of variation and movement initiation which is located in the centre of country(main animal market)
-Spatial analysis modelling play an important role in identification of targets
-Control strategies for controlling variations should be revised, otherwise the current situation is unchangeable
slide title (2/20)
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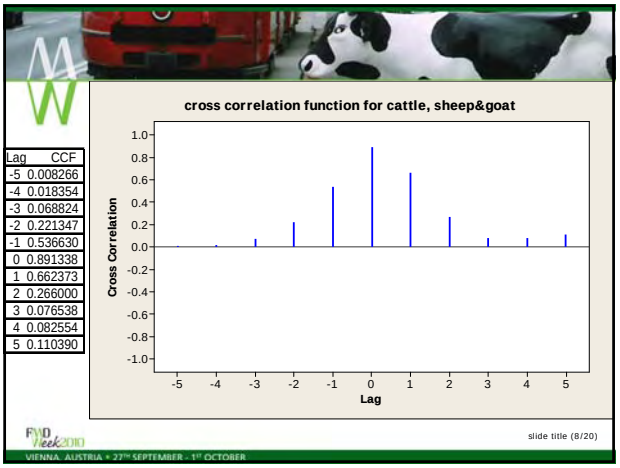

Cattle , Sheep ang goat outbreak evolution in I.R.Iran (06-10)
slide title (3/20)
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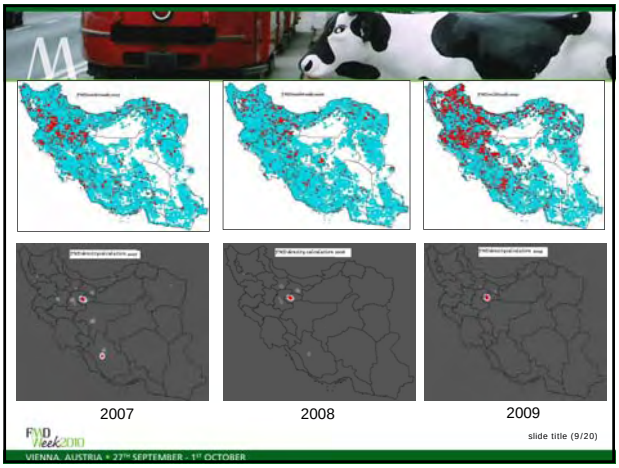

trend anlysis plot for cattle
Linear Trend Model
Yt = 40.3 + 2.50*t
cattle
Index
Variable
Actual
Fits
Forecasts
Accuracy Measures
MAPE 118.42
MAD 67.70
MSD 9427.60
slide title (4/20)
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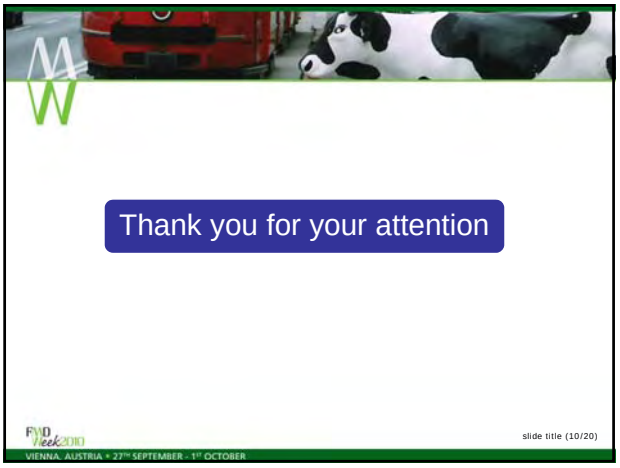

trend anlysis plot for sheep&goat
Linear Trend Model
Yt = -29.1 + 3.83*t
sheep&goat
Index
Variable
Actual
Fits
Forecasts
Accuracy Measures
MAPE 289.5
MAD 81.9
MSD 22504.2
slide title (5/20)
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moving average plot for cattle
cattle
Index
Variable
Actual
Fits
Forecasts
95.0% PI
Moving Average
Length 5
Accuracy Measures
MAPE 40.89
MAD 43.17
MSD 4841.05
slide title (6/20)


moving average plot for sheep&goat
sheep&goat
Index
Variable
Actual
Fits
Forecasts
95.0% PI
Moving Average
Length 5
Accuracy Measures
MAPE 67.9
MAD 47.4
MSD 13315.2
slide title (7/20)


cross correlation function for cattle, sheep&goat
Lag CCF
-5 0.008266
-4 0.018354
-3 0.068824
-2 0.221347
-1 0.536630
0 0.891338
1 0.662373
2 0.266000
3 0.076538
4 0.082554
5 0.110390
Cross Correlation
Lag
slide title (8/20)


2007
2008
2009
slide title (9/20)


Thank you for your attention
slide title (10/20)

Detection of FMDV in carrier buffalo in South East Asia

Blesilda Verin[1&2], John Edwards[2], Aravindh Babu[1], Antonello Di Nardo[1], Santina Grazioli[3], Emi Brocchi[3], David Paton[1], Carolyn Benigno[4], Keith Sumption[5] and **Satya Parida[1&2]**

1. Institute for Animal Health, UK
2. Murdoch University, Australia
3. IZSLER, Italy
4. FAO regional office for Asia and the Pacific
5. EU FMD Secretariat, FAO, Rome

Murdoch UNIVERSITY

Aims of the study

1. Detection of infection in swamp buffalo using NS antibody tests
2. To find out the carrier status in swamp buffalo
3. Duration of persistence of virus and its potential role in disease transmission?
4. Epidemiological study of FMDV in South East Asia

Study design/Survey details





Province	Survey	No Collected
Ayeyarwady	1st	100
	2nd	86
Yangon	1st	101
	2nd	81
	3rd	121
Magway	1st	84
	2nd	44
	TOT	617

Province	Survey	No Collected
Vientiane Prefecture	1st	178
	2nd	84
	3rd	77
	TOT	339

Lecture and Field sampling Demo to Lao PDR animal health staffs









Laos

Overall prevalence by test

		No/No Tested	% of Detection [95% CI]
PrioCHECK	+ve	229/339	67.55% [62.38% - 72.33%]
	-ve	110/339	32.45% [27.67% – 37.62%]
Brescia 3ABC Elisa	+ve	188/339	55.46% [50.12% - 60.67%]
	-ve	151/339	44.54% [39.33% - 49.88%]
Chekit	+ve	175/339	51.62% [46.30% - 56.91%]
	-ve	164/339	48.38% [43.09% - 53.70%]
UBI	+ve	60/338	17.75% [14.03% - 22.20%]
	-ve	278/338	82.25% [77.80% - 85.97%]
IgA	+ve	144/340	42.35% [37.20% - 47.68%]
	-ve	196/340	57.65% [52.32% - 62.80%]
PrioCHECK + IgA	+ve	253/341	74.19% [69.28% - 78.56%]
	-ve	88/341	25.81% [21.44% - 30.72%]

Adjusted Wald test = 61.9085 P = 0.000

		IgA +ve	IgA -ve	TOT
PrioCHECK	+ve	120	109	229
	-ve	24	86	110
	TOT	144	195	339

Kendall's T-b = 0.290 ASE = 0.049

Myanmar

Overall prevalence by test

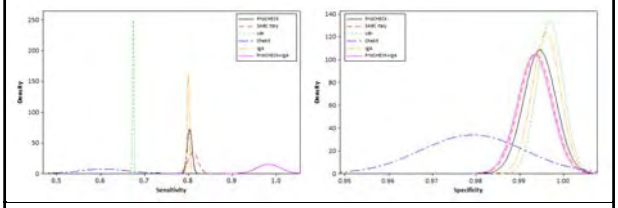
		No/No Tested	% of Detection [95% CI]
PrioCHECK	+ve	353/616	57.31% [53.36% - 61.16%]
	-ve	263/616	42.69% [38.84% - 46.64%]
Brescia 3ABC Elisa	+ve	300/616	48.70% [44.77% - 52.65%]
	-ve	316/616	51.30% [47.35% - 55.23%]
Chekit	+ve	266/616	43.18% [39.32% - 47.13%]
	-ve	350/616	56.82% [52.87% - 60.68%]
UBI	+ve	127/615	20.65% [17.63% - 24.04%]
	-ve	488/615	79.35% [75.96% - 82.37%]
IgA	+ve	174/584	29.79% [26.22% - 33.63%]
	-ve	410/584	70.21% [66.37% - 73.78%]
PrioCHECK + IgA	+ve	386/641	60.22% [56.37% - 63.94%]
	-ve	255/641	39.78% [36.06% - 43.63%]

Adjusted Wald test = 68.5315 P = 0.000

		IgA +ve	IgA -ve	TOT
PrioCHECK	+ve	141	172	313
	-ve	32	214	246
	TOT	173	386	559

Kendall's T-b = 0.344 ASE = 0.036

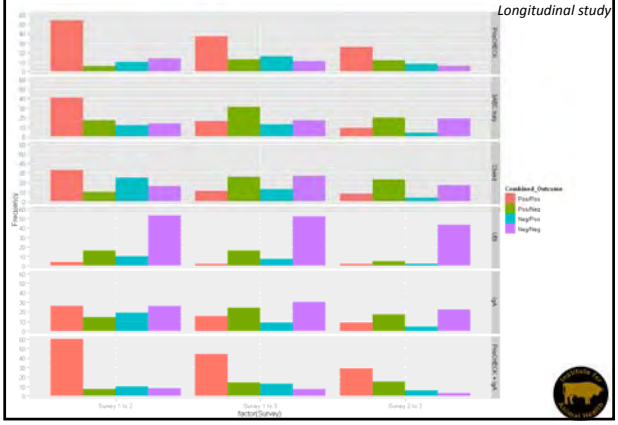
Characterisation of test performances by bayesian framework



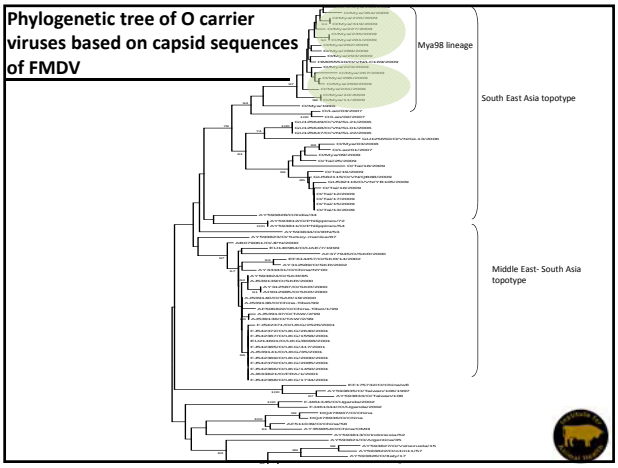
	Se [95% BPI]	Sp [95% BPI]	PPV [95% BPI]	NPV [95% BPI]	LR+ [95% BPI]	LR- [95% BPI]
PrioCHECK	0.803 [0.797-0.818]	0.995 [0.987-1]	99.65% [99.18%-100%]	72.64% [71.32%-74.28%]	9175.5 [63.68-3988]	0.1981 [0.1833-0.1997]
3ABC Italy	0.811 [0.798-0.839]	0.994 [0.987-1]	99.61% [99.19%-99.99%]	72.95% [70.11%-76.48%]	747.4 [63.21-2889]	0.1907 [0.1610.2039]
UBI	0.674 [0.673-679]	0.997 [0.990-1]	99.67% [99.13%-100%]	62.01% [61.52%-62.41%]	2047 [60.72-8660]	0.3266 [0.322-0.330]
Chekit	0.608 [0.524-0.716]	0.979 [0.960-0.999]	98.91% [97.39%-99.95%]	44.43% [27.80%-64.02%]	160.5 [14.34-574.6]	0.4001 [0.2882-0.4879]
IgA	0.800 [0.797-0.806]	0.996 [0.988-1]	99.74% [99.22%-100%]	72.57% [71.98%-73.27%]	5344 [68.21-6970]	0.2011 [0.1945-0.2044]
PrioCHECK + IgA	0.983 [0.894-0.999]	0.993 [0.987-0.999]	99.68% [99.36%-99.99%]	96.33% [76.13%-99.99%]	1707 [76.32-2953]	0.017 [0-0.1072]

Laos

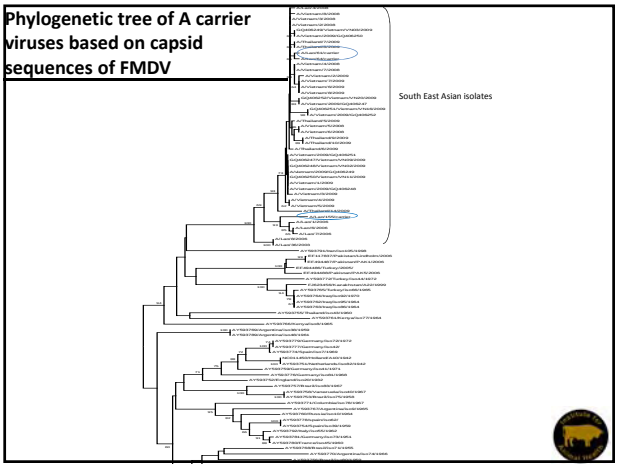
Longitudinal study



Phylogenetic tree of O carrier viruses based on capsid sequences of FMDV



Phylogenetic tree of A carrier viruses based on capsid sequences of FMDV



Conclusions

1. NS antibody tests could detect infection in swamp buffalo with required sensitivity and specificity
2. Swamp buffalo may act as FMDV carrier
3. This preliminary work showed virus may persists at least for 2 years
4. Transmission of FMDV from carrier animals to naïve may be a speculation at this stage and needs more focused work.
5. Type O Myanmar 98 lineages (of SEA topotypes) circulates in SEA and Type A carrier viruses are very closely related to recent SEA isolates

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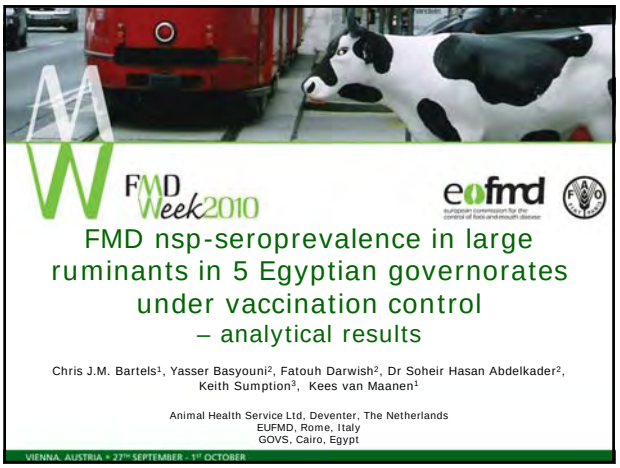
AB-CRC, Australia

FAO, Regional office, Bangkok

OIE regional office, Bangkok

EUFMD, FAO, Rome

Disconvac, FP7



FMD Week2010

FMD nsp-seroprevalence in large ruminants in 5 Egyptian governorates under vaccination control
– analytical results

Chris J.M. Bartels[1], Yasser Basyouni[2], Fatouh Darwish[2], Dr Soheir Hasan Abdelkader[2], Keith Sumption[3], Kees van Maanen[1]

Animal Health Service Ltd, Deventer, The Netherlands
EUFMD, Rome, Italy
GOVS, Cairo, Egypt

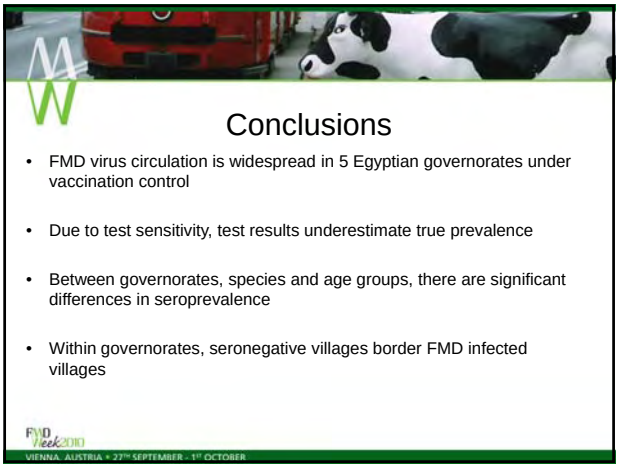
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In addition to descriptive results

- Estimates for true prevalence
  - Rogan-Gladen
  - Bayesian analysis
- Risk-factor analysis (logistic regression-GEE)
- Spatial distribution

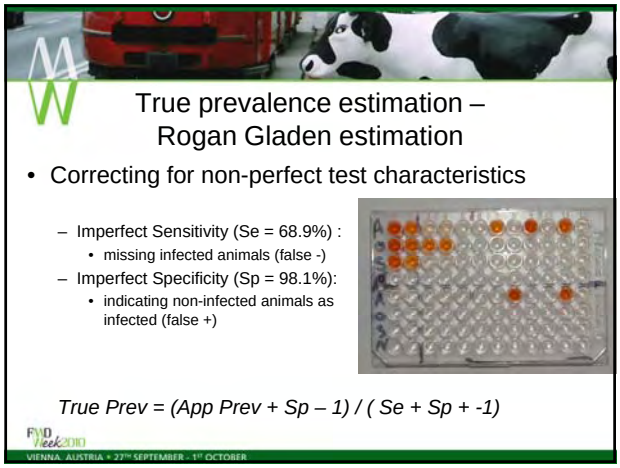
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Conclusions

- FMD virus circulation is widespread in 5 Egyptian governorates under vaccination control
- Due to test sensitivity, test results underestimate true prevalence
- Between governorates, species and age groups, there are significant differences in seroprevalence
- Within governorates, seronegative villages border FMD infected villages

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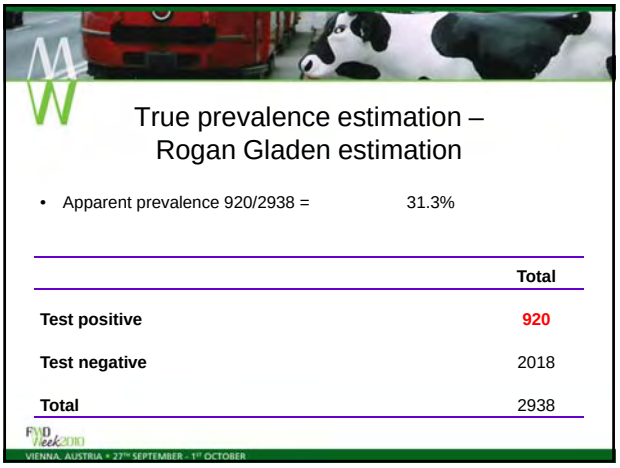


True prevalence estimation – Rogan Gladen estimation

- Correcting for non-perfect test characteristics
  - Imperfect Sensitivity (Se = 68.9%) :
    - missing infected animals (false -)
  - Imperfect Specificity (Sp = 98.1%):
    - indicating non-infected animals as infected (false +)

True Prev = (App Prev + Sp – 1) / (Se + Sp + -1)

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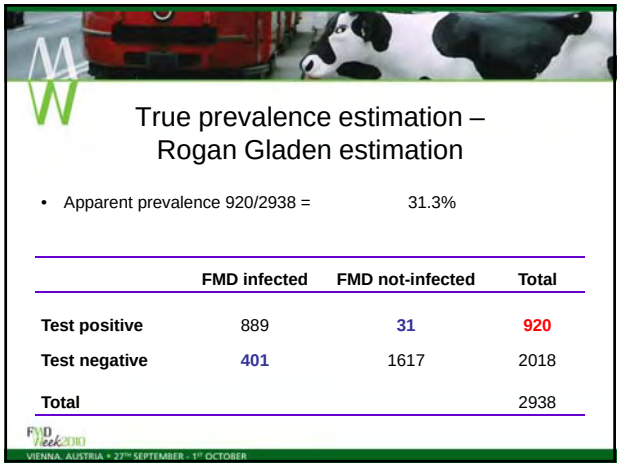


True prevalence estimation – Rogan Gladen estimation

- Apparent prevalence 920/2938 = 31.3%

	Total
Test positive	920
Test negative	2018
Total	2938

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True prevalence estimation – Rogan Gladen estimation

- Apparent prevalence 920/2938 = 31.3%

	FMD infected	FMD not-infected	Total
Test positive	889	31	920
Test negative	401	1617	2018
Total			2938

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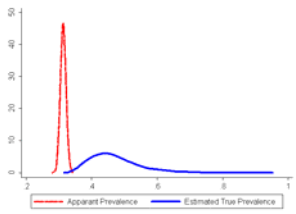
True prevalence estimation – Rogan Gladen estimation

- Apparent prevalence 920/2938 = 31.3%
- Estimated true prevalence: 1290/2938 = 43.9%

	FMD infected	FMD not-infected	Total
Test positive	889	31	920
Test negative	401	1617	2018
Total	1290	1648	2938

True prevalence estimation – Bayesian analysis

- Correcting for non-perfect te
  - Taking prior information on Se
  - As distribution from which is s
    - 95% confidence interval Se: (
    - 95% confidence interval Sp:
  - Accounting for uncertainty and
- Wider confidence intervals



True prevalence estimates

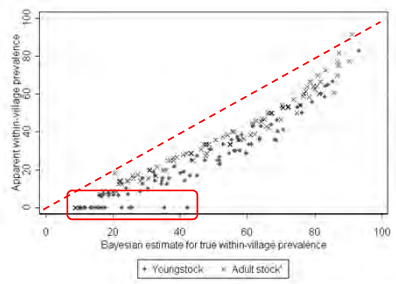
	Apparent	True estimate RG	True estimate BA
Village prevalence	101/105		105/105
Animal prevalence [95%CI]	31.3 [29.6-33.0]	43.9 [41.4-46.4]	46.6 [35.9-64.6]
Within village prevalence (Range)	29.8 (0 – 92%)	*	47.4 (7-95%)

*For estimation of within-village prevalence, the RG is not applicable

Comparison Apparent and True prevalence

Bayesian estimates higher than apparent results
➔ Sensitivity

Villages with test-negative results are considered seropositive
➔ effect of limited sample size and Se



Comparison two methods to estimate true prevalence

Rogan Gladen estimator
+ Quick and easy

- uses Se and Sp estimates as point values
- not applicable at within-village level

Bayesian estimates
+ use of prior knowledge
+ including uncertainty and variation
+ can be applied at all levels

- requires good prior information
- requires more statistical background

Result logistic regression - main effects

- Outcome: test result: positive (1) or negative (0)
- Modeling outcome in relation to age, breed and governorate:

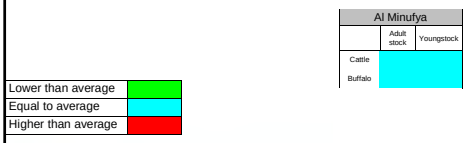
$$Test\ result = constant + \beta_1_species + \beta_2_age + \beta_3_govern + clustering + error$$

- Odds ratio: odds of testing seropositive
  - OR > 1; greater probability
  - OR < 1; smaller probability

Result logistic regression - main effects

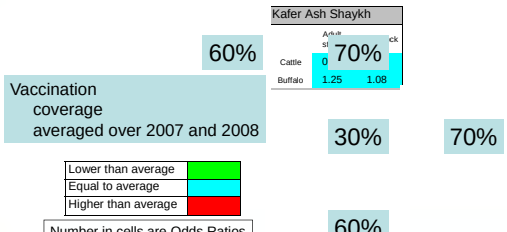
Variable	Category	Frequency	Odds Ratio	95% confidence interval	P-value
Age	Adult stock	55.5	Reference		
	Young stock	**44.5**	**0.50**	**0.43-0.59**	**< 0.01**
Species	Cattle	59.8	Reference		
	Buffaloes	**40.2**	**1.56**	**1.33-1.83**	**< 0.01**
Governorate*	Al Buhayrah	23.6	0.84	0.62-1.14	0.26
	Kfer Ash Shaykh,	11.2	0.90	0.60-1.33	0.59
	Ash Sharqiyah	25.0	1.12	0.84-1.49	0.43
	Al Minufiyah,	15.3	0.78	0.54-1.11	0.17
	Al Minia	**24.9**	**1.52**	**1.14-2.02**	**< 0.01**

Result logistic regression - interaction terms



Lower than average	
Equal to average	
Higher than average	

Result logistic regression - interaction terms



60%

70%

Vaccination coverage averaged over 2007 and 2008

30%

70%

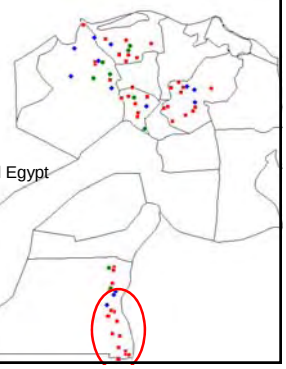
Lower than average	
Equal to average	
Higher than average	

60%

Number in cells are Odds Ratios indicating odds on seropositivity compared with random adult or random youngstock

Spatial distribution of infected villages

- FMD infected villages spread across non-FMD infected villages
- Highest prevalence of FMD infection in Al Minia and Ash Sharqiyah, central Egypt
- Only 69 out of 105 villages
- For Al Buhhayrah, significant few 'negative villages



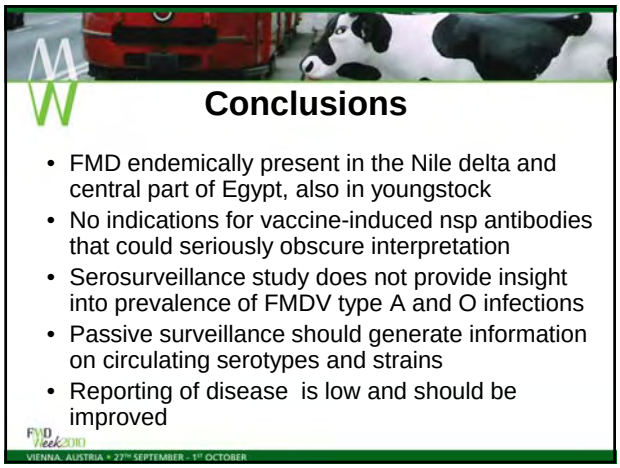
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- FMD virus circulation is widespread in 5 Egyptian governorates under vaccination control
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- Within governorates, seronegative villages border FMD infected villages

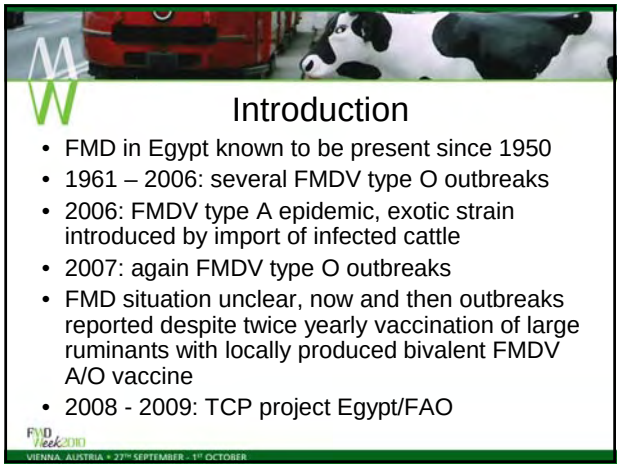
Discussion

- Risk factors – animal level
  - age, species
- What risk factors at village level?
  - Contact structure within and between villages
  - Village practices regarding grazing and feeding.
- What risk factors at national level?
  - Vaccination strategy
  - Market chain mechanisms and animal movement patterns
  - Role of sheep and goats
- 2010/2011: nationwide survey, including inventory of possible risk groups and collection of additional information


FMD Week2010
eufmd
FMD NSP-seroprevalence in large ruminants in 5 Egyptian governorates under vaccination control - descriptive results
Kees van Maanen, Yasser Basyouni, Sayed Salem, Ahmed Habashi, Fatouh Darweesh, Hassan Soheir, Keith Sumption and Chris Bartels
VIENNA, AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER


Conclusions
• FMD endemically present in the Nile delta and central part of Egypt, also in youngstock
• No indications for vaccine-induced nsp antibodies that could seriously obscure interpretation
• Serosurveillance study does not provide insight into prevalence of FMDV type A and O infections
• Passive surveillance should generate information on circulating serotypes and strains
• Reporting of disease is low and should be improved
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Introduction
• FMD in Egypt known to be present since 1950
• 1961 – 2006: several FMDV type O outbreaks
• 2006: FMDV type A epidemic, exotic strain introduced by import of infected cattle
• 2007: again FMDV type O outbreaks
• FMD situation unclear, now and then outbreaks reported despite twice yearly vaccination of large ruminants with locally produced bivalent FMDV A/O vaccine
• 2008 - 2009: TCP project Egypt/FAO
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Overall project objectives

OVERALL OBJECTIVES

- *Reduction of economic losses and consequently milk and meat production improvement through effective prevention and control of vesicular diseases in general and FMD in particular.*

IMMEDIATE OBJECTIVES

- **Objective 1** Improve preparedness and planning to contain quick spread of vesicular diseases in general and FMD in particular.
- **Objective 2** Prevent future outbreaks in livestock.
- **Objective 3** Improve detection of FMD and vesicular diseases in cattle.
- **Objective 4** Improved outbreak investigation and local containment measures.
- **Objective 5** Improve the quality of locally produced vaccines by continuous and regular evaluation and continuous sero-typing of local strains.
- **Objective 6** Improvement of Animal Disease Reporting System.

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Aims serosurveillance study

- Determine apparent prevalence rates of old and recent FMD infections in cattle and buffalos
- Estimate true prevalence rates of old and recent FMD infections in cattle and buffalos
- Explore risk factors that can be used for redesigning current control strategies

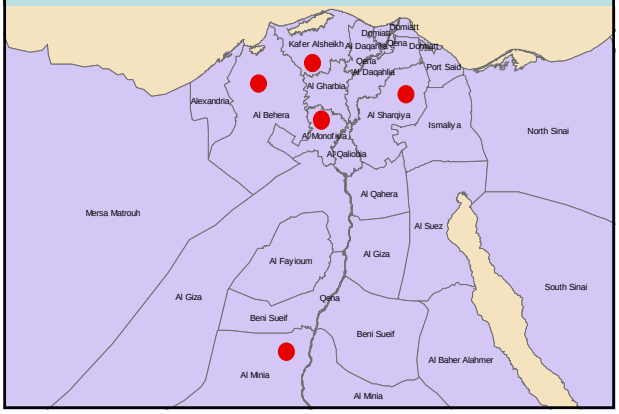
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Serosurveillance study

- Five pilot governorates
- Two-stage sampling design
- 105 villages ad random selected
  - Blood samples from young animals < 2 year (n=14)
  - Blood samples from older animals > 3 year (n=14)
  - Detection of old and new infections by NS-ELISA on village level
  - Estimation of apparent prevalence in pilot governorates (village as an epidemiological unit)
- PrioCHECK FMDV NS ELISA, two different cut-offs
  - 50% inh. (Se 68.9%; Sp 98.1%) and 60% inh. (Se 62.3%; Sp 99.2%)
- Sera from multi-vaccinated cattle from two FMD free farms were investigated to exclude vaccine-induced nsp antibody responses

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PROPOSED PILOT GOVERNORATES

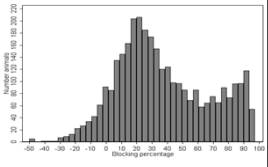






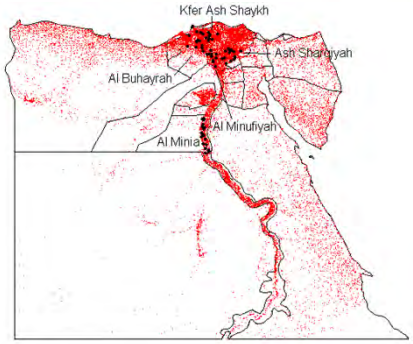
Descriptives

- 2938 ELISA testresults
  - 105 villages
  - Age
    - 1306 youngstock
    - 1632 adult cattle
  - Species
    - 1757 cattle
    - 1181 buffalos



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Selection of 4 governorates in Nile Delta and 1 in central Egypt



Descriptive results overall FMD seroprevalence

Cutoff	# positively tested	# tested	Apparent prevalence	95% Confidence interval
50	**920**	**2938**	**31.3%**	**[29.6 - 33.0%]**
60	738	2938	25.1%	[23.6 - 26.7%]

FMD therefore a common infection:
at least 25% of sampled animals nsp seropositive!!!!

Results by age

Age	Cutoff	# positively tested	# tested	Apparent prevalence	95% Confidence interval
Youngstock	**50**	**313**	**1306**	**24.0%**	**[21.7 - 26.4%]**
	60	235	1306	18.0%	[15.9 - 20.2%]
Adult cattle	**50**	**607**	**1632**	**37.2%**	**[34.8 – 39.6%]**
	60	503	1632	30.8%	[28.6 – 33.1%]

Results by species

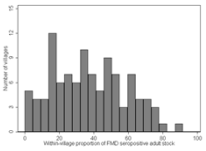
Species	Cutoff	# positively tested	# tested	Apparent prevalence	95% Confidence interval
Cattle	**50**	**486**	**1757**	**27.7%**	**[25.6 - 29.8%]**
	60	372	1757	21.1%	[19.3 – 23.2%]
Buffalos	**50**	**434**	**1181**	**36.8%**	**[34.0 – 39.6%]**
	60	366	1181	31.0%	[28.4 - 33.7%]

Results by governorate

Governorate	Cutoff	# positively tested	# tested	Apparent prevalence
Behaira	50	176	692	25.4
El-Sharqya	50	233	735	31.7
Kafer El-Sheikh	50	90	330	27.3
Menia	50	292	733	39.8
Mounofya	50	129	448	28.8

Within-village prevalence

Adult cattle and buffaloes

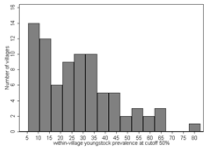


Zero positive-tested animals: 5 villages

Median: 36%

Maximum: 92%

Youngstock

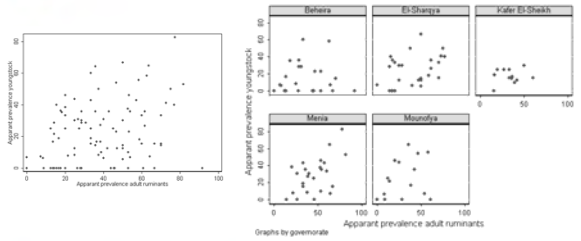


Zero positive-tested animals: 23 villages

Median: 26%

Maximum: 83%

Relation Youngstock and Adult cows



Low correlation (r = 0.12 (Baheira) – 0.53 (Menia) between seroprevalence in youngstock and in adult cattle within villages

Conclusions

- FMD endemically present in the Nile delta and central part of Egypt, also in youngstock
- No indications for vaccine-induced nsp antibodies that could seriously obscure interpretation
- Serosurveillance study does not provide insight into prevalence of FMDV type A and O infections
- Passive surveillance should generate information on circulating serotypes and strains
- Reporting of disease is low and should be improved

Acknowledgements

- FAO, in particular Toni Ettel
- AHRI
- GOVS
- National consultants
- Local veterinarians

Thank you for your attention!



Factors associated with within-herd transmission in cattle of serotype A foot and mouth disease virus in Argentina, 2001

B. Brito*,1, A. Perez[1], G.Konig[2], L. Rodriguez[3]

[1] FMD lab, Center for Animal Disease Modeling and Surveillance, UC Davis, Davis, CA 95616, USA
[2] Instituto de Biotecnologia, CICVyA, INTA, Buenos Aires, Argentina
[3] United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Orient, NY USA

EU FMD Week- September, 2010, Vienna, Austria.

Conclusion

- Factors associated with transmission: herd size, days to detection and vaccination
- Improved understanding of disease spread
- Field study serotype A
- Epidemiologic factors- targeted resources

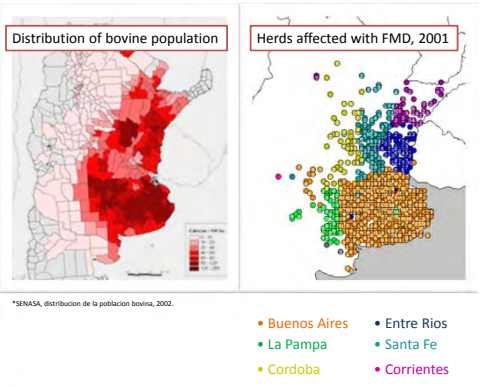
Introduction

FMD in Argentina



- 1870 - Introduced
- 1996 - FMD free-with vaccination
- 2000 - FMD free-without vaccination
- 2000 - FMDV serotype A and O epidemic
- 2001- new serotype A extensive epidemic

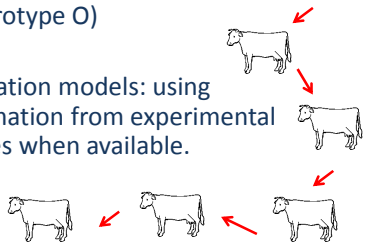
Argentina 2001 Epidemic



Within herd transmission

- Experimental studies: controlled variables (in serotype O)
- Simulation models: using information from experimental studies when available.





Objectives

- To estimate the intra-herd transmission coefficient (β)
- To estimate the association with potential epidemiological factors for the disease.

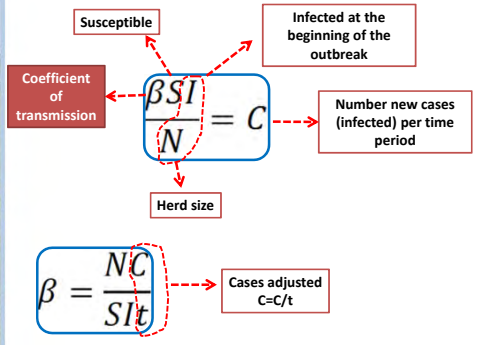
Materials & Methods

Database

- Outbreak (case): herd officially recognized by SENASA as infected.
- Records : demographic variables and disease/dates records.

Coefficient of Transmission

Estimating β- "Mass action" frequency-dependent



Multivariate logistic regression

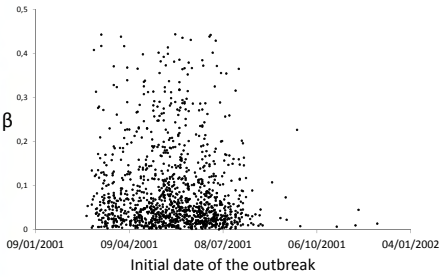
- Dependent variable: β categorized

  1 = > β median
  0 = ≤ β median

- Independent variables
  - Latitude and longitude
  - Duration of the outbreak
  - Herd size
  - Days initial infection-detection
  - Predominant age group
  - Days initial infection-vaccination

Results

Distribution of β Over Time



Multivariate Logistic Regression

Variable	Category	n	OR	95% CI	P-value
Herd size (100 animals)		1134	1.01	(1.00, 1.02)	0.04
Days to detection[a]		1134	0.95	(0.92, 0.98)	0.001
Vaccination					
	Non vaccinated	747	1	*	Reference
	0-4 days after[a]	25	0.59	(0.26, 1.34)	0.211
	1-3 days before[a]	35	0.43	(0.21, 0.88)	0.021
	4-7 days before[a]	65	0.52	(0.31, 0.88)	0.015
	8-14 days before[a]	55	0.58	(0.33, 1.01)	0.054
	15-28 days before[a]	56	0.7	(0.40, 1.21)	0.205
	>28 days before[a]	151	0.51	(0.36, 0.74)	<0.001

[a] days in relation to the estimated day of first infection in the herd.

Discussion

Within Herd Transmission Associations

- Herd size
  - ↑ Susceptible
  - ↑ Density?
- Time to detection
  - o ↑Time→ Low Density Herds → Lβ
  - o ↓time→ High Density Herds → Hβ



Within Herd Transmission Associations

- VACCINATION- DAYS TO VACCINATION
  - o Decreased spread in vaccinated herds
  - o Vaccination >28 days before outbreak
  - o Decreased transmission in short vaccination-challenge time
  - o Vaccine A/Arg/00 vs A/Arg/01
  - o Parallel dynamics of spread-immunity development

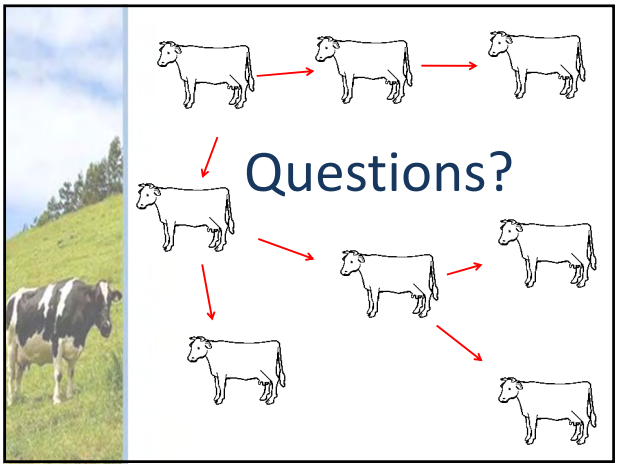
- Variables not associated with β
  - o Duration of the outbreak
  - o Predominant age group in the herd
  - o Latitude and longitude

Conclusion

- Improved understanding of mechanisms for disease spread
- SEROTYPE A
- Epidemiologic factors- targeted resources

Acknowledgements

- Plum Island Animal Disease Center Research Participation Program fellowship, ORISE.
- CADMS, University of California, Davis



TEMPORAL, SPATIAL, AND PHYLOGENETIC ASSESSMENT OF FULL-SEQUENCED SEROTYPE A FOOT AND MOUTH DISEASE VIRUSES FROM ARGENTINA, 2001

Introduction

- The FMDV produce a devastating epizootic that affected the Argentine herd in 2000-2002.
- The A Argentina 2001 FMDV strain is considered the responsible for most of the 2561 outbreaks that affected the country during the epizootia.
- Unvaccinated and vaccinated animals were affected by the disease.

Sampling

- A location with good epidemiologically information, high attack rate, limited duration over time was selected to conduct the analysis (Mar Chiquita from Buenos Aires province).
- The protocol for the selection of samples made use of a factorial design to stratify the selection, so that samples are representative of the temporal and spatial distribution of outbreaks, attack rate and vaccination status.
- Sixteen samples were selected from 41 outbreaks occured from May to July 2001 with viral isolates and good epidemiological data.

Hypothesis

- The FMD virus was introduced once in Mar Chiquita and all the subsequent outbreaks derived from the index case (first outbreak in Mar Chiquita).
- The genetic evolution of the virus is related to the spatial, temporal, demographic or immunological characteristics of the infected herd.

Materials and Methods

- Protocols and primers to produce seven overlapping PCR products were designed and 45 primers were selected with the aim of obtaining a full genome FMDV sequence of each isolate.
- Phylogenetic analyses were completed with MEGA, Model Test, PAUP, Phy-ML, Split tree and Network software.
- Association between virus evolution and selected epidemiological factors was assessed using Mantel correlation technique.

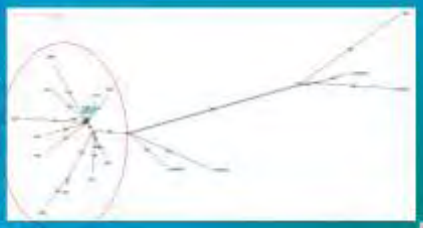
Results

- 15 complete genome sequenced were already obtained
- As expected they group together and showed a cluster association among them



Results

- Split decomposition network, with bootstrap (100x). Complete genome



Results

- Genealogical analysis of Vp1 sequences
- ML tree made in PHYML (as obtained in Modeltest), using model GTR +I +G. Bootstrap values for a low support



Results

Multiple correlation performed in program FSTAT with 10000 permutations

Distance		0.149
Group		0.758

No dependence of genetic to geographic distance or time between premises.

Conclusion

- Multiple introduction of FMDV in Mar Chiquita ?
- No correlation with time or distance ?

Support:

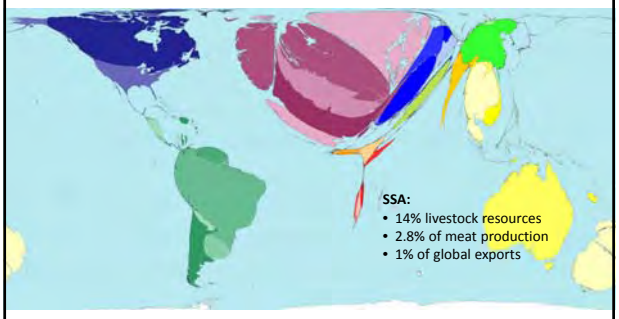
+SCyA ARS-INTA / Argentina Agreement Number 58-1940-7-248
Developing profiling tools for prevention of FMD
+BMGF 57286-PC-T 11 / Estudio de la evolución molecular del VFA
+PE Fisher Affinity Models I Epidemiología Molecular

Thank you for your attention



Context

CBT- moving away from area-based sanitary requirements for trade



Territory size shows the proportion of worldwide **net exports** of meat (in US$) originating from there.

© Copyright SASI Group (University of Sheffield) Fao,2002

Elements of interest

Main AH implications of the new CBT market access derive from a shift towards more **formal trade** in **products**:

- Change in movements of commodities and associated potential for disease spread.
- Nature of commodities traded.
- And other critical unknowns: market focus? safety requirements? supply routes?

CBT concept

Country-based trade → Commodity-based trade

Loose definition:

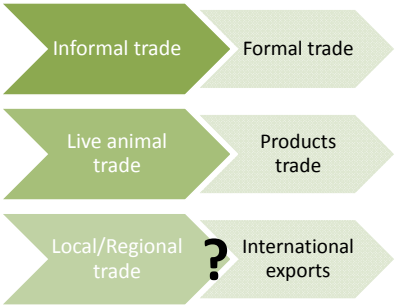
- Healthy animals/ adequate processing
- +/- quarantine/vacc.
- Traceability /certification

Many market access scenarios:

- Export markets
- Regional trade
- Domestic urban markets

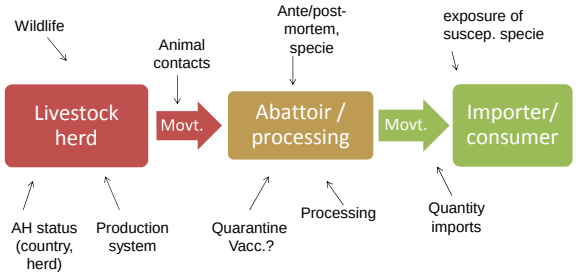
Thomson, Leyland, Donaldson, 2008
Scoones et al., 2010

Implications of CBT



CBT Implementation requirements

CBT trade context – link to AH



Pathway for disease transmission

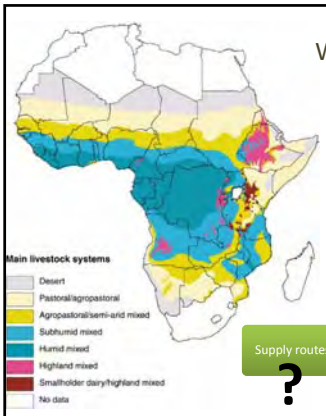
Platon et al., 2010

CBT- Main AH implications

- **Formal trade**
  - investment AH services
  - CBT requirements
  - more controlled movts.
  - facilitated mkt. access / illegal trade
- **Change in movement patterns**
  - reorientation towards approved plants
  - mkt. focus ~degree of change
  - production systems
- **Animals to Products**
  - Δ nature disease risk
  - transport (less animal contacts, greater distance)
  - multiple safety layers possible
- **Wildlife**
  - New industry focus? (ex. Game)
  - Compatible with TFCAs

Ryan et al., 2008

Where would products come from?

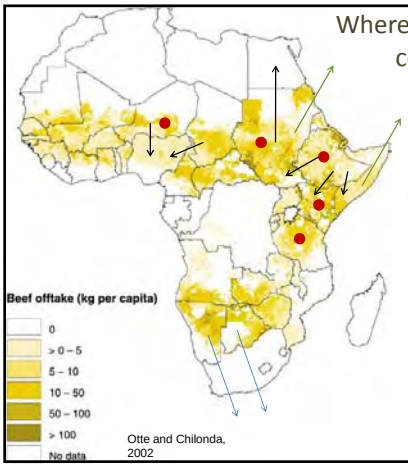


Supply routes ?

- Use surplus/ ineligible
  - ↑numbers of cattle
  - ↑productivity
- ↑offtake
  - ↑Efficiency of mkt chain
- Diversify supply base
  - New industry focus (ex. Game)
  - Niche products

Otte and Chilonda,2002

Where would products come from?



Otte and Chilonda, 2002

MC14

CBT offtake zones: relative potentials

- Sizable herd

Middle East
EU
Within Africa

CBT offtake zones

- Higher offtake zones
- Mostly informal trade in animals
- Uneven use of potential

→

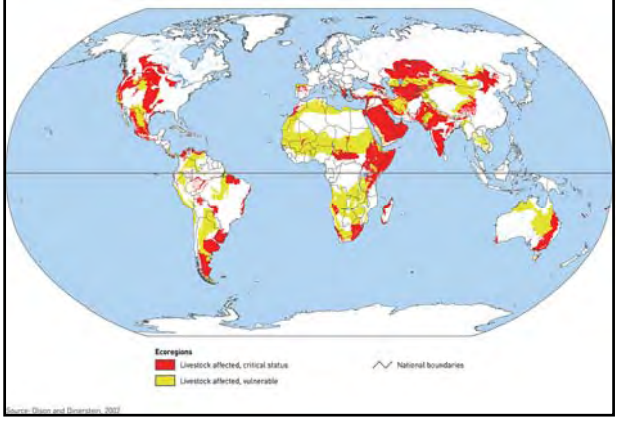
- identify C BT potential
- partial indicators of mvt
- highlights limitations

Other factors affecting commodity movement:

- Infrastructure restrictions
- Private standards
- Productivity / competitiveness(!)
- Information network, bilateral agreements, national policies…

Perry and Dijkman, 2010
Rich and Perry, 2009
Aklilu and Catley, 2009

Parting thoughts



Thank you



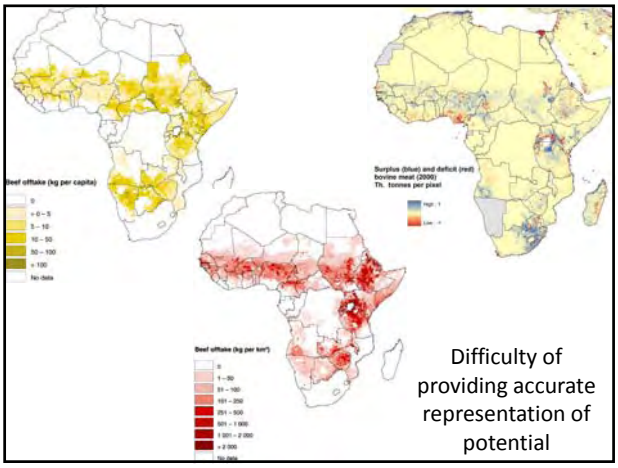
South Africa, 2006

Partial Bibliography

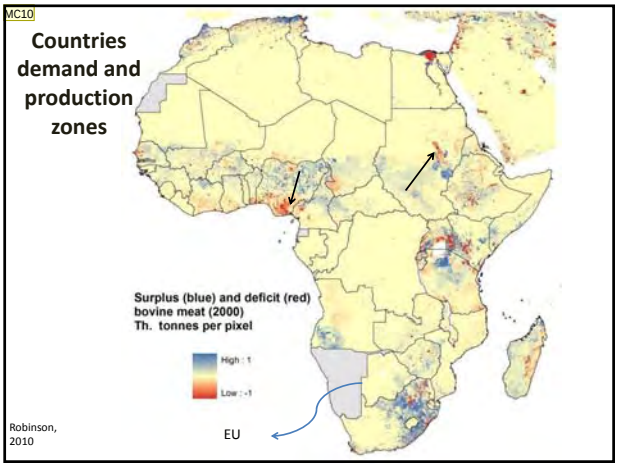
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Difficulty of providing accurate representation of potential



Foot and mouth disease in Mali and West Africa

September 2010, Abdallah TRAORE. Mali

Introduction

- The Republic of Mali is a landlocked country of approximately 1,204,201 km2. The country spans three major climatic zones: a large central band of semi-arid (Sahelian area), Soudanian area, with rain falls only from mid-June to October and north by the Sahara desert.
- Mali has one of the largest cattle populations in West Africa (approximately 8.385.700), as well as a large number of small ruminants (24.522.400).
- The cattle population consists principally of indigenous breeds (Mere cattle, N'Dama cattle, Zebu peulh, Zebu Maure, Zebu Azawak).
- However, attempting to improve livestock productivity by importing exotic breeds for crossing-breeding is becoming a common practice in Mali.

Introduction

- Disease such as foot and mouth disease (FMD) require particular attention. The long campaign to eradicate rinderpest diverted attention and effort from FMD control in West Africa. Furthermore, the economic impact of FMD, particularly the reduction in milk production and depreciation in the value of meat (due to weight loss in the animal and the reduction in meat quality), has been overlooked or is not well understood by livestock-owners. In Mali, the loss (milk, meat, draught, cost of treatment) have been evaluated to $600 000 by year.
- These factors, combined with the low mortality rate of the disease, may explain the relative lack of attention to FMD infections in Mali, in particular and throughout the African continent as a whole.

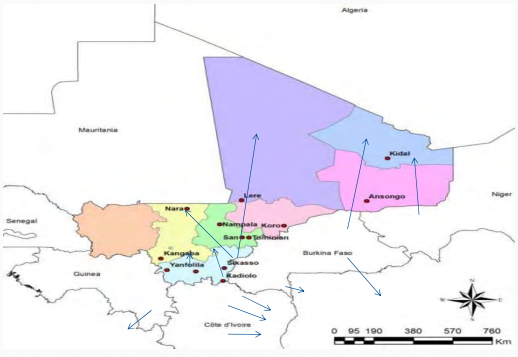
Foot-and-mouth disease in Mali and West Africa

- Three of the seven serotypes have been found circulating in Mali: these include serotype A, O and SAT 2. FMD was first reported in Mali in 1991 in outbreaks attributed to type SAT 2 virus and, subsequently, other serotypes were identified (A, 1999; O, 2005). Although no extensive studies have been conducted in Mali, the onsets of FMD seem to follow a seasonal occurrence: during June-July-August (rain season) in the entire country, and during January-February (dry season) in target areas of Gao and Timbouctou regions.

Foot-and-mouth disease in Mali and West Africa

- The livestock husbandry in Mali is divided between three different systems: the sedentary system in the Soudanian area, where focal points of transhumance occur during the rain season; the semi-nomadic system in the Sahelian area; and the nomadic system in the Saharian area. It should be noticed that, however, the pastoral system is the predominant husbandry system in sub-Saharian Africa and many of these flocks traverse national borders in search of water and better pastures without any recourse to effective quarantine or movement control measures.

Cross-border transhumant movement in Mali



Foot-and-mouth disease in Mali and West Africa

- Most of the cattle and small ruminat population enter into Mali from Mauritania, Guinea, Ivory Coast, Burkina Faso and Niger; on the other hand transboundary movement also exists from Mali to the above countries and also to Algeria. The figure 1 shows the main transboundary movement in Mali by regions. Therefore, the regular congregation of herds from different sources without essential health check predisposes all herds to FMD and to the introduction and spreading of new strains.
- In Mali, there are regularly FMD outbreak. In 2007 ten outbreak were found, amongst 33182 animals contaminated. According the retrospective study conducted in the seven countries of West Africa (Burkina Faso, Mali, Niger, Benin, Côte d'Ivoire, Ghana, Togo), over the periode 1970-2003, FMD became enzootic in theses countries.

Foot-and-mouth disease in Mali and West Africa

- According to the OIE notifications, 898 outbreak of FMD were declared over the period 2000-2004 by these seven countries. According to the same study, within the seven countries, 198 biological samples from identified outbreak, have permitted to confirm FMD outbreak with the follow serotypes: O: 62 outbreaks; A: 32 outbreaks; SAT1: 18 outbreaks; SAT2: 88 outbreaks.
- Even though under estimted, these results show clearly the importance of FMD in West Africa. The diffusion of FMD in the subregion is facilitated by the rearing mode of livestock dominated by the perpetual and non control movementof livestock (transhumance, commerce, nomadism).

Strategy to control foot and mouth disease in Mali

- Before the start of the national program for the control of foot and mouth disease, we want start study for all regions of Mali for serotypes screening.
- The study will assess the prevalence of FMD in Mali using serological and clinical disease survey based on a random sampling approach by the means of applied Geographic Information System (GIS) method. Furthermore, Participatory Epidemiology (PE) component will be integrated within the survey activities in order to gather relevant epidemiological information on the past events of FMD, the seasonal variation in the disease occurrence, and to investigate the risk associated with livestock movement and FMD prevalence.

Strategy to control foot and mouth disease in Mali

The data collected by combining quantitative epidemiological tools would provide useful information in exploring the FMD serotypes circulating in the region, the spatio-temporal pattern of the disease occurrence, the livestock movement, the contact rate and the dynamics of aggregation and dispersion between herds, with the ultimate aim of identifying potential presence of hotspots and endemic foci of FMD within the region.

Strategy to control foot and mouth disease in Mali

- Preventing and controlling FMD requires a good diagnostic laboratory, good quarantine facilities and qualified personnel. The Central veterinary laboratory of Bamako (CVL) has need serological and polymerase chain reaction (PCR) facilities. Moreover, the CVL routinely conducts ELISAs, PCR, qRT-PCR to detect antibodies, antigen and nucleic acid.

Number of outbreaks reported in West Africa from 2005 to 2008

Country/Years	2005	2006	2007	2008
Benin	15	10	-	-
Burkina Faso	-	34/5	-	-
Ivory Coast	36	2	3	-
Gambia	3/1	3/3	2/1	5
Ghana	4	2	-	-
Guinee Conakry	-	2/2	2/2	-
Mali	4/4	37/3	20/2	18/2
Nigeria	28	11	2/2	10/8
Senegal	1/1	47	8	40
Togo	69	20/8	13	3



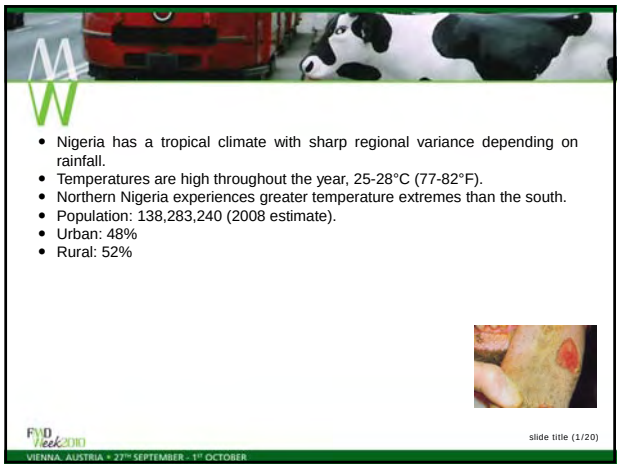




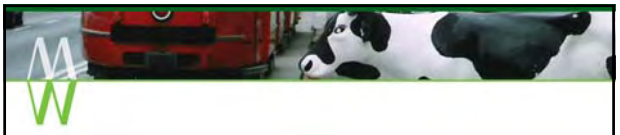


FMD VIRUS SITUATION IN NIGERIA

D. D. Lazarus*., S. S. Adamu., D. Shamaki., F. O. Fasina



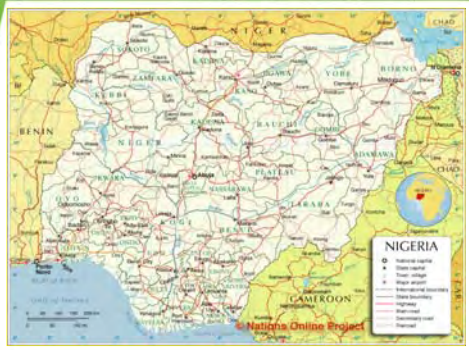
- Nigeria has a tropical climate with sharp regional variance depending on rainfall.
- Temperatures are high throughout the year, 25-28°C (77-82°F).
- Northern Nigeria experiences greater temperature extremes than the south.
- Population: 138,283,240 (2008 estimate).
- Urban: 48%
- Rural: 52%



- Cattle population: 15,239,647 (WAHID, 2008) aside the countless heads of cattle that cross into the country daily.
- Agriculture accounts for 23% GDP.
- Agriculture has contributed to >75% of the national export earnings before 1970.
- Livestock sector: dominated by Fulani Pastoralists.
- In 1983, the industry was devastated by the rinderpest outbreak.







INTRODUCTION Contd.

- First reported outbreak in 1920, untyped; 1924,serotype O.
- Thereafter; A, SAT1 and SAT 2.
- Nigeria shares land borders with Republic of Benin, Chad & Cameroon and Niger.
- The country serves as a meeting point for most of the cattle arriving from West & Central African countries in view of the abundant feed resources, enormous population (1/5 African population) and relative wealth/purchasing power in the sub-region.



INTRODUCTION Contd.

- It should be understood that transhumance production is the predominant system of management in sub-Saharan Africa.
- Many of these individuals traverse national borders without any recourse to quarantine and control measures.
- Over the years, there has been no coordinated national control policy.



INTRODUCTION Contd.

- No surveillance activities on ground.
- No full cost implication of FMD.
- The role of wildlife and carrier animals in the epidemiology of FMD in Nigeria is still unclear.
- Research reports has always been regionalised and fragmented.
- In 2007, we started the collection of samples from outbreak cases.



INTRODUCTION Contd.

- This is in order to determine the circulating serotypes in the country.
- In the meantime, we are collecting samples across the country for typing and molecular studies.
- This is to recommend a national control strategy for implementation.



MATERIALS AND METHODS

- Nigeria is divided into three agro-ecological zones.
  - . Forests
  - . Savannahs
  - . Montane land
- FMD surveillance in Nigeria is undertaken by NADIS and NVRI.
- During 2007-2009, areas that reported outbreaks were visited.
- Team of experts were dispatched to conduct a disease investigation.



MATERIALS AND METHODS

- Within the period under review, 10 outbreak cases were reported to the NVRI Vom.
- Samples were collected.
- Epithelial tissues, oral swabs and vesicular lesions from clinically sick animals were collected.
- The samples were transported to the laboratory in virus transport media on ice.



MATERIALS AND METHODS

- In the laboratory samples were processed and packaged according to International standard for the transportation of infectious materials affecting animals (UN2900).
- The samples were sent to the WRLFMD, IAH Pirbright, UK for confirmatory diagnosis and serotyping.
- Other samples collected by a postgraduate student were sent to PIADC-FADDL for confirmatory diagnosis and serotyping as well.



MATERIALS AND METHODS

- Virus was isolated in primary bovine thyroid cell culture and serotyped using antigen capture ELISA, and molecular analyses were performed at the WRL according to standard procedures.



RESULTS



- 48 samples tested positive for FMD; 36 were typed while the rest 12 were positive at PCR with no virus detected in cell culture.
- Sero-typing 36 (75.0%) sero-positive samples; O (4.2%), A (52.1%) and SAT 2 (18.7%).
- No SAT 1 was recovered from the recent outbreaks.
- From the 30 tissues submitted to PIADC-FADDL, 21/30 (70%) were positive and typed as serotype A.
- Phylogenetic analysis of the PI and the VPI regions revealed close identity to A21 Kenya 1984 virus and Cameroon 2000 virus respectively.

RESULTS

- In Nigeria, both serotypes O, A and SAT 2 co-circulated between 2007 and 2009.
- Sequence analyses indicated that the serotype O that occurred in 2007/2008 as well as the 2009 are closely related to the Sudan 2004 and 2005 isolates respectively.
- Similarly, the SAT 2 viruses were closely related to Sudan 2007 and Niger Republic 2005 outbreaks.



SAMPLE DISTRIBUTION

	SAMPLE PER ZONE (%)	SAMPLE +VE PER ZONE (%)	SAMPLE –VE PER ZONE (%)
NORTH CENTRAL	55 (59.8)	33 (60.0)	22 (40.0)
NORTH EAST	21 (22.8)	10 (47.6)	11 (52.4)
SOUTH WEST	16 (17.4)	5 (31.3)	11 (68.7)



REGIONS/DISTRICTS WHERE SAMPLES WERE COLLECTED

ZONE	DISTRICT
NORTH CENTRAL	MINNA, JOS, BUKURU, BARIKIN-LADI, VOM, SHENDAM
NORTH EAST	BAUCHI, NABORDO, TAFAWA BALEWA, YOLA
SOUTH WEST	ABEOKUTA, IBADAN, EKITI



PROPORTION OF SEROTYPE POSITIVE BY ZONE 2007-2009

ZONE	OVERALL	O	A	SAT 2	NVD
NORTH CENTRAL	33/55 (60.0%)	1/33 (3.0%)	22/33 (66.7%)	8/33 (24.2%)	2/33 (6.1%)
NORTH EAST	10/21 (47.6%)	2/10 (20.0%)	3/10 (30.0%)	1/10 (10.0%)	4/10 (40.0%)
SOUTH WEST	5/16 (31.3%)	0/5 (0.0%)	0/5 (0.0%)	0/5 (0.0%)	5/5 (100.0%)



SUMMARY OF OUTBREAKS

PERIOD	NUMBER OF REPORTED OUTBREAK
2007	1
2008	4
2009	5
TOTAL	10



DISCUSSION

- Nigeria is one among the least countries sampled for FMD.
- Very little efforts have been made in time past to control FMD in Nigeria.
- However, FMD still continues to gain importance as livestock farming becomes more intensive in the global fight against food security.
- The serotypes O, A and SAT 2 isolated from Nigeria were from locations along the nomadic trade routes.



DISCUSSION

- These locations serve as exchange points of shared infections as the vegetation is good for livestock husbandry.
- As majority of these animals are rarely sedentary, the location of isolation may significantly differ from the point of infection.
- From this study, genetic sequences have confirmed high degree of relatedness.

DISCUSSION

- Samples collected in 2007 and 2008 were submitted to the WRLFMD for the first time in many years, resulting in the identification of serotypes O and SAT 2.
- Interestingly, isolates of both serotypes were genetically closest to previously characterised isolates from Sudan (pool 4) obtained between 2005-07.
- More studies are required to define the relationships between viruses of pool 4 and 5.



DISCUSSION



- FMDV types O and A were isolated from samples in 2009.
- A single type O virus belonged to the EA-3 topotype and was most closely related to viruses of 2007 and Sudan (2004-08).
- Eight type A viruses belonged to the AFRICA topotype (lineage G-I), but fell into two distinct sub-lineages, one of which clustered with Kenyan viruses.
- Four type A viruses all belonged to the AFRICA topotype G-IV lineage but fell into two distinct sub-lineages.
- Our analyses revealed a pattern of FMDV serotypes that traverses beyond boundary of Nigeria.
- It is thus necessary to carry out a comprehensive assessment of the recent FMD situation in Nigeria.

SUMMARY OF REPORTED OUTBREAKS IN NIGERIA

YEAR OF OUTBREAK	SEROTYPE IDENTIFIED
1920	untyped
1924	O
1961	A
1962	A
1963	SAT 1, SAT 2, O
1964	SAT 1, A
1965	A
1966	A
1967	A
1968	SAT 1, A
1970	SAT 1, A
1971	A



REPORTED OUTBREAKS

1972	SAT 1, A
1973	SAT 1, SAT 2
1974	SAT 2, A
1975	SAT 1, SAT 2, A
1976	SAT 1, A
1979	SAT 1, A
1980	SAT 1
1981	SAT 1, SAT 2
1982	SAT 2
2007	O, SAT 2
2008	SAT 2
2009	O, A

Source :**WRLFMD, TADP OVI, FAO,OIE.**



DISCUSSION

- Successful control of FMD in Nigeria relies strictly on quarantine and movement control at the border states and vaccinations.
- Though vaccines have limitations;
  - .Custom made vaccines
  - .Cold chain
  - .Cost
  - . Incorrect application



ACKNOWLEDGEMENT

- We thank N. J. Knowles, J. M. Hammond and their staff for the confirmation of these outbreaks.
- We acknowledge field staff for their support in sample collection.
- The management of National Veterinary Research Institute, Vom.
- National Animal Disease Information System (NADIS), Nigeria.
- The EuFMD for funding my trip and expenses to attend this event.



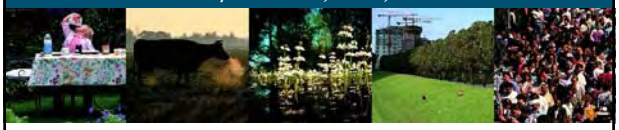
THANK YOU FOR LISTENING!



Serological evidence of foot-and-mouth disease virus serotype C & SAT-1 infections in Eritrea

Tesfaalem T. Sebhatu, Aldo Dekker, Rob J.M. Moormann

Eufmd, 2010 programme
30 September 2010, Vienna, Austria



CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

Objectives of this study

- Information on the distribution and prevalence of antibodies against FMDV serotypes
- Design future plan to conduct country wide FMD sero-surveillance study
- Enable and improve the capacity of FMD diagnosis in Eritrea

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR



CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

Livestock Population in Eritrea

- Cattle: 2.1 million
- Sheep: 2.3 mil.
- Goats: 5.0 mil.
- Camels (Dromedary): 0.4 mil.
- Equine: 0.5 mil.

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

History of Foot-and-mouth disease in Eritrea

- Repeated waves of infection every year;
  - ✓ in the traditional extensive transhumance and pastoralist cattle herds
  - ✓ uncontrolled animal movement is key factor for the spread of FMD across the permeable borders.
- The national indigenous cattle herd has never been vaccinated against FMD.

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

FMDV genotyped at OIE/FAO WRLFMD Pirbright, U.K. from outbreaks in Eritrea 1996 – 2009

Serotype	Year	Lineage / Topotype
O	1996	Not known
A	1997-1998	Central Africa 2 / V
SAT-2	1997-1998	VII
O	2004	EA-3/I
A	2006-2009	AFRICA / G-IV

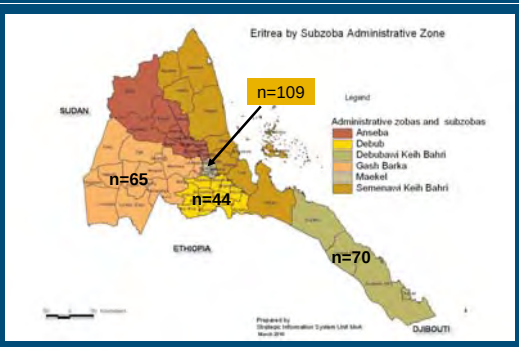
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Conti.

- In outbreaks of 2006 - 2009, the VP1 gene sequence and phylogenetic analyses of type A viruses showed high sequence identity with Sudanese (>96.4%) and Cameroon (>89.83%) virus isolates indicating transmission of FMDV within the Sahelian region between East and West Africa (WRLFMD, Pirbright Report, 2010).

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Materials & Methods: Serum sample collection sites



CIDC-LELYSTAD WAGENINGEN UR

Materials and Methods

FMD NS ELISA:

- All sera (n=218 + 70) were screened for non-structural protein (NSP) antibodies using the FMDV NS kit (PrioCHECK FMDV NS, Prionics)

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Materials and Methods

- Serum samples (n=74 CVI Lelystad):

from experimentally vaccinated and/or challenged animals tested using virus neutralization test (VNT) to validate possible cross-neutralization reactions.

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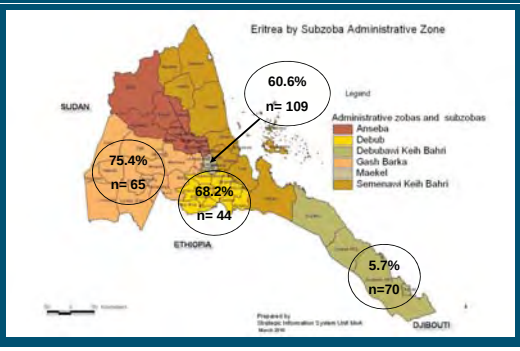
Materials and Methods

VNT:

- The neutralizing activity of serum samples against 100 TCID50 of virus was determined in BHK-21 cells for the 7 FMDV serotypes.
- Each test was performed in duplicate and results were recorded as the mean 10 log titres of the serum dilution.
- Interpretation of VNT results for FMD as described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2008.

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Results: FMD NS ELISA positives



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Percentage of positive field serum samples ≥ 10 log 1.65 VNT titres

Region	Total sera	A	O	C	SAT-1	SAT-2	SAT3	Asia1
Debub	44	14	17	31	12	8	-	4
G. Barka	65	13	50	32	15	3	-	3
Maekel	109	45	79	65	51	6	1	7
Total	218	72	146	128	78	17	1	14

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VNT Results on experimentally vaccinated and/or challenged animals to FMDV (n=74)

No. of sera	A	O	C	SAT-1	SAT-2	SAT-3	Asia1	Vacc.	Chall.
2	2	2	2	0	0	0	2		A, C & Asia1
17	8	4	0	0	2	0	1	A	A
6	0	0	2	0	1	0	6	Asia1	Asia1
49	0	40	1	1	11	0	1	O	O

Cross-neutralization reactions indicated with red colour

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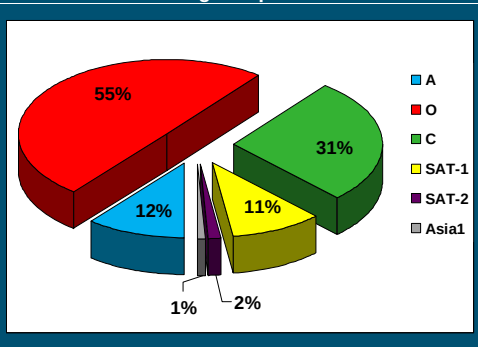
Results of VNT from the 74 experimental serum samples

- Significant cross-neutralization reactions was observed among the FMDV serotypes
- Some sera showed higher VNT titre with the heterologous than the homologous virus.

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Results

No. of field sera with the highest positive VNT titre



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Conclusion

- Most field serum samples were positive for more than one FMDV serotype which could be due to repeated rounds of infection with the different FMDV serotypes.
- Nevertheless, cross-neutralization reactions can not be overlooked as it has been observed in experimental challenge infections.

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Conclusion

- FMD serotype C and SAT-1 viruses, however, have never been detected in Eritrea, but the VNT demonstrates that infections with type C and SAT-1 could have been occurred.
- Further FMD surveillance with extensive epidemiological investigation including virus isolation from probang and epithelial tissue samples is imperative for a detailed image on the disease in Eritrea.

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Acknowledgments

CVI, Wageningen UR, Lelystad
- Mr Klaas Weerdmeester
- Mrs Froukje van Hemert-Kluitenberg

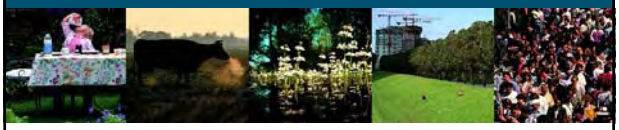
Ministry of Agriculture, Eritrea
- Animal Health staff

- This study was sponsored by the International Atomic Energy Agency (IAEA), Vienna, Austria

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Serological evidence of foot-and-mouth disease virus serotype C & SAT-1 infections in Eritrea

Tesfaalem T. Sebhatu, Aldo Dekker, Rob J.M. Moormann



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ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS IN AFRICAN BUFFALO HERDS IN DIFFERENT NATIONAL PARKS IN UGANDA (2001-2008)

Chrisostom Ayebazibwe, Frank Norbert Mwiine, Kirsten Tjørnehøj, Sheila Nina Balinda, Vincent B Muwanika, Ademun Anna Rose Okurut, Graham J Belsham, Charles Masembe, Hans Redlef Siegismund, Soren Alexandersen



Introduction

- FMD is endemic in Uganda, multiple serotypes and possibly strains of FMDV. Multiple risk factors including livestock-wild life interactions
- No much substantive epidemiological data. Vaccination and quarantine are the major methods employed to control the spread of FMD
- African buffalos (*Syncerus caffer*) believed to play a major role as reservoirs of the SAT-serotypes

National Parks in Uganda



10 national parks and 10 game reserves –unfenced, with over 15,000 buffalos.

Livestock populations in Uganda (2008)





Cattle = 11.4 millions, Goats = 12.5 millions, Sheep = 3.4 million, Pigs = 3.2 million

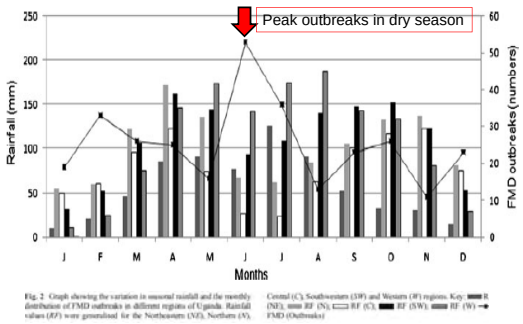
Note the size of herds

FMDV serotypes in Uganda

Time	O	A	C	SAT 1	SAT 2	SAT
1950-	1955-59	1953,		1953,	1956,	
1960-	1961-69	1960-69		1961	1966-69	
1970-	1970-76,	1970-76	1970-71	1970-74,	1970-76	1970
1980-						
1990-	1998			1997,	1991,	1997
2000-	2000,	2002			2002	

WRLFMD, 2009. *Only from African buffalos

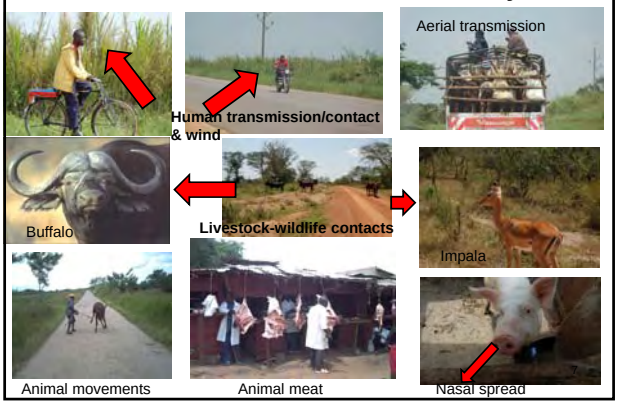
Patterns of FMD outbreaks in Uganda -2001-2008



FMD outbreaks were reported over different years, months and regions

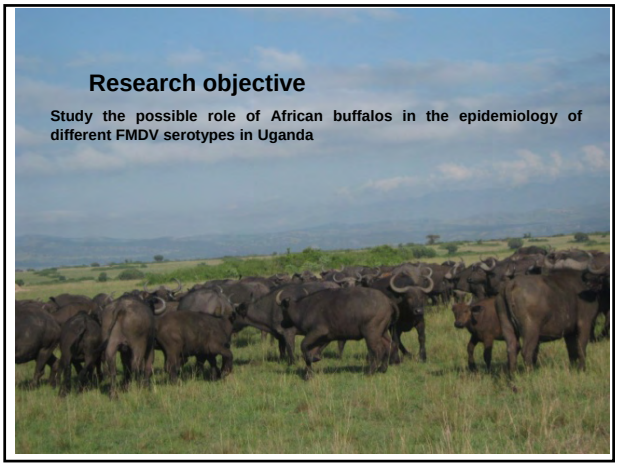
6

FMD transmission/risk factors are many

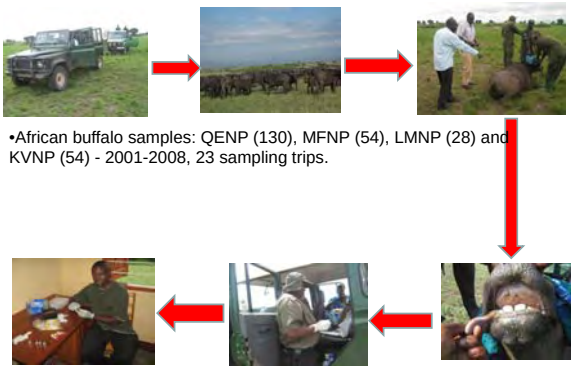


Research objective

Study the possible role of African buffalos in the epidemiology of different FMDV serotypes in Uganda



Field sampling



•African buffalo samples: QENP (130), MFNP (54), LMNP (28) and KVNP (54) - 2001-2008, 23 sampling trips.

Laboratory methods

- Ceditest® FMDV type O ELISA
- Ceditest® FMDV NS ELISA
- Serotyping: SPBEs. Samples were considered positive at dilutions≥80.

Results

National Park	Sampling Trip	Total Samples	Antibody screening results		Serotypes† identified at high dilutions (≥80)				
			NSP+	SP+	O	C	Sat 1	Sat 2	Sat 3
KVNP	SEPT. 02	12	10/12	5/12	0/2	0/4	4/6	0/6	1/6
	SEPT.05	21	10/21	3/10	*	*	*	*	*
	OCT.06	8	7/8	3/7	*	*	*	*	*
	NOV.06	10	9/10	3/10	0/1	**	1/1	*	**
	JUL.07	3	0/3	*	*	*	*	*	*
		54	36/54	14/39	0/3	0/4	5/7	0/6	1/6
LMNP	NOV.03	3	0/1	0/1	0/0	0/2	1/2	1/2	**
	JAN.06	6	6/6	0/6	0/1	**	**	3/3	**
	JAN.07	7	7/7	2/7	0/2	**	1/2	**	**
	APR.07	6	5/5	4/5	1/4	0/2	3/4	2/4	1/4
	OCT.08	6	*	*	0/4	0/6	4/4	0/5	1/5
		28	18/19	6/19	1/11	0/10	9/12	6/14	2/9
MFNP	SEPT.02	1	0/1	0/1	0/1	**	**	**	**
	OCT.05	20	20/20	7/20	2/3	0/3	3/3	3/3	3/3
	NOV.06	19	17/19	5/18	0/3	**	2/3	1/3	2/3
	OCT.07	14	14/14	7/14	1/4	0/2	3/4	2/4	4/4
		54	51/53	19/52	3/11	0/5	8/10	6/10	9/10
QENP	JUL.01	29	18/24	6/26	4/12	1/4	7/18	8/14	8/18
	JAN.06	3	0/3	**	**	**	**	**	**
	JAN.07	9	9/9	2/9	**	**	1/1	1/1	**
	APR.07	30	25/29	4/30	0/1	**	**	1/2	**
	JUL.07	12	11/12	0/12	1/2	**	1/1	1/1	**
	AUG.07	17	16/17	9/16	2/3	0/3	3/3	3/3	3/3
	SEPT.07	7	5/7	0/5	**	**	**	**	**
	JAN.08	16	15/16	6/16	**	**	**	**	**
	OCT.08	7	*	*	0/7	0/4	2/2	2/4	2/4
		130	99/117	27/114	7/25	1/14	14/25	16/25	13/25
Total		**266**	**204/243**	**66/221**	**11/50**	**1/30**	**36/54**	**28/55**	**25/50**

*: Not done
**: All tested negative
†: The numbers of samples titrated depended on the results of serotype screening in Solid Phase Blocking ELISA (results not shown) and the availability of sufficient sample amounts

- 84.0% seropositivity for the NSP antibodies
- 29.5% seropositivity for SP O antibodies
- FMDV seroprevalence: SAT 1 (36/54) > SAT 2 (28/55) > SAT 3 (25/50) > O (11/50) > C (1/30).
- Consistent detection of antibodies against FMDV among all the sampling trips (n ≥ 3) in the different national parks for all the years 2001-2008.
- Mixed antibodies against different FMDV serotypes were detected during 12/23 (52%) sampling trips.



Conclusions & Recommendations

- African buffalos may play a role in the epidemiology of SAT FMDVs in Uganda
- Also, may play a role as hosts for serotypes O and even perhaps C, although further studies are needed –including virus characterization and molecular epidemiological studies

Acknowledgement

This is part of DANIDA funded collaborative research project on Livestock-Wildlife Diseases in East Africa by 4 Institutions:

1. National Animal Disease Diagnostics and Epidemiology Centre, Ministry of Agriculture Animal Industry and Fisheries, Entebbe, Uganda
2. Makerere University Institute of Environment and Natural Resources, Kampala, Uganda
3. National Veterinary Institute, Danish Technical University, Lindholm, Denmark
4. Department of Biology, University of Copenhagen, Denmark

Technical University of Denmark DTU

Thanks to EU FMD Commission for organizing the meeting

Bayesian Evolutionary Analysis of Foot-and-Mouth Disease Type A Virus in Africa

Nick J. Knowles, Jemma Wadsworth, Rebecca J. Midgley and Donald P. King

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BBSRC

1

Introduction

- Foot-and-mouth disease (FMD) type A viruses have been classified into three topotypes (AFRICA, ASIA and EURO-SA) based on the nucleotide distances between their VP1 gene sequences.
- However, such methods are not based on an evolutionary model and virus isolation dates are not taken into account.
- It is therefore difficult to deduce the evolutionary history of the viruses being examined.
- We have used Bayesian evolutionary analysis to try and resolve these problems.

2

Materials & Methods

- FMDV RNA extraction, reverse transcription, VP1 amplification and DNA sequencing was carried out using standard methods (Knowles et al., 2009; Transbound. Emerg. Dis. 56, 157-169).
- A phylogenetic tree was generated using the Neighbor-joining (N-J) method with bootstrap re-sampling (MEGA 4.0).
- Within-lineage regression analysis of sequence distances was used to estimate origin dates.
- Bayesian evolutionary analysis was performed using the BEAST software package. The Bayesian Markov Chain Monte Carlo (MCMC) method, using virus isolation dates, was used to generate a tree.

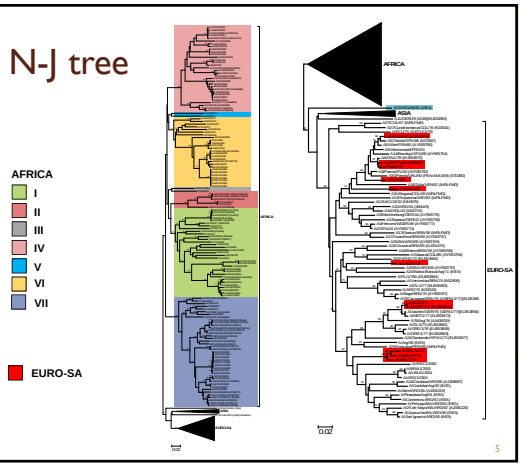
3

Results

- Complete VP1 nucleotide sequences of 152 African FMD type A viruses, isolated between 1964 and 2009, were compared with each other and with reference virus strains.
- All viruses belonging to the AFRICA topotype fell into seven genetic clusters (I to VII).
- 11 African viruses fell into the EURO-SA topotype.
- One virus could not be placed into any of the three topotypes,

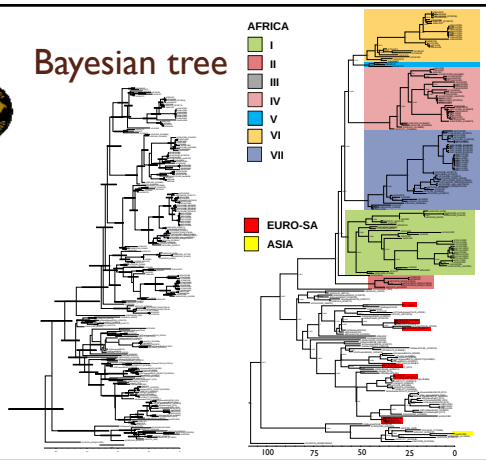
4

N-J tree



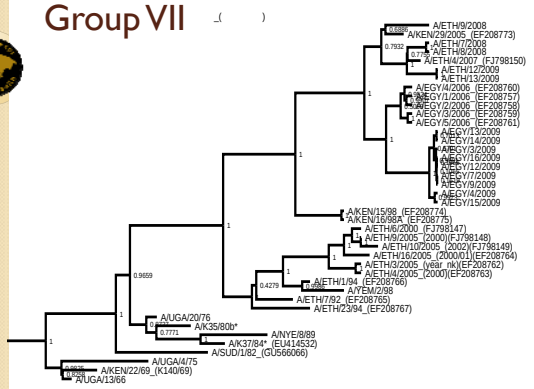
5

Bayesian tree



6

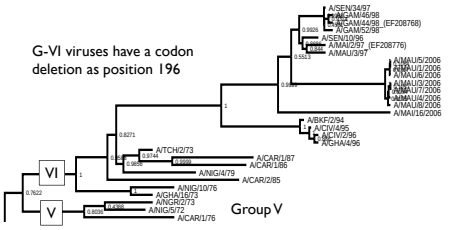
Group VII



7

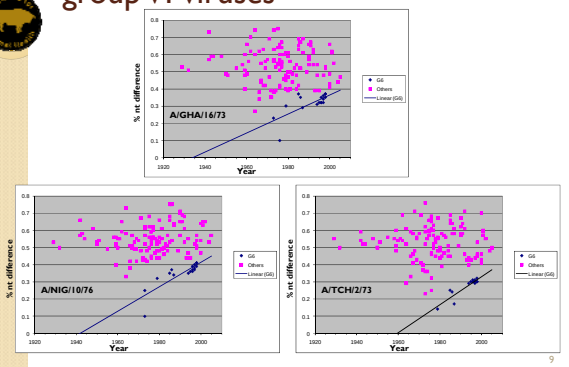
Groups V & VI

G-VI viruses have a codon deletion as position 196



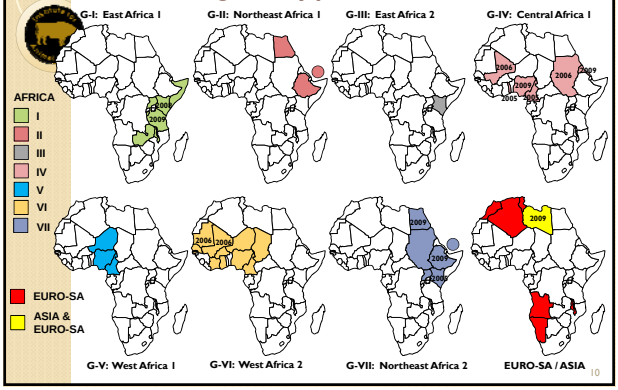
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Regression analyses of FMDV A group VI viruses



9

FMDV A genotypes



10

Conclusions

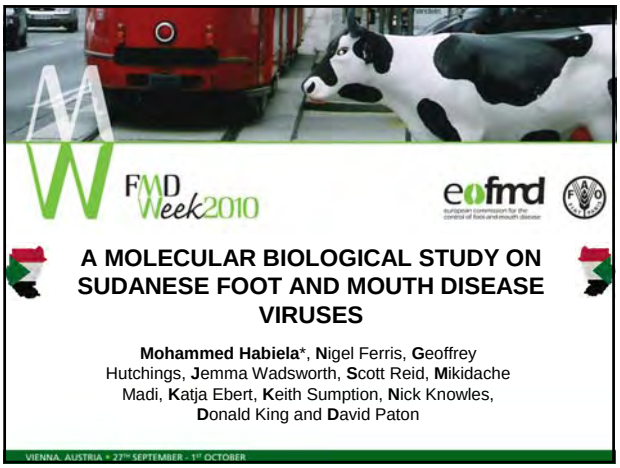
- Using the N-J tree, seven FMDV A genotypes (G-I to G-VII) were defined within the AFRICA topotype.
- Bayesian evolutionary analysis broadly agreed with the groupings defined using the N-J tree, although G-V and G-VI could be considered as a single cluster.
- No recent examples of G-II, G-III and G-V were found therefore these genotypes may be extinct.
- Bayesian analysis suggested that the seven African genotypes had a common ancestor 60-75 years ago.
- Regression analyses of within-lineage sequence distances were consistent with this dating confirming the relatively recent origin of most FMDV A's in Africa.

11

Conclusions

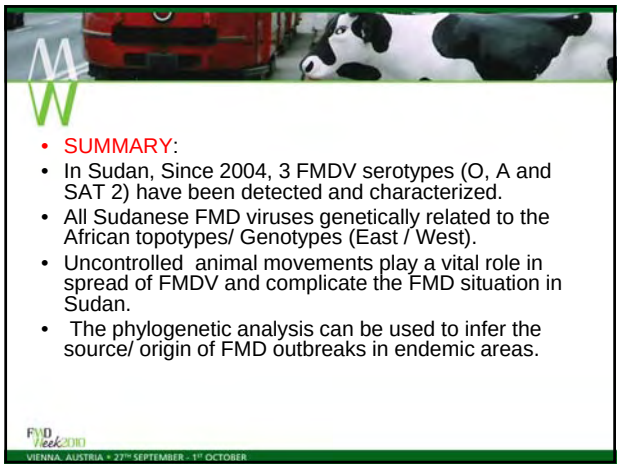
- A single isolate (A23/Kitale/KEN/64) could not be reliably placed within any of the three FMDV A topotypes and appeared to have diverged from other type A viruses some 74-112 years ago.
- Between 1967 and 1983 FMD type A viruses belonging to the EURO-SA topotype were introduced into North Africa (Algeria, Libya, Morocco and Tunisia) and southern Africa (Angola, Malawi and Namibia) on at least five occasions.
- African type A viruses are not found outside the continent (except for occasional incursions into the Yemen Arab Republic).

12

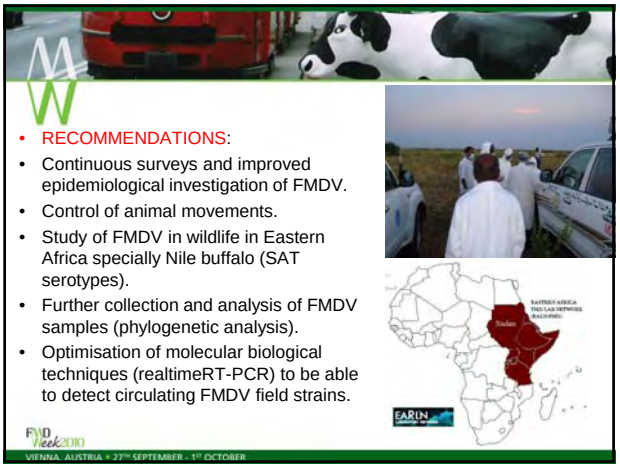


A MOLECULAR BIOLOGICAL STUDY ON SUDANESE FOOT AND MOUTH DISEASE VIRUSES

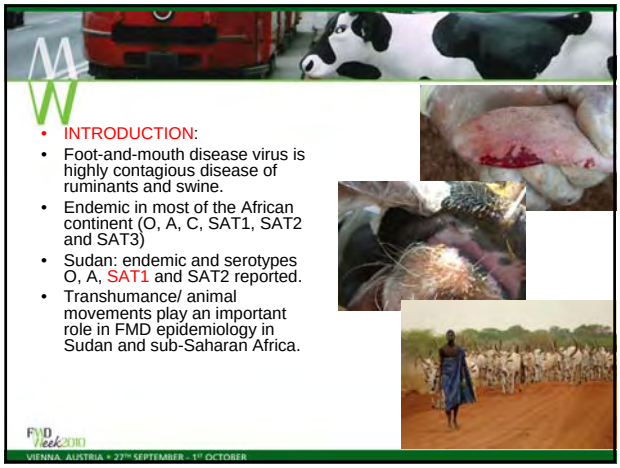
Mohammed Habiela*, **N**igel Ferris, **G**eoffrey Hutchings, **J**emma Wadsworth, **S**cott Reid, **M**ikidache Madi, **K**atja Ebert, **K**eith Sumption, **N**ick Knowles, **D**onald King and **D**avid Paton



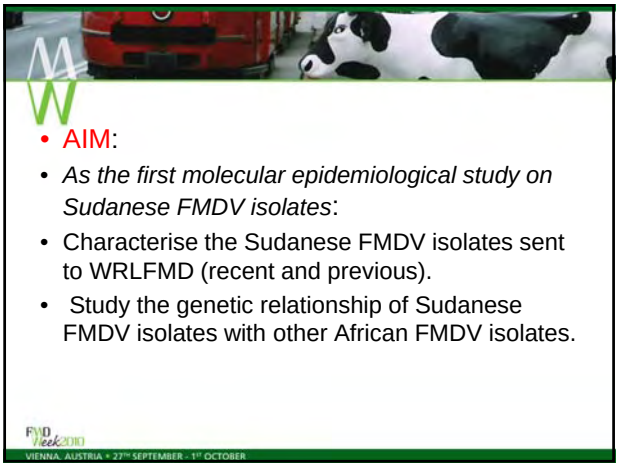
- SUMMARY:
- In Sudan, Since 2004, 3 FMDV serotypes (O, A and SAT 2) have been detected and characterized.
- All Sudanese FMD viruses genetically related to the African topotypes/ Genotypes (East / West).
- Uncontrolled animal movements play a vital role in spread of FMDV and complicate the FMD situation in Sudan.
- The phylogenetic analysis can be used to infer the source/ origin of FMD outbreaks in endemic areas.



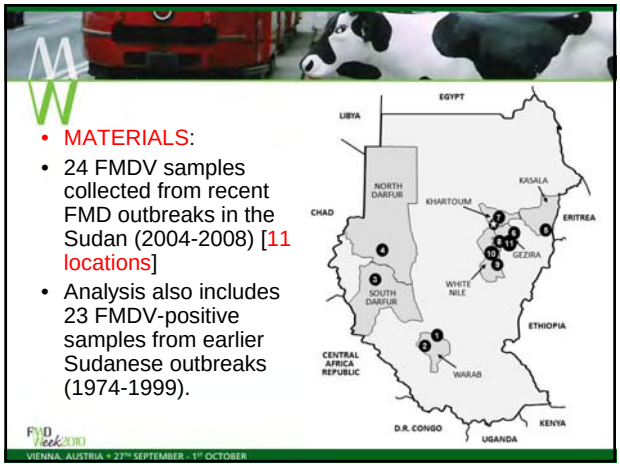
- RECOMMENDATIONS:
- Continuous surveys and improved epidemiological investigation of FMDV.
- Control of animal movements.
- Study of FMDV in wildlife in Eastern Africa specially Nile buffalo (SAT serotypes).
- Further collection and analysis of FMDV samples (phylogenetic analysis).
- Optimisation of molecular biological techniques (realtimeRT-PCR) to be able to detect circulating FMDV field strains.



- INTRODUCTION:
- Foot-and-mouth disease virus is highly contagious disease of ruminants and swine.
- Endemic in most of the African continent (O, A, C, SAT1, SAT2 and SAT3)
- Sudan: endemic and serotypes O, A, SAT1 and SAT2 reported.
- Transhumance/ animal movements play an important role in FMD epidemiology in Sudan and sub-Saharan Africa.



- AIM:
- *As the first molecular epidemiological study on Sudanese FMDV isolates*:
- Characterise the Sudanese FMDV isolates sent to WRLFMD (recent and previous).
- Study the genetic relationship of Sudanese FMDV isolates with other African FMDV isolates.



- MATERIALS:
- 24 FMDV samples collected from recent FMD outbreaks in the Sudan (2004-2008) [11 locations]
- Analysis also includes 23 FMDV-positive samples from earlier Sudanese outbreaks (1974-1999).

- METHODS:
- VI (BTY/IB-RS-2), FMD-Antigen ELISA, rRT-PCR, Phylogenetic analysis were carried out.

❖ ***Some samples have been tested by the CVRL, (Khartoum, Sudan) using virus isolation (VI) and antigen-detection ELISA prior to submission to the WRLFMD. (Habiela et al., 2008).***

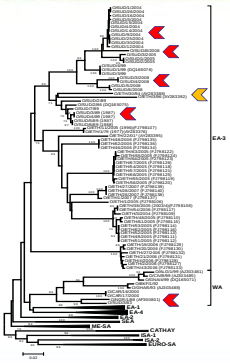




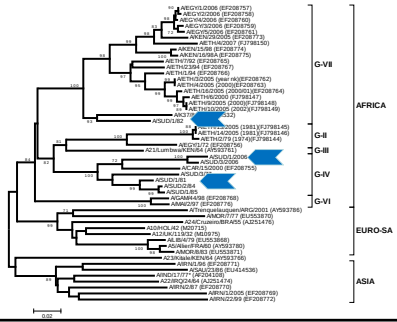
- PCR &Sequencing:
- FMDV one-step RT-PCR (5UTR & 3D) were carried out.
- One-step RT-PCR Oligonucleotide primers for sequencing (VP1) were selected based on results from Ag-ELISA.
- For sequencing, a set of primers (forward and reverse) specific for each serotype (O, A &SAT2) were used.

- RESULTS:
- FMD Antigen ELISA:
- The twenty-four FMDV samples, collected during 2004-2008, were typed as:
- Serotype O (n = 19),
- Serotype SAT2 (n = 3) ,
- Serotype A (n = 2).
- realtime RT-PCR:
- All of the samples were positive using the 3D assay, in contrast, the 5' UTR generated poorer signal (O & SAT2).

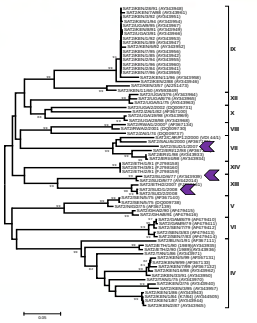
- Results (cont.):
- Genetic relationship:
- Serotype O:
- The 19 recent serotype O isolates, and all of the archived FMD serotype O isolates, were EAST AFRICA-3 [EA-3] topotype.
- [EA-3] group also contained two older viruses from Ethiopia (O/ETH/30/94 and O/ETH/3/96).
- The exception was a single isolate (O/SUD/3/83) that was related to viruses from Nigeria in WEST AFRICA [WA] topotype.



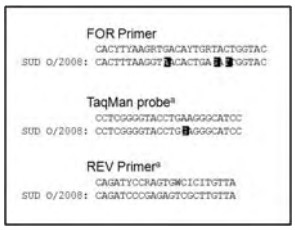
- Serotype A:
- Two samples of this serotype were detected (A/SUD/1/2006 and A/SUD3/2006), and fall within the G-IV genotype of the AFRICA topotype with the older FMDV isolates from the country beside an isolate from Cameroon (A/CAR/15/2000), .
- For archived samples: a single Sudanese (A/SUD/1/82) was characterised as a member of the G-VII genotype



- Serotype SAT2:
- The two isolates collected during 2008 (SAT2/SUD/1/2008 and SAT2/SUD/2/2008) were closely related to each other and to SAT 2 virus from Ethiopia (SAT2/ETH/2/2007) .
- These viruses were grouped together with older viruses collected during 1977 from Sudan in topotype[XIII].
- A further recent SAT 2 virus from Sudan (SAT2/SUD/1/2007) was grouped in topotype [VII].
- Viruses from [VII] lineage have also been identified from several other countries in sub-Saharan Africa (Cameroon, Eritrea [Bastos et al., 2003], Nigeria, Niger and Libya) and beyond (Saudi Arabia).



- Sequencing of FMDV O/SUD/2008 isolates:
- Sequencing of 5' UTR of these viruses showed nucleotides mismatches with some primers used in realtimeRT-PCR.

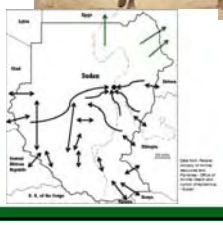


nt mismatches highlighted

- **CONCLUSION:**
- Since 2004, 3 FMDV serotypes (O, A and SAT 2) have been detected and characterized.
- These viruses were related to isolates from Sudan/ Africa.
- Serotype O was the most frequently recovered FMDV serotype (79 % of contemporary isolates and 70% of the historical samples).
- Sudanese type O isolates belong to EA-3 and WA, Type A to G-IV and G-VII, and SAT2 to VII and XIII.
- The phylogenetic analysis may infer how FMD viruses might be dispersed between countries in the region.
- The sampling density of FMD outbreaks is generally low in Sudan/across sub-Saharan Africa.

- Conclusion (cont):
- Uncontrolled animal movements play a significant role in spread of FMDV (SAT2-2008).
- Since 1976, no FMDV- SAT1 viruses has been detected in Sudan.
- previous SAT1 isolates were found to be related to other FMD viruses from West Africa within topotype [VI].
- Reintroduction of SAT1 to Sudan remains a possibility [e.g. SAT 1 outbreaks in Ethiopia, in 2007 (Ayelet et al., 2009)].
- There are clear gaps in some countries that neighbour Sudan where FMD outbreaks are rarely reported.





- ACKNOWLEDGEMENT:
- ARRC-CVRL & FMARF (Sudan).
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- WRLFMD- Pirbright Lab (UK).
- *Special thanks*:
- *Prof. Musa Tibin (ARRC-CVRL).*
- *EUFMD.*
- This study was funded by ARRC, FAO and DEFRA.



** THANK YOU FOR LISTENING **

A review of foot-and-mouth disease viruses collected in Tanzania from 1967 to 2009

Christopher J Kasanga
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Sokoine University of Agriculture
Central Veterinary Laboratory
Institute for Animal Health

Summary and conclusion

- The aim of this paper was to review the FMD viruses collected in Tanzania from 1967 to 2009
- Serotypes A, O, SAT1 and SAT2 viruses prevail in different regions of Tanzania, and contribute to FMD outbreaks
- However, the epidemiology and factors associated with outbreaks remain unclear and need to be investigated
- Improved FMD surveillance, genetic and antigenic characterisation of FMDV field strains is recommended to understand endemicity and hence rational control measures of the disease

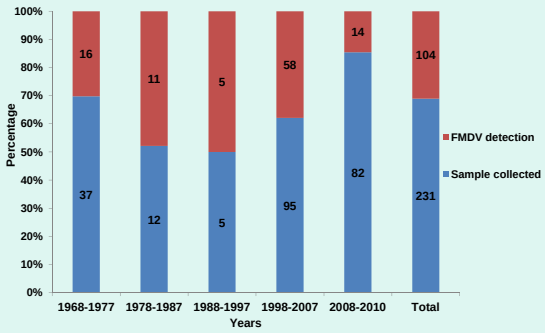
FMD in Tanzania

- FMD is endemic in Tanzania
- First FMD outbreak reports ~ 1954
- Animal affected: Cattle, Pigs, small ruminants, wild animals
- Outbreaks occur in different geographic regions
- Factors associated with outbreaks are not clearly known

Samples and analysis

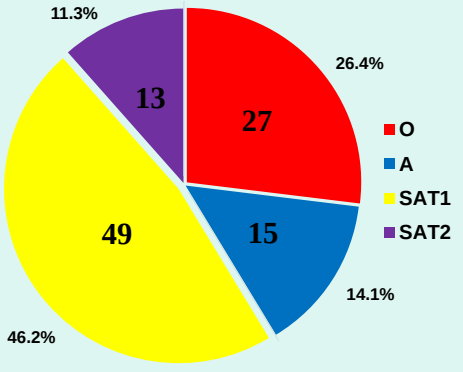
- 231 samples (epithelial tissues, probang samples, and whole blood) were submitted to the WRLFMD from 1967 to 2009 for analysis
- Lab analysis for FMDV was conducted by VI, CF, Antigen ELISA, RT-PCR and sequencing of the VP1 gene
- Phylogenies of VP1 sequences were determined by Neighbor-joining method

Detection rate of FMDV

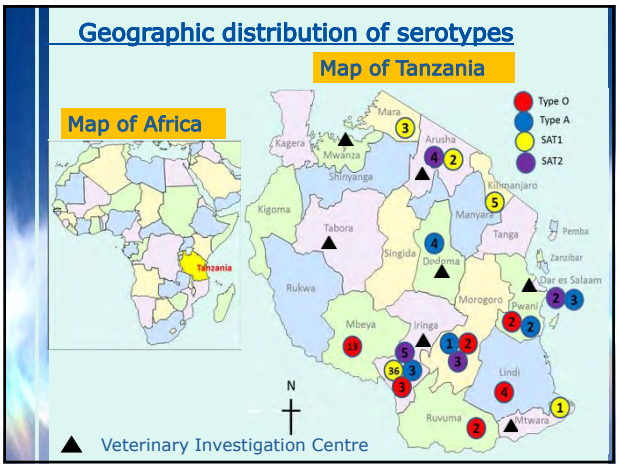


- Detection rate varied with time and geographic location
- Are the correct samples being collected?
- Preservation of the samples?

Detection frequency of FMDV serotypes



Geographic distribution of serotypes

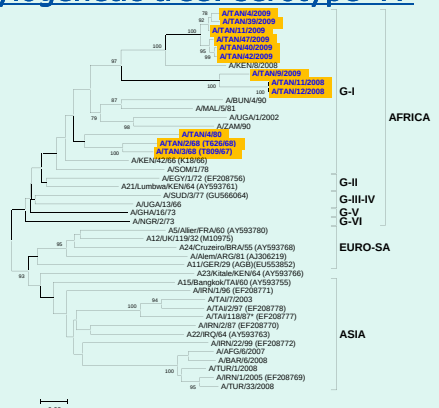


Years and zones of FMDV detection

Serotype	Years detected	Zones in Tanzania
O	1971, 1980, 1984, 1985, 1996, 1998, 2004, 2008, 2009	Southern and Eastern-Coastal
A	2008, 2009	Eastern, Southern, Central and Eastern-Coastal
SAT1	1971, 1972, 1977, 1980, 1996, 1999, 2008	Southern, Northern, Northern-lake, Southern-Coastal
SAT2	1970, 1972, 1975, 1986, 1999, 2004, 2009, 2010	Southern, Northern, Eastern and Eastern-Coastal

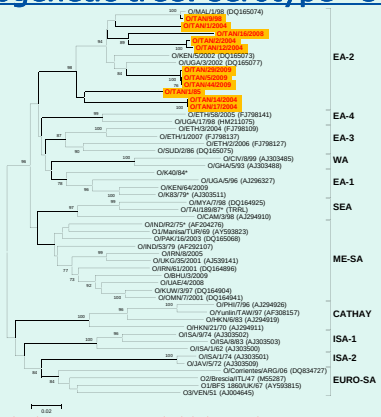
- No FMDV isolates from Western zone???

Phylogenetic tree: serotype "A"



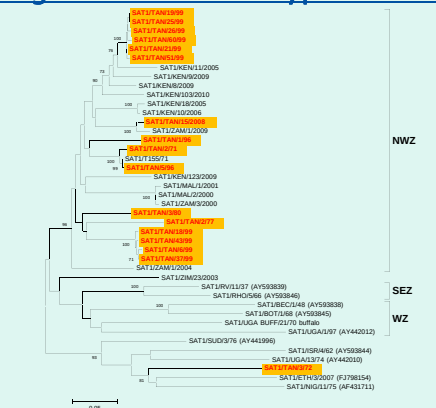
Coloured blue: Tanzania strains, which belong to the Africa G-I

Phylogenetic tree: serotype "O"



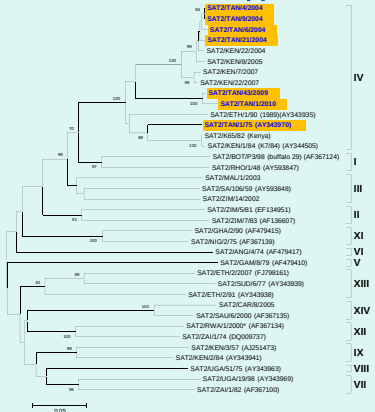
Coloured red: Tanzania strains, which belong to the EA-2 topotype

Phylogenetic tree: serotype "SAT1"



Coloured red: Tanzania strains, which belong to the NWZ and other topotype

Phylogenetic tree: serotype "SAT2"



Coloured blue: Tanzania strains, which belong to the IV topotype

Discussion and conclusion

- Serotypes A, O, SAT1 and SAT2 are the main cause of FMD outbreaks in Tanzania
- FMDV isolates so far detected in Tanzania are genetically related to lineages and topotypes from West and East Africa
- Presence of multiple serotypes and topotypes complicates FMD control in the region
- Understanding the spatio-temporal distribution, epidemiology, genetic and antigenic characteristics of circulating FMDV is a pre-requisite for control of FMD in Tanzania and sub-Saharan region

Recommendations

- FMD outbreak investigation:
  - More sample collection and appropriate diagnosis is needed
- Research to describe the complex epidemiology and endemicity of FMD in Tanzania and sub-Saharan Africa is needed
- Molecular characterisation and analysis of many FMD samples is needed to elucidate the phylodynamics and evolutionary nature of FMDV
- Cross-protection and vaccine-matching of the field isolates to available vaccines is required

Future work

- Collaborative research on FMD in Tanzania by SACIDS, BBSRC-CIDLID (UoG and IAH) and SADC-TADs projects will be conducted focusing at:
  - Improving surveillance and diagnostic capacity
  - Molecular, antigenic and evolutionary characteristics of circulating FMDV
  - Understanding livestock-wildlife interface on FMD epidemiology
  - Developing appropriate control strategies

Acknowledgements

wellcome trust

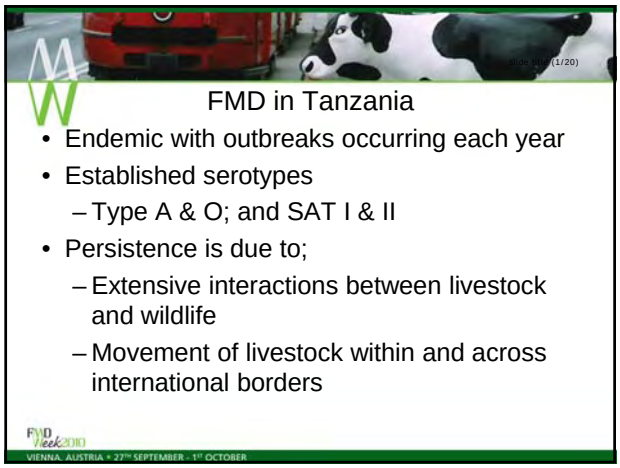
Don King
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Philemon Wambura
Chanasa Mpelumbe
Mmeta Yongolo
Mark Rweyemamu



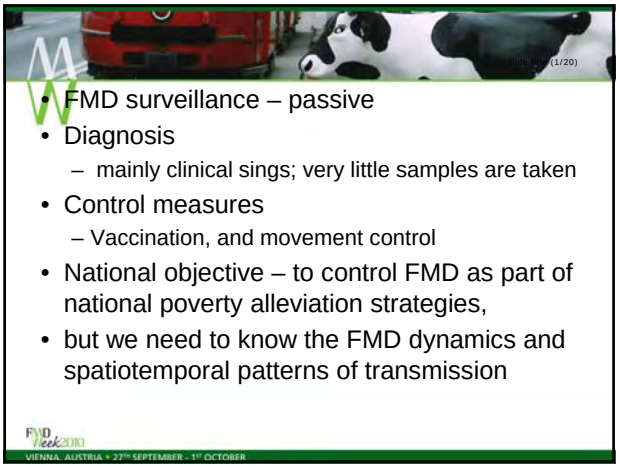
Descriptive and Spatiotemporal Analyses of Foot-and-Mouth Disease in Tanzania From 2001 to 2006

Kivaria F. M.



FMD in Tanzania

- Endemic with outbreaks occurring each year
- Established serotypes
  - Type A & O; and SAT I & II
- Persistence is due to;
  - Extensive interactions between livestock and wildlife
  - Movement of livestock within and across international borders



- FMD surveillance – passive
- Diagnosis
  - mainly clinical sings; very little samples are taken
- Control measures
  - Vaccination, and movement control
- National objective – to control FMD as part of national poverty alleviation strategies,
- but we need to know the FMD dynamics and spatiotemporal patterns of transmission

Study methodology

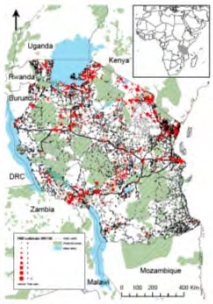
- **Objective**
  - **Improve the current knowledge on the dynamics and factors related to FMD occurrence, so control measures can be implemented more efficiently.**
- **Data**
  - **i. Passively collected FMD data – 2001 – 2006**
  - **ii. GEOnet – list of all villages in Tz**
  - **iii. World data base on protected areas**
  - **iv. ArcGIS & TADinfo**

Methodology

- **Analyses**
  - **i. Villages were the unit of analysis**
  - **ii. Extraction maps- used to determine high density distribution of FMD outbreaks**
  - **iii. Spatio-analyses were conducted using the package;**
    - **SatScan**

Results

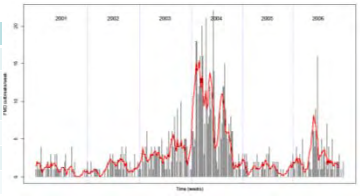
- 878 – FMD outbreaks in 605 villages were reported
- The number of outbreaks per location ranged from 0-9 and;
- FMD affected villages were mainly located on the borders, northern and central areas;
- Very few outbreaks were reported in the south or inside protected areas



Results

- Variable outbreaks

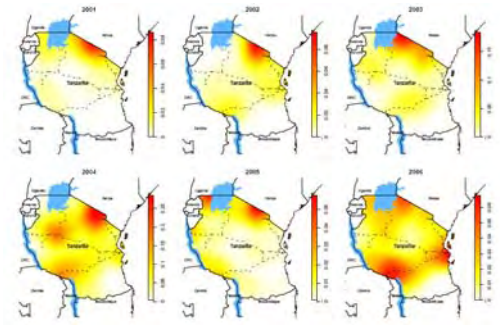
Year	FMD outbreaks
2001	52
2002	62
2003	160
2004	410
2005	59
2006	135

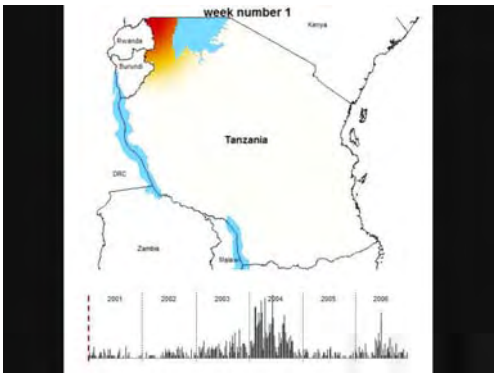


Temporal distribution of FMD outbreaks for the 2001 – 2006 reporting period

Results

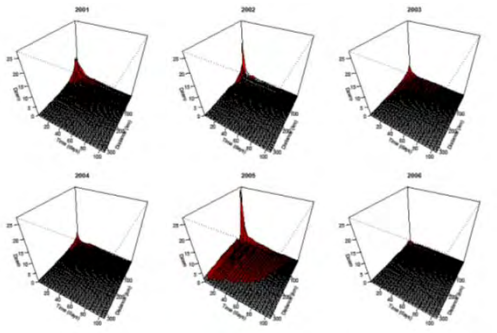
- **Spatial distribution**
  - i. Uneven
  - ii. Highest density recorded in 2003 & 2004;
  - iii. Located mainly in border with Kenya





The spatio-temporal interaction

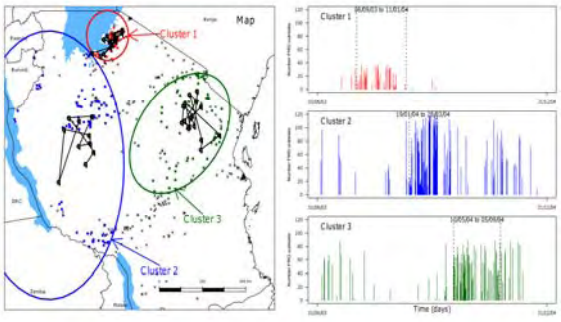
- Significant ($P \leq 0.01$)
- FMD affected villages were clustered at 80-100km in 2001 and 2002, but 2001 had a larger temporal component (50days)
- In 2003 there was an increase of the clustering in both dimensions which would indicate an increase of the infectiousness in time (65days) and space (200km)
- 2004 limited clustering ≈ 2001
- 2005 intense clustering at shorter distances (30km) and time (5days)
- 2006 – generalised distribution of FMD affected villages



Satscan analysis

- Three statistically significant clusters
  i. From 08/09/2003 to 11/01/2004; affecting 47 villages (RR = 7.97; $P \leq 0.001$)
  ii. From 19/01/2004 to 28/03/2004 affecting 99 villages (RR = 2.76; $P \leq 0.01$)
  iii. From 10/05/2004 to 05/09/2004 affecting 86 villages (RR = 2.72; $P \leq 0.01$)
- The spatio-temporal clusters were consecutive in time

SatScan + Mean centre



Discussion

- Data limitation – underreporting + clinical
- First time passive surveillance data used to model spatiotemporal dynamics in Africa
- Wildlife may play a role in maintaining and spreading the disease in the region BUT
- Clustering in border areas and communication networks would indicate that FMDV transmission was primarily related to human activity.

- The observed complex epidemiological dynamics → control is difficulty
- Control measures → regional approaches
- Many control options but zoning is probably the best option
  – Using the major railway lines as reference four major zones could be delimited;

FMD zone 1

- **FMD endemic area**
  – North of the central railway line (the Maasai ecosystem and the lake Victoria basin)
  – FMD control is challenging
  – Efforts to improve the knowledge on FMDV persistence in the pastoralists herds

FMD zone 2

- **FMD epidemic area**
  – Between the central and Tanzania-Zambia railway lines, where the number of cases increase during epidemic phases.
  – Surveillance need to be improved to detect the disease at early stages and
  – control of propagation along the international borders and communication networks

FMD zone 3

- Low density FMD area
  - South of the Tanzania-Zambia railway line, especially the Mtwara corridor
  - Potential FMD free zone
  - Strict active surveillance
  - Strict movement control
  - Vaccination of all susceptible livestock in the area

FMD zone 4

- FMD free area
  - The islands in the Indian ocean
    i. Pemba
    ii. Zanzibar
    iii. Mafia
- FMD free zone or holiday resorts?



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Jordi Casal - UAB – IRTA, Barcelona, Spain
Dirk Berkvens - Institute of Tropical Medicine, Antwerp, Belgium



Thank you
Merci
Dankeschön
Muito Obrigado
La Ringrazio
Muchas Gracias

DEVELOPING A PARTNERSHIP FOR FMD RESEARCH IN SOUTHERN AFRICA

Mark Rweyemamu, Tony Musoke, Francois Maree, Misheck Mulumba, Gavin Thomson, George Mathlo, Philemon Wambura, Mmeta Yongolo, Swithine Kabilika, Julius Keyyu, Koos Coetzer, Donald King, David Paton, Satya Parida, Sarah Cleaveland, Joe Brownlie and Keith Sumption

SADC Priority TADs and Zoonoses

(SADC CVOs 2003)

1. FMD
2. CBPP
3 - 5. RVF; LSD; ASF
6 - 12. ND/IBD/ FOWL POX/ Salmonellosis
13 - 14. CCPP; ruminant Salmonellosis
15 – 16. Bovine TB – Brucellosis - Rabies
17-18. Rinderpest; PPR
19. HPAI
20. BSE
21+. etc

2

Using Community of Practice Approaches to foster Collaborative Research Partnerships with Others

-

An Example of Foot-and-Mouth Disease

Key Qn: What contributes to FMD endemicity in Southern and East Africa and what options for its risk management?

FMD – Poverty Impacts in Africa



Understanding the impact of FMD on the livelihoods of livestock-reliant communities in Africa has been largely neglected

But pastoralists in East Africa consider FMD as one of the most important diseases of livestock

- reduced productivity (milk production, mortality of young stock)
- draft power
- losses contribute to declining livestock production base
- impacts upon poverty and nutrition

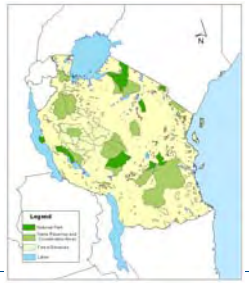
Partnership for FMD Research in Southern Africa:

Contribution from the following projects:
SACIDS (Wellcome Trust), CIDLID (BBSRC-DfID), SADC TADs, National Govts e.g. ASDP

- **Tanzania:**
  - *Central Veterinary Laboratory (CVL)*
  - *Sokoine University of Agriculture (SUA)*
  - *Tanzania Wildlife Research Institute (TAWIRI)*
  - *National Epidemiology Unit (NEU)*
- **Zambia:**
  - *University of Zambia (UNZA)*
  - *Central Veterinary Research Institute (CVRI)*
- **SADC SECRETARIAT**: *SADC TADs Regional Project*
- **Republic of South Africa**: *ARC-Onderstepoort Veterinary Institute* (OVI)
- **Botswana:** *Botswana Vaccine Institute (BVI*
- **United Kingdom:**
  - *Royal Veterinary College, London (Professor Brownlie)*
  - *University of Glasgow (Dan Haydon, Sarah Cleaveland, Richard Reeve)*
  - *World Reference Laboratory for FMD, Pirbright (IAH)*
  - *University of Edinburgh (Professor Brian Perry)*

Mapping the Diversity of Viruses in Understanding endemic FMD

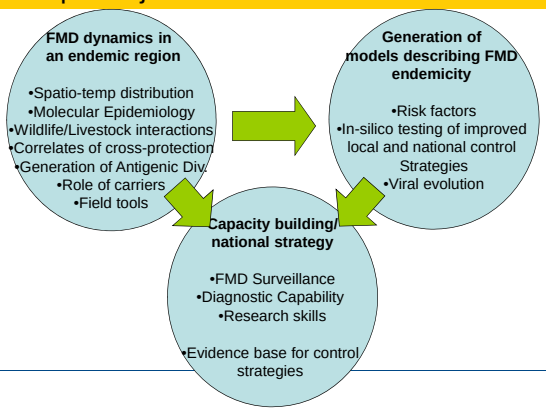
Wildlife, particularly buffalo, are likely to be important reservoirs of FMD in Tanzania





Sampling wildlife and livestock around protected areas will allow us to map the genetic diversity of FMD viruses across Tanzania

Principal Objectives





Livestock sampling: Tanzania, April 2010

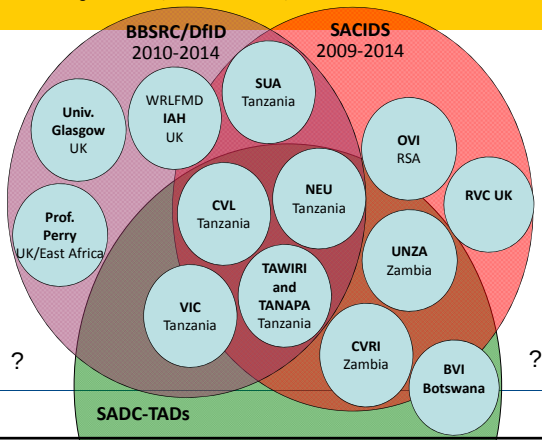


Buffalo sampling: Serengeti, Tanzania April 2010



Laboratory visit: Dar-es Salaam, Tanzania April 2010

Proposed FMD Program: SACIDS/CIDLID BBSRC-DfID/SADC-TADs Partners



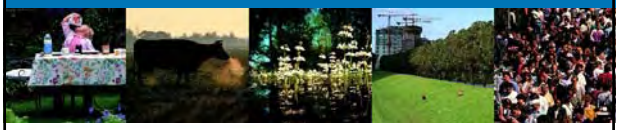


Sponsors: Google.org, Global Health Corps, Rockefeller Foundation, Wellcome Trust

Secretion and excretion of Foot-and-Mouth-Disease Virus: A preliminary quantitative risk assessment

C. Bravo de Rueda [a, b]*, A. Dekker[a], P.L. Eblé[a], M.C.M de Jong[b]

[a] Central Veterinary Institute (CVI) of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands
[b] Department Quantitative Veterinary Epidemiology, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands



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- Aim: To identify risk factors associated with the maximum amount of FMDV in different secretions and excretions
- How?
  FMD quantitative data review
- Why?
  - The contribution of contaminated fomites in the spread of the disease
  - The importance of other variables on virus excretion

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Relevance

- Gives awareness on the different secretions and excretions
- The importance of a silent spread between animals (within farms)
  - Max. titres are found mostly at 2 DPI* (1 - 3.5 dpi)
- Disinfection protocols
- Contingency plans
- Model parameters

* mostly when transmission occurs

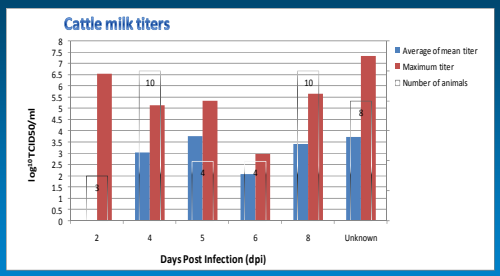
CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

Collection and extraction of data

- +40 articles reviewed (1963 – 2009)
- Data ordered by serotype, route of infection, dose of infection, stage of disease, DPI, animal species, type of secretion/excretion
- Secretes (milk, semen, saliva) and excretes (faeces, material from the respiratory tract, urine)
- Units correction (House and Yedloutschnig, 1994; Murhammer, 2007) e.g. PFU/ml->TCID50/ml

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Example



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Maximum titres (overview)



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Observations

- High titres in blood, airborne excretion, semen, faeces and urine when no clinical signs are apparent
- High in OPF and milk until 7 dpi
- In nasal discharge and saliva – higher during initial clinical signs
- Meaning that: **high* FMDV titres could be found when no clinical signs are apparent**

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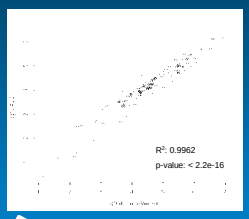
Model

- To find the effect of different variables on the maximum amount of virus
- How?
  - multivariate linear regression analysis

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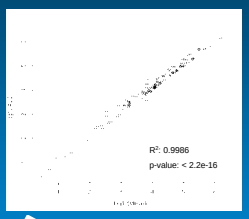
Response variable selection

lm(formula = log10(AUC*) ~ log10(DPImaxVimax) - 1, data = auc[AUC > 0,])



UC is higher

lm(formula = log10(AUC) ~ log10(VImax) - 1, data = auc[AUC > 0,])



UC is covered

* Area under the curve and max. titres were calculated from own laboratory data

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Explanatory variables

Table . Description of the variables used in the multivariable linear regression model

Variable	Type	Description
type of secretion/excretion	Categorical	airborne, blood, faeces, milk, nasal discharge, OPF, saliva, semen, urine
route of exposure	Categorical	intranasal, mixed (lung infiltration, IV, airborne, oral, and contact exposure)*, airborne, oral, udder, contact
animal	Categorical	cattle, swine, sheep, goats
serotype	Categorical	A, O, Others (A+O, Asia-1, C, SAT 1-3)
stage	Categorical	not clinical, clinical
infection dose	Numerical	From 1.5 to 10.15 TCID50/ml
days post infection (DPI)*	Numerical	From day 0.3 to 28 days post infection

* DPI was included as a factor during the analysis. The model fitted better when including this variable as a factor and not as a numeric variable

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- We considered variable "stage of the disease" as when clinical signs are apparent or not apparent
- We classified factor DPI into different classes
- Excluding 21 and 28 DPI
- Sheep and goats-> small ruminants
- Interactions were included

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Best fitting model

- √ animal+secretion+stage+serotype+ animal*secretion
- √ References: cattle, OPF, serotype O, clinical stage
- √ Diagnosis OK
- √ No correlation
- √ Comparison with observations
- √ Biologically relevant

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Results: secretions and excretions

- √ Airborne: pigs are major secretors (2 TCID50) (p-value<0.05)
- √ OPF: cattle>other species
- √ Milk and upper respiratory samples>>faeces (p-value<0.05)

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Results: virus serotype and stage

- √ Serotype O>A (1.2 TCID50) and others (p-value<0.001)
- √ In not clinical stage, titre is reduced in 0.70 TCID50 (p-value<0.05)

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Next steps

- Model validation
- Experimental quantification
- Correlation with transmission of FMD





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"The research leading to these results have received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 226556 (FMD-DISCONVAC)."

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FMD Control
Learning from the Recent Events in FMD-Free countries in Asia
---
The challenges of high density swine/ruminant areas

Sherrilyn Wainwright
Christopher Hamilton
Julio Pinto

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Animal Production and Health Division

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Short Summary

- Recent events of FMD outbreaks
- Countries previously FMD-free without vaccination
- High density livestock industries
- Real challenges and risks in response

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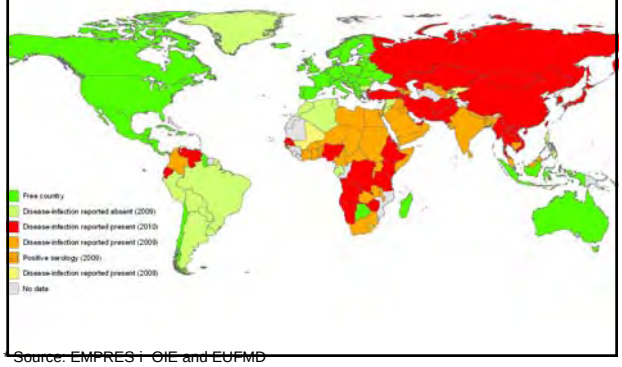
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2010 FMD Outbreaks in Southeast Asia



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World Foot and Mouth Disease reported outbreaks 2008 through September 2010*



* Source: EMPRES i, OIE and EUFMD

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FMD Outbreak

- Essentially plunged into the Progressive Control Pathway (PCP) Stage 0
- If surveillance and stoppage of movement can be accomplished , there may be quick progression through Stages 1, 2 and Stage 3
- Decision of whether to use vaccination will be one of key decisions to be taken
- And then, economic decisions of whether to work through Stage 4 or, through culling of those vaccinated, move back into Stage 5
- Actually, former FMD-free countries will follow the OIE regulations to return to FMD-free status.

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Conclusions

- Livestock sector continues to change in response to globalization and the growing demand for animal food products
- The expanding trade in livestock and livestock products is constantly under the threat of disease outbreaks
- A risk analysis is needed for the response to outbreaks of highly infectious transboundary animal diseases in dense livestock populated areas which must be incorporated in business plans of governments and private industry

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	2004	2009	Growth Compared to 2004 (%)
Human population	6,456,443,080	6,755,987,239	4.64
Inventory			
Pigs (million)	894	941	5.26
Cattle (million)	1,348	1,382	2.52
Production			
Pig meat (thousand tonnes)	96,650	106,069	9.74
Cattle meat (thousand tonnes)	58,085	61,838	6.46
Milk (cattle) (thousand tonnes)	527,180	580,482	10.11

Source: FAOSTAT

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Material and Methods

FAO's

- Global Early Warning and Response System for Major Animal Diseases, including Zoonoses (GLEWS) team's disease tracking activities
- Disease situation reports from countries affected by FMD during 2010
- Emergency Prevention and Early Warning System (EMPRES) Animal Health
- EMPRES-i: Global Animal Disease Information System
- Global Livestock Production and Health Atlas (GLiPHA)

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FMD-free without vaccination

- The risk of FMD entry is low through legal trade of animals and animal products from zones officially recognized as FMD-free by the OIE
- However, there consistently is evidence of animals and animal products entering FMD free countries by various routes "under the radar," some which carry infectious virus

FAO Focus on FMD, 2005 – 2006 report

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Countries which have achieved FMD-free status without vaccination incur potential risks related to this success:

- Over time, there may be decreased political support and funding to maintain the vigilance required and resources required for response
- A loss of a sense of urgency to include FMD as a differential to be ruled out
- There is too much at risk if FMD is the last disease on the differential list after other endemic diseases are first ruled out
- Risk of continued unrecognized spread
- Especially if in a high "mixed livestock species" dense area

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There are inherent risks associated with areas of high livestock density

- **Disease transmission**
  - Neighborhood spread: movement of people (including response teams) - mechanical
  - Biosecurity measures not uniform on all farms
  - Feed trucks and other vehicles due to the lack of disinfection between uses (movement of livestock, dead cattle to processors, litter/waste, etc)
  - Visitors and goods coming from FMD infected countries
  - Wildlife

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Disease Control Measures

- Detection to identify the actual extent of the outbreak
- Huge economic / commercial constraints to stop movement
  - manure removal
  - swine industry
  - dairy industry
  - beef industry
- "Animal welfare" issues with stoppage of movement
  - vehicles such as feed trucks

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Human and equipment resources for the response

- Sampling suspect and exposed animals
- Clinical observation
- Laboratory testing
- Vaccination
- Euthanasia
- Movement of carcasses
- Burial of carcasses
- Disinfection
- Public relations
- Industry relations
- Providing information to the government policy-makers

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Delays in response due to:

- Lack of appropriate legislation to respond as needed
- Knowing the actual location of and an idea of the numbers of farms and animals that will need to be known even for estimates of what it will take in resources to respond
- The above is a key challenge of private versus public good
- Weather, such as heavy rains or winds
- Lack of identified and committed financial resources to address compensation concerns
- Lack of compliance
- Emotional toll on animal owners and the responders

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Response Challenges

- There is a clear need for well informed:
  - livestock sector planning
  - policy development and analysis
- There is a paucity of reliable and accessible information on the:
  - Distribution
  - Abundance
  - Uses of livestock

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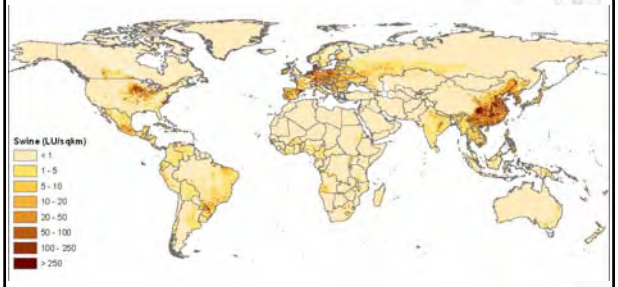
FAO's GLiPHA and GLIMS

- Global Livestock Production and Health Atlas (GLiPHA), user-friendly electronic atlas which draws on sub-national data managed within the Global Livestock Impact Mapping System (GLIMS)
- The interface uses the 3rd generation of the Key Indicator Data System (KIDS -3g)
- Gridded livestock of the world – FAO, 2007
  - LU: *Livestock unit;* conversion factors: cattle (0.65), buffalo (0.70), sheep and goats (.10) and pigs (0.25)
- Better data is needed

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Estimated World Density of Pigs*

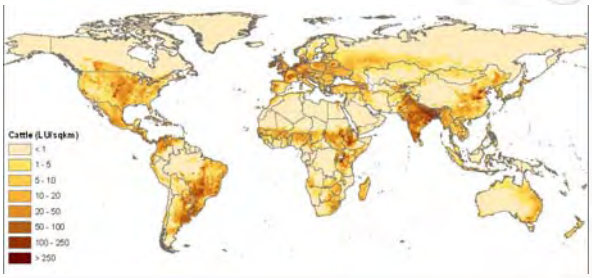


*Gridded livestock of the world – FAO, 2007

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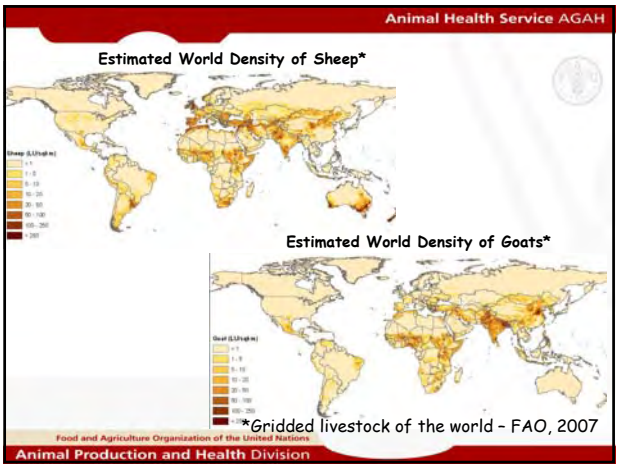
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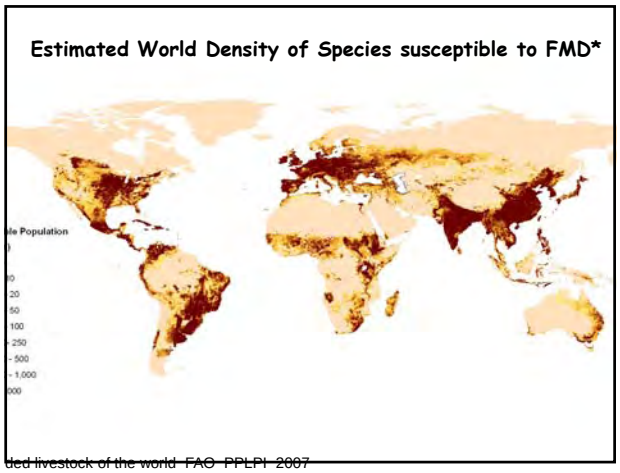
Estimated World Density of Cattle*

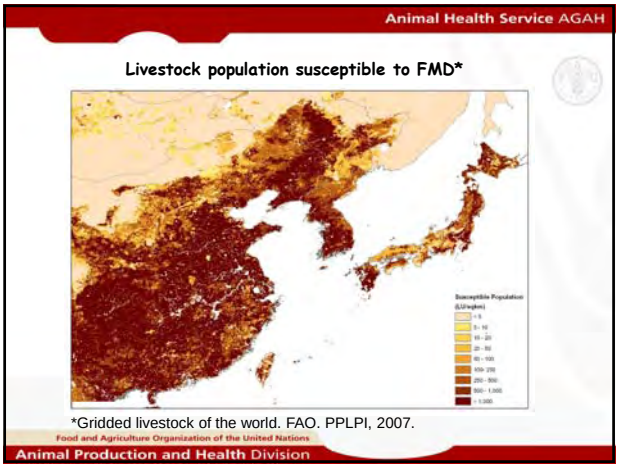


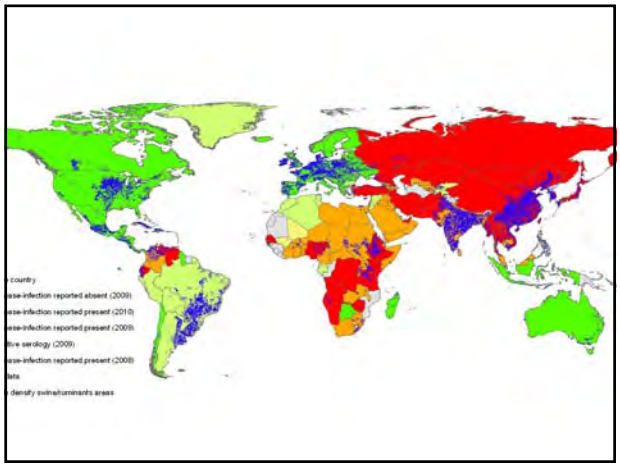
*Gridded livestock of the world – FAO, 2007

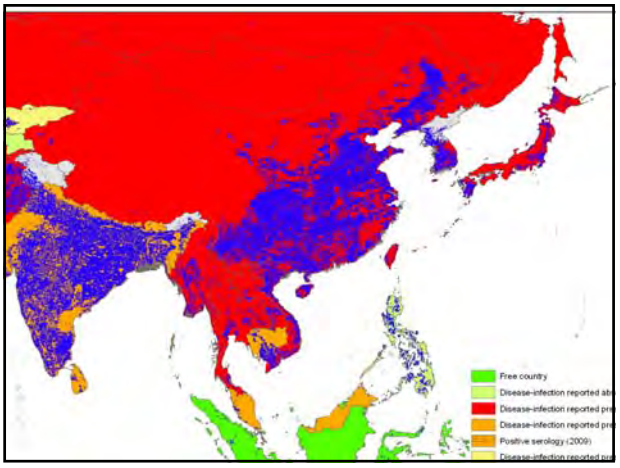
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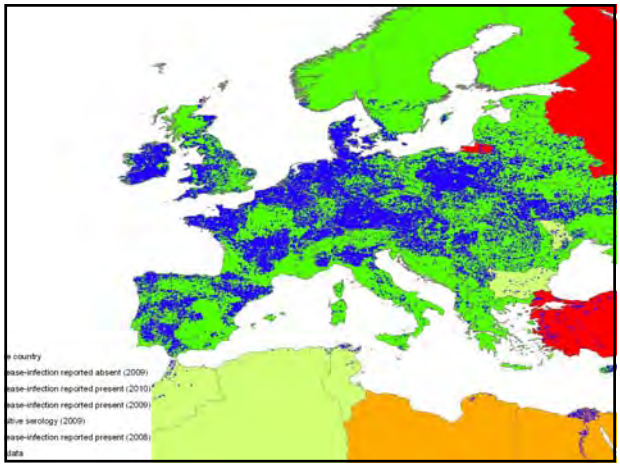

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Estimated World Density of Sheep*
Estimated World Density of Goats*
*Gridded livestock of the world – FAO, 2007
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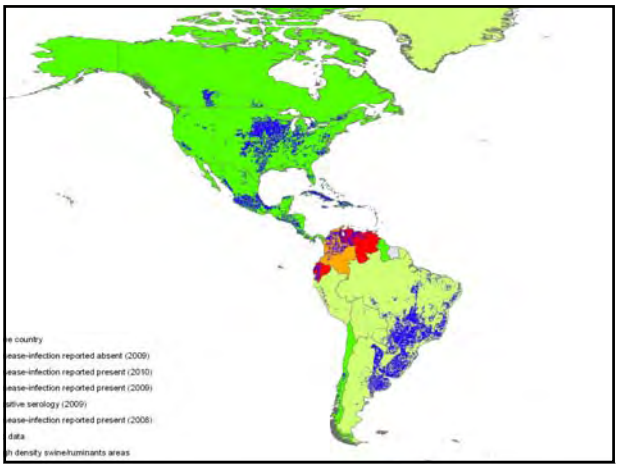

Estimated World Density of Species susceptible to FMD*
ded livestock of the world- FAO- PPLPI- 2007

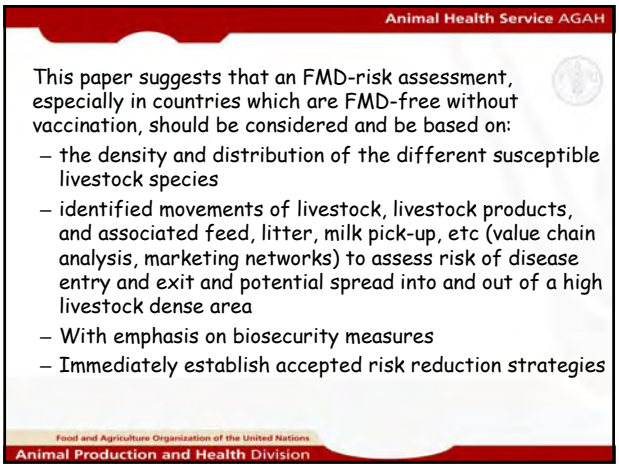

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Livestock population susceptible to FMD*
*Gridded livestock of the world. FAO. PPLPI, 2007.
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Free country
Positive serology (2009)




e country
ease-infection reported absent (2009)
ease-infection reported present (2010)
ease-infection reported present (2009)
itive serology (2009)
ease-infection reported present (2008)
data
h density swine/ruminants areas


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This paper suggests that an FMD-risk assessment, especially in countries which are FMD-free without vaccination, should be considered and be based on:
– the density and distribution of the different susceptible livestock species
– identified movements of livestock, livestock products, and associated feed, litter, milk pick-up, etc (value chain analysis, marketing networks) to assess risk of disease entry and exit and potential spread into and out of a high livestock dense area
– With emphasis on biosecurity measures
– Immediately establish accepted risk reduction strategies
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Some elements of disease preparedness for highly contagious emerging and re-emerging diseases in Germany

Matthias Kramer*, Thomas Fröhlich², Josef Diekmann', Doris Kerschhofer³, Rolf Krieger** & Bernd Haas*

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'State Office for Consumer Protection and Food Safety of Lower Saxony
³Central Bureau of the Task Force of Animal Disease Control, Bonn, **Federal Ministry of Food, Agriculture and Consumer Protection, Bonn, Germany

Summary

- An animal disease preparedness system is established in order to improve the rapid response in case of FMD outbreaks or other highly contagious emerging, re-emerging and exotic diseases.
- Key-elements:
  - Mobile crisis and management centre (MCMC)
  - Emergency equipment stock (EES)
  - Vaccine and diagnostics bank (exclusively for FMD)
  - Expert groups (task forces)
  - Training system
- Appropriate disease preparedness systems can be successfully installed, also in decentralized countries or in federal structured countries with a high degree of independence of their regions.

Structure of the German veterinary services (VS)

- Federal organised and decentralized structure
- Clear separation of competencies and tasks between federal government and states (Länder)
  - Federal government – e.g. veterinary legislation under certain conditions, foreign affairs, collection of animal health data on federal level, coordination of measures
  - States: law enforcement, veterinary legislation
- Administrative structure of VS
  - Veterinary issues on federal level
    - Ministry of Food, Agriculture and Consumer Protection
    - Federal institutions including National Ref. Labs
  - 16 federal states
    - 22 governmental districts in some states
    - 431 local veterinary offices in rural districts and towns
    - 36 regional veterinary labs

Administrative boundaries concerning infrastructure of VS



Some lessons to be learned from previous epidemics in Europe

- Rapid response is required in case of an animal disease outbreak in order ensure a harmonised approach of measures
- Vaccine & test kit banks (FMD) needed
- System of sharing lab and rendering capacities in disaster situations
- One crisis management software available in all veterinary authorities
- Mobile crisis management centre (MCMC)
- Lack on equipment, increased prices - Emergency equipment stock (EES)
- Expert groups on national and regional level
- System of training at all administrative levels of VS at the national emergency training centre

Mobile crisis management centre (MCMC)

- Experiences used from other countries like the UK, Netherlands and France
- Transportable infrastructure needed to support the local disease control centres (LDCC)
- Use for emerging, re-emerging and exotic animal diseases
  - Highly contagious,
  - high zoonotic and or economic importance
- Only in nation-wide crisis situations

LDCC



- Planning and enforcement
- Issuing measures/orders
- Providing staff and equipment
- Surveillance
- Sitreps
- Public relations

MCMC



- Clinical investigations
- Sampling
- Tracing
- Valuing/ killing
- Disposal
- Cleansing and disinfection
- Vaccination

MCMC

Briefing and De-briefing, Protection clothes Sampling kits and Set for clinical investigations



Reception of samples, Sampling logistics

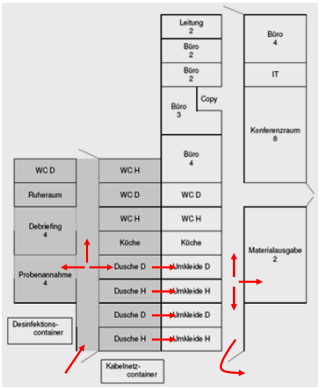


Cleansing, decontam. of staff/ equipment



Data management & processing





- 37 office containers and sanitary containers + 12 corridor segments
- Servers and working stations.



In case of an outbreak



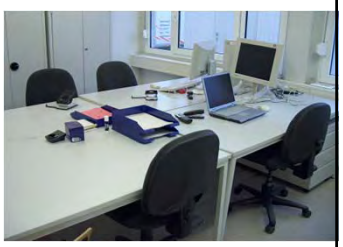






MCMC office equipment

- About 20 PC working stations and laptops, server, interface for accessing regional and central databases
- 7 network printer
- Enlargement up to 100 PC working stations foreseen
- Phone system (VoIP)



Emergency equipment stock (EES) for protection of the staff

- ✓ 20.000 sets of protection cloths (L – XXL)
- ✓ 18.000 pair wellingtons
- ✓ 2.000 pair PVC boots with steel cap
- ✓ 1.000 FFP3 masks
- ✓ 1.000 goggles
- ✓ 1.000 PVC rain coats



Emergency equipment stock (EES) for sampling and vaccination

- ✓ 4.200 Sampling sets
- ✓ 4.200 Vaccination sets
- ✓ 4.200 Capture devices for pigs and cattle
- ✓ 100 Transport boxes for dangerous goods
- ✓ 600 vaccination sticks (90 cm)



Additional key-elements (1)

- National vaccine/stock bank (FMD)
  - 10 FMD strains with 1,75 Mill. doses each
- Diagnostics bank (foot and mouth disease)
  - 50.000 kits (NSP antibody ELISA) ready for use
  - After 4 weeks of the outbreak, sampling kits for 200.000 samples per week available
  - Detection kits for altogether 2 Mill. samples stored

Additional key-elements (2)

- Expert groups
  - Veterinary experts established on federal and state level
  - currently about 50 persons (ready to send nation-wide)
  - Tasks.
    - Supporting directly the LDCC's with expertise in the control of emerging, re-emerging and exotic diseases
    - Giving advices and recommendations
- Training measures
  - Desktop simulation exercises on federal level for about 30 local veterinary authorities
  - Real-time exercises al Federal state and district level
  - Training sessions for senior veterinary officers at governmental level (states)
  - Continuous training measures with the expert group by simulation or real-time exercises

Conclusions

- The system of disease preparedness is still not perfect, but we feel better prepared now.
- Appropriate disease preparedness systems can be successfully installed, also in decentralized countries or in federal structured countries with a high degree of independence of their regions.
- A long-lasting maintenance and sustainability is required.

Quadrilateral Animal Health Committee

Relative validation of simulation models of FMD for informing decision makers

Katie Owen

On behalf of the Quadrilateral Epidemiological Working Group (EpiTeam)

Conclusion

The study

- Demonstrated the value in comparing results when using identical input data and exploring reasons for any differences in interpreting and gaining confidence in the outputs
- Reinforced the merit in international collaboration in building and deploying these types of models.
- Highlighted the challenges in obtaining suitable data

Overview



- Introduction
- Method
- Example results
- Key points in discussion
- Conclusion

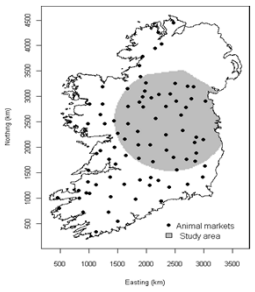
Problem: It is all about uncertainty

- When used well models provide valuable evidence to inform control decisions
- Decision makers need confidence in the model outputs
- Methods for verifying and validating disease models are lacking
- Epidemics are rare and data is sparse so cannot compare results with reality

Introduction: The Project

- "Relative" validation
- 3 epidemiological models:
  - InterSpread Plus (NZ)
  - AusSpread (Australia)
  - NAADSM (N. America)
- Stage 1: Formal description
- Stage 2: Simple scenarios with hypothetical population (Dube et al 2007)
- **Stage 3: Complex scenarios based on real farm data and actual movement and market data**

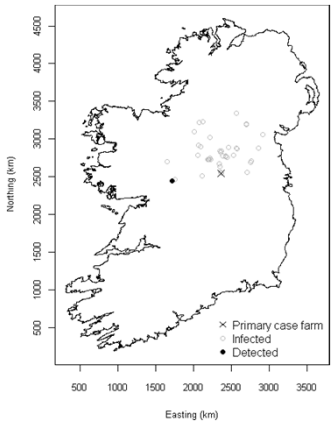
Materials and methods: Population



- Real farm data from the Republic of Ireland
- 50125 Farms
- 90km radius
- 90 Markets

Materials and Methods: Parameters

Parameter type	Derivation
Movement	90 d excerpt from Irish movement data for study farms
Direct spread probability of transmission	Published papers
Indirect spread (by farm type	Scaled from direct transmission using adjusted NZ survey data
Local spread	Analysis of 2001 UK epidemic
Airborne spread	Run of NAME (UK) based on weather observations over 4 months
Control policies	Literature review expert elicitation, legal and policy requirements



Input for scenarios 2-5

- 41 infected premises were present when first premises detected on day 17

Materials and Methods: Scenarios

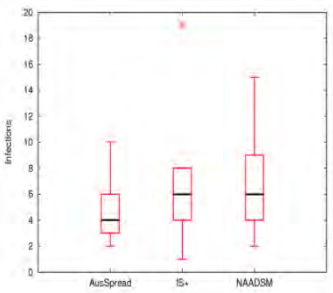
Scenario	Initiating conditions	Control policies
Scenario 1	A single farm in the centre of the study area.	No controls. Ran for 60 days.
Scenario 2	Single farm identified on day 17 post infection of primary case farm in centre of the study area (41 infected and undetected farms at time of detection of index case) +/- Airborne spread	Standard EU-aligned stamping out policy: depopulation, movement controls, surveillance (as per Council Directive 2003/85/EC
Scenario 3	As for Scenario 2	Standard EU-aligned stamping out policy plus 0.5 km contiguous cull around IPs.
Scenario 3	As for Scenario 2	Standard EU-aligned stamping out policy plus 3 km suppressive ring vaccination starting 7 days after the date of first detection. 60 teams/5 herds per day on day 8 ramping to 80-100 teams.
Scenario 4	As for Scenario 2	Standard EU-aligned stamping out policy plus 3 km suppressive ring vaccination with delayed implementation (21 days after the date of first detection).

Materials and Methods: Statistics & spatial analysis

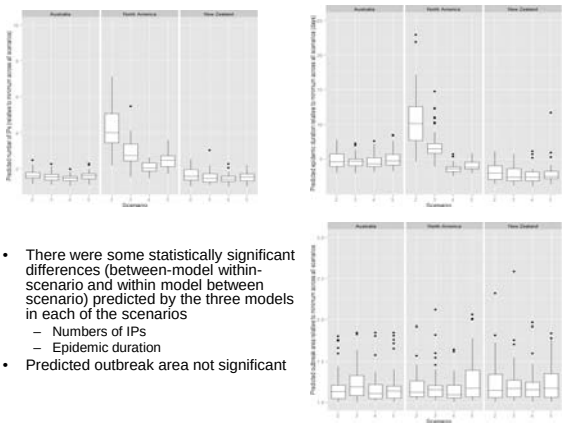
For each scenario and for each model:

- the **predicted numbers of IPs** and the **duration** of the epidemics were compared using the Kruskal-Wallis test.
- The **sizes of predicted outbreak areas** were assessed by constructing a minimum convex hull around each set of IPs. The resultant areas of these hulls were compared using the Kruskal-Wallis test.
- The **spatial distribution** of the risk of premises becoming infected was estimated using kernel smoothing techniques.

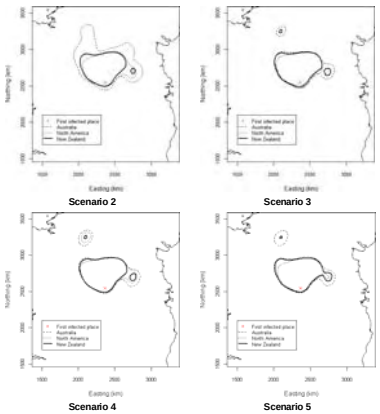
Results: Scenario 1



Number of infected premises predicted by each model 2 weeks into the silent spread phase



- There were some statistically significant differences (between-model within-scenario and within model between scenario) predicted by the three models in each of the scenarios
  - Numbers of IPs
  - Epidemic duration
- Predicted outbreak area not significant



Contour plots delineating areas where the risk of a farm premises being a case was beyond that expected by chance ($p<0.05$) at simulation day 60

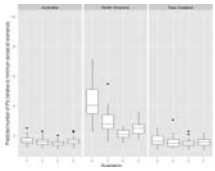
Key findings: developing parameters

- Opportunity for each of the modeling teams to take an in-depth look at the way core functions were implemented in their models

Challenges- devising rules for e.g.:

- Differentiating farm types
- Determining epidemiological units
- Market mixing
- Implementation of each of the control measures
- Order of events on farm

Why did the North American model predict larger outbreaks and greater benefits in controls?



- Differences explored using simulate- brainstorm-revise-repeat
- Programming decisions, e.g.
  - North American model always selected non-detected farms to receive movements
  - Lower effectiveness of surveillance

What about the policy decisions?

- Relative within-model between-scenario changes resulting from the application of different control strategies showed consistent patterns
- Any advice provided to decision makers in terms of the relative merits of different control strategy options would have been similar.

Conclusion

The study

- Demonstrated the value in comparing results when using identical input data and exploring reasons for any differences in interpreting and gaining confidence in the outputs
- Reinforced the merit in international collaboration in building and deploying these types of models
- Highlighted the challenges in obtaining suitable data

Acknowledgements

- Quads CVOs and EMWG
- Ministry of Agriculture and Forestry NZ
- EpiTeam members
  - Neil Harvey, Caroline Dube (Canada/NAADSM)
  - Mark Stevenson, Robert Sanson (InterSpread)
  - Graeme Garner (AusSpread)
- Tim Davies and Martin Hazelton (Stats)

Sanson et al. Foot-and-mouth disease model verification and 'relative validation' through a formal model comparison OIE Scientific and Technical Review - Vol. 30 (1), April 2011 In Press.





London's water distribution map (used by John Snow in his 1855 studies) and some of his plots, which identified the source of the cholera epidemic (the pump)

Classic vs. connectivity-based control strategies in FMD and other viral epidemics

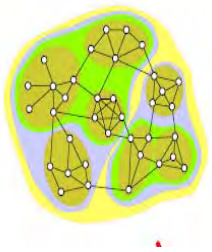
Ariel L Rivas, Jeanne M Fair, J Mac Hyman, Steve Smith, Folorunso Fasina (e-mail: alr4 @cornell.edu)
North Carolina State University, Los Alamos National Laboratory, Tulane University, USA

Can we demonstrate network properties in epidemics?

a) In 1998, Watts & Strogatz predicted that epidemics should reveal networks properties. Networks are composed of nodes connected by lines.

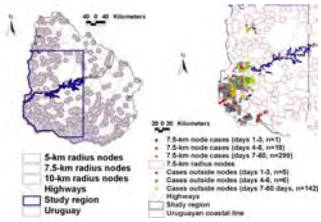
b) To demonstrate Network properties in epidemics, the data should meet two criteria: (i) clinical (observable) cases, and (ii) it should be known when and where the epidemic started (time and place of primary cases).

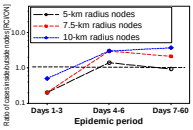
c) We have data that meet those criteria: (i) the 2001 Uruguayan foot-and-mouth disease (FMD) epidemics. and (ii) the 2006 Nigerian avian influenza (AI H5N1).



"Infection" vs. "epidemic": Networks in exotic/emerging epidemics.

From abstract to measurable **epidemic nodes**: the **FMD** case



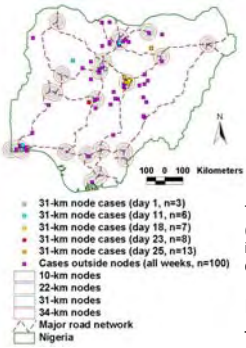


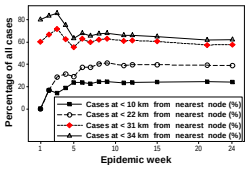
We investigated ***not any cluster of*** FMD cases but ***disease clusters associated*** with a ***pre-existing, contact network***.

The FMD **case density** was highest within **7.5 km-radius circles** centered on **highway intersections** (**epidemic nodes**) .

Except the first 3 days, **epidemic nodes** contained **~ 70% of all** FMD **cases**.

Assessing reproducibility –the AI case





Throughout the epidemic, epidemic nodes (31-km radius circles centered on road intersections and with the highest case density) included ~60% of all AI cases.

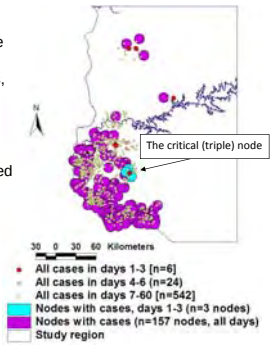
Both epidemics showed **nodes** –a property predicted by Network theory.

Finding the critical epidemic node in FMD epidemics

A **triple node** contained one of the 6 cases reported in the first 3 epidemic days, i.e., the first replication cycle of the virus (primary cases). By involving 3 highway intersections, this node offered the virus 3 opportunities to disseminate.

The index case was one of the 6 first reported cases. Only primary cases with connectivity can disseminate –regardless of whether the index case is one of them.

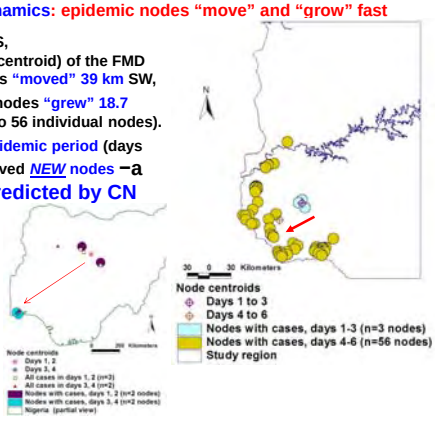
→**The first reported case (the index case) is not necessarily the most influential case.**



Epidemic dynamics: epidemic nodes "move" and "grow" fast

WITHIN 3 DAYS, the epicenter (centroid) of the FMD epidemic nodes "moved" 39 km SW, and epidemic nodes "grew" 18.7 times (from 3 to 56 individual nodes). The second epidemic period (days 4- 6) only involved *NEW* nodes –a property predicted by CN theory.

The node epicenter also moved (700 km) in the second period of the AI epidemic



The role of connectivity (FMD

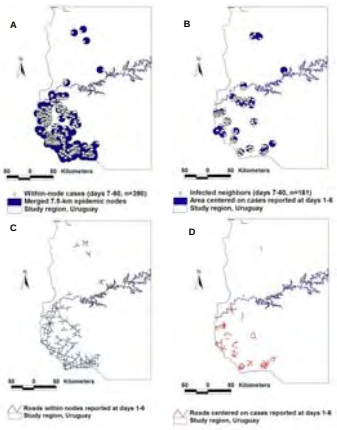
After two replication cycles:

(**A**) circles that **included epidemic nodes** captured **390** cases,

(**B**) circles that **did not consider epidemic nodes (connectivity was ignored, all cases were assumed to have equal influence)** captured **181** cases,

(**C**) circles that **included epidemic nodes** showed a less disrupted and **longer road length,**

(**D**) circles that **did not consider epidemic nodes** showed a more disrupted and **shorter road length.**



The role of connectivity (AI)

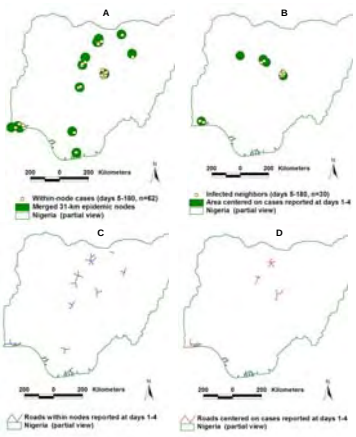
After two replication cycles:

(**A**) circles that **included epidemic nodes** captured **62** cases,

(**B**) circles that **did not consider nodes** captured **30** cases,

(**C**) circles that **included epidemic nodes** showed a less disrupted and **longer road length,**

(**D**) circles that **did not consider nodes** showed a more disrupted and **shorter road length.**



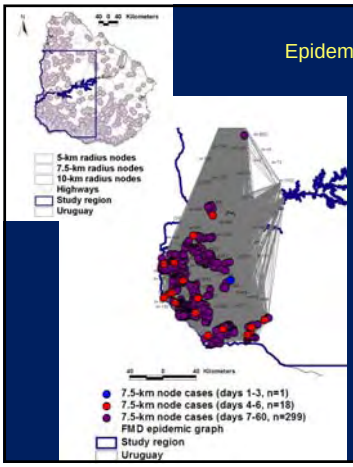
Not all disease clusters are equal:
→*connected* disease clusters are more influential

Because connected disease clusters (nodes) "move" rapidly,

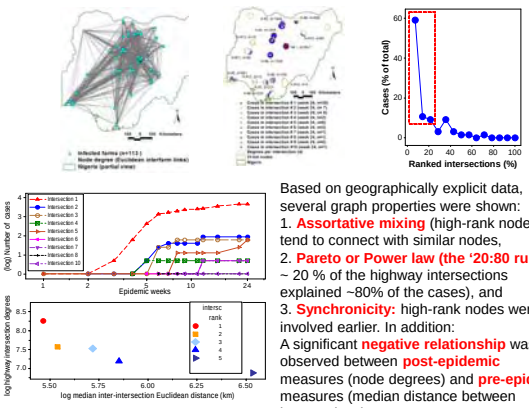
→ *static* clustering analysis is not adequate to assess epidemic *dynamics*

→ connectivity should be measured in each viral replication/transmission period).

Epidemic graphs



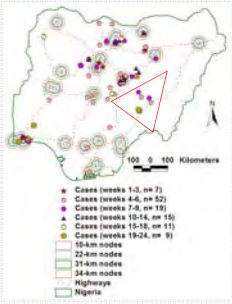
1. Determine critical radius of epidemic nodes (cases centered on highway intersections),
2. Determine the portion of the road network that coincides with epidemic nodes.
3. Determine attributes of epidemic graph (e.g., 'degrees').
4. Rank highway intersections according to the epidemic graph degrees'.
5. Determine other network or graph properties.

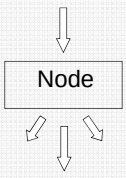


Based on geographically explicit data, several graph properties were shown:
1. **Assortative mixing** (high-rank nodes tend to connect with similar nodes,
2. **Pareto or Power law (the '20:80 rule"):** ~ 20 % of the highway intersections explained ~80% of the cases), and
3. **Synchronicity:** high-rank nodes were involved earlier. In addition:
A significant **negative relationship** was observed between **post-epidemic** measures (node degrees) and **pre-epidemic** measures (median distance between intersections)

IMPLICATIONS

Epidemic nodes are not only recipients but also disseminators





Conclusions

- Epidemic nodes and links *(connectivity) can be measured with geo-referenced data.*
- Because *connectivity predates contacts,* in rapidly disseminating epidemics, connectivity is likely to be a pre-existing condition, e.g., road networks can be explored *before epidemics occur*.
- The combination of *population density* (animal and human) and *connecting structures* likely to act as 'epidemic nodes' may be determined before epidemic occur.



Protective vaccination to combat FMD in Finland – Does it pay?

Jarkko K. Niemi & Heikki Lehtonen
MTT Agrifood Research Finland, Economic Research

Tapani Lyytikäinen, Leena Sahlström & Terhi Virtanen
Finnish Food Safety Authority (Evira)

1

Summary

- Question: Does protective vaccination reduce economic losses due to FMD outbreak in Finland?
- Answer: Generally no!
- Why?

2

Introduction

- The rationality of vaccination-to-live policy vs. stamping-out policy has been debated in the EU
- Little research focusing on countries where farm density and other production environment is similar to the Nordic countries
- Finland has been free from FMD since 1959
- Our goal was to study whether protective vaccination could be economically rational disease control policy

3

Data and models

- Results from epidemiological Monte Carlo simulations (n=100 000) are used as input in this study
  - Spatial farm data (n=23 439)
  - Explicit event-based animal movements data
  - Other contact information
- Economic simulations
  - Price, import, export and consumption statistics
  - Production costs for pigmeat, beef and six dairy product groups
  - Direct costs of disease eradication based on Finnish sources and the 2001 UK outbreak
  - Minimize indirect losses conditional on uncertainty

4

Models

The primary infected farm (PIF) in the country

↓

Epidemiological simulations

↓ Outbreak duration: Trade scenarios

↓ Outbreak size: Infected farms, Affected farms

↓

Dynamic partial-equilibrium model

↓

Economic welfare and supply effects conditional on PIF

5

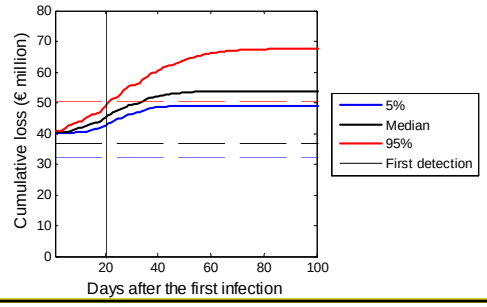
Results

- Typical outbreak
  - Approximately 5 infected farms
  - Duration 1-2 months
  - Losses under stamping-out policy €24 million
- In the subset of 5.5% largest epidemics
  - The protective vaccination resulted in up to 45% higher losses than stamping-out policy when trade losses are accounted for
  - If only direct costs are taken into account, the costs of protective vaccination still exceed those of stamping-out policy
  - In 95% of cases no losses could be reduced after some 60 days after the first infection
- Vaccination was not able to reduce epidemic size significantly because the earliest possible time to vaccinate was too late!

6

Costs are accumulated early during an epidemic

Curves represent the costs of protective vaccination policy (median, range where 90% of losses are situated) for **5.5% most severe outbreaks**. Horizotal dashed lines represent the *final* costs of stamping-out policy for a similar situation as curved lines of the same colour.



7

Why protective vaccination can increase epidemic costs?

- Large number of vaccinated animals, implementation costs
- Only few farms, if any, could be saved
  - Difficult to identify situations where benefits could be available
- Time required to obtain protective effect
- Non-EU countries unlikely to accept products originating from vaccinated animals in their markets
  - Prolonged trade distortions matter, because exported products have limited demand on the domestic market
  - In Finland >35% of milk (milk equivalent) and 20% of pigmeat are exported
- Similar conclusion also if trading partners would fully accept vaccination-to-live policy

8

Thank you for your attention!

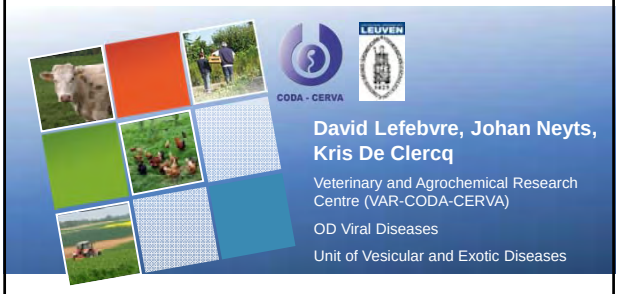
jarkko.niemi@mtt.fi

Check out also the poster:
Emergency vaccination is not a feasible option in control of FMD outbreak in Finland

We gratefully acknowledge funding from the Ministry of Agriculture and Forestry and the National Emergency Supply Agency.

9

DEVELOPMENT OF AN FMDV INFECTION MODEL IN SEVERE COMBINED IMMUNODEFICIENT MICE FOR THE PRELIMINARY EVALUATION OF ANTIVIRAL DRUGS



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Veterinary and Agrochemical Research Centre (VAR-CODA-CERVA)

OD Viral Diseases

Unit of Vesicular and Exotic Diseases



OPEN SESSION OF THE EuFMD STANDING TECHNICAL COMMITTEE

01 10 2010

Short Summary

- Introduction:
  - Emergency vaccination and antivirals against FMD
  - Development of antiviral drugs
  - FMDV infections in mice
- Aim
- Materials and Methods
  - FMDV titrations in mice
  - Clinical and virological follow-up
- Results
- Conclusions
- Acknowledgements

Conclusions

- FMDV can quickly induce a severe, generalized disease in mice
- Clinical outcome of the infection depends on FMDV serotype and/or strain, inoculation dose and mouse strain and/or age
- Infections of SCID mice with A_{22} Iraq may represent an appropriate model for the preliminary *in vivo* evaluation of antiviral drugs

Introduction: emergency vaccination

- Council Directive 2003/85/EC: Emergency vaccination against FMD
- Emergency FMD vaccines:
  - Induce protective, neutralizing antibodies 4 to 7 days post vaccination ("immunity gap")
  - Serotype- and subtype-specific (7 FMDV serotypes, multiple subtypes)
- Serotype-independent antiviral drugs can close the "immunity gap"

Introduction: development of antivirals

- Screening of chemical compounds and identification of antiviral activity *in vitro*
- Optimization of antiviral activity *in vitro*
- Study of safety, pharmacokinetics and antiviral activity in a *rodent model*
- Study of safety, pharmacokinetics and residue-analysis in *cloven-hoofed animals*
- Study of antiviral activity in *cloven-hoofed animals*: clinical protection and effect on transmission

Introduction: FMDV in mice

- The ideal rodent model for the evaluation of sub-optimized antiviral drugs:
  - 100% infectivity and 100% disease
  - Low inoculation dose
  - Slow disease development
- Literature: FMDV in mice: precarious results:
  - No infection, sub-clinical infection, generalized disease
  - FMDV serotype and/or strain dependent
  - Inoculation dose dependent
  - Mouse strain and/or age dependent

Aim

To develop an FMDV infection model in mice that is suited for the evaluation of early antiviral leads

Materials and methods

- I.P. inoculations of 10-fold dilution series of FMDV
- Reference strains of the Eurasian serotypes:
  - O_1 Manisa
  - A_{22} Iraq
  - C_1 Noville
  - Asia1 Shamir
- Mean time of death or euthanasia
- Clinical symptoms
- Evolution of body weight
- Quantification of viraemic peak at 2 days post inoculation (dpi) by qRT-PCR

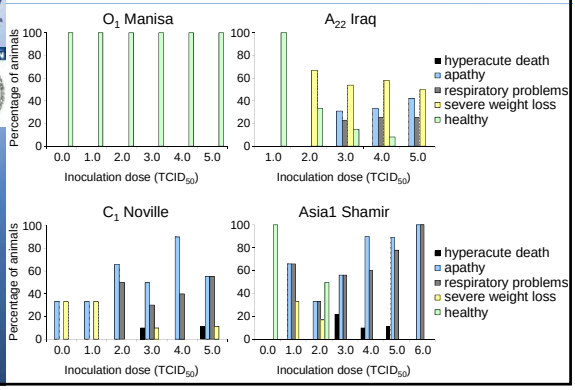
Results

- 7- to 9-week-old Balb/c mice:
  - O_1 Manisa: no clinical disease
  - C_1 Noville: no clinical disease
  - Asia1 Shamir: † at 3-4 dpi: too fast to assess the antiviral activity of early antiviral leads
- Switched to 3- to 4-week-old SCID mice:
  - Innate immune response
  - No functional B- and T-cell responses
  - Frequently used for the development of antiviral drugs in human medicine
  - % of protected mice correlates with the antiviral activity

Results: mortality in SCID mice

Virus strain	Inoculum ($TCID_{50}$)	Mortality (Ratio)	Mortality (%)	No. of days until †
A_{22} Iraq	1	0 / 3	0	-
	2	2 / 3	67	7.0 ± 0.0
	3	11 / 13	85	5.9 ± 1.5
	4	11 / 12	92	5.7 ± 1.8
	5	12 / 12	100	5.6 ± 1.9
C_1 Noville	0	3 / 3	100	6.0 ± 3.5
	1	3 / 3	100	4.7 ± 2.1
	2	6 / 6	100	3.8 ± 0.4
	3	10 / 10	100	3.9 ± 1.3
	4	10 / 10	100	3.6 ± 0.5
	5	9 / 9	100	3.4 ± 1.5
Asia1 Shamir	0	0 / 3	0	-
	1	3 / 3	100	5.3 ± 4.0
	2	3 / 6	50	4.3 ± 2.3
	3	9 / 9	100	2.1 ± 0.6
	4	10 / 10	100	2.5 ± 0.7
	5	9 / 9	100	2.7 ± 0.5
	6	3 /3	100	3.0 ± 0.0

Results: clinical symptoms in SCID mice



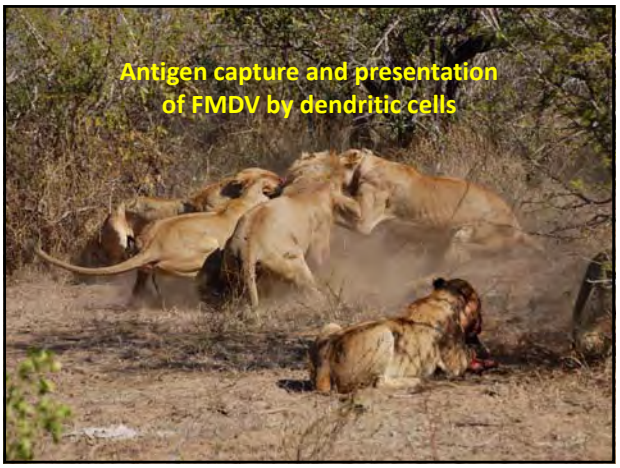
Conclusions

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- Clinical outcome of the infection depends on FMDV serotype and/or strain, inoculation dose and mouse strain and/or age
- Infections of SCID mice with A_{22} Iraq may represent an appropriate model for the preliminary *in vivo* evaluation of antiviral drugs

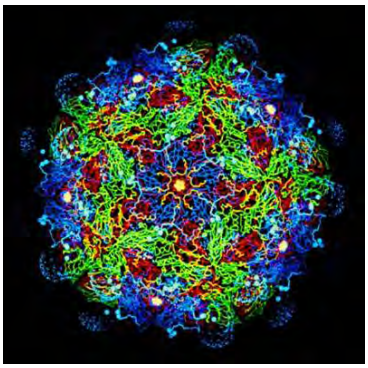
Acknowledgements

- The Belgian Federal Public Service for Health, Food Chain Safety and Environment (RF 6203)
- DG Research of the European Commission
  - EC 6th Framework Programme (EC-EPIZONE FOOD-CT-2006-016236, www.epizone-eu.net)
  - EC 7th Framework Programme (grant agreement n°226556, www.fmddisconvac.net)
- Staff at the VAR and the Rega Institute

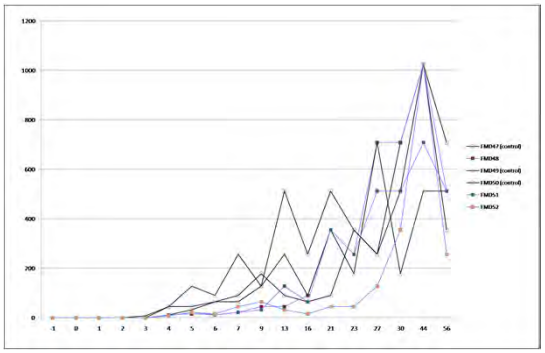
- Questions?
- Remarks?



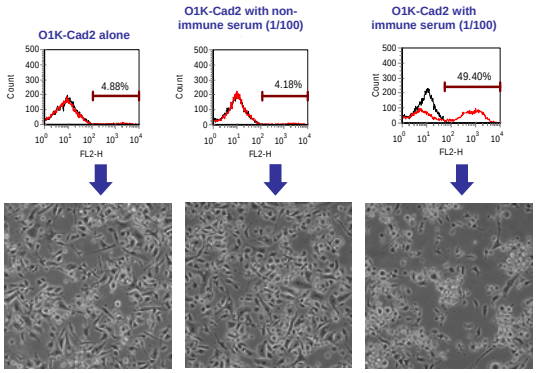
FMDV capsid: T independent and T dependent epitopes



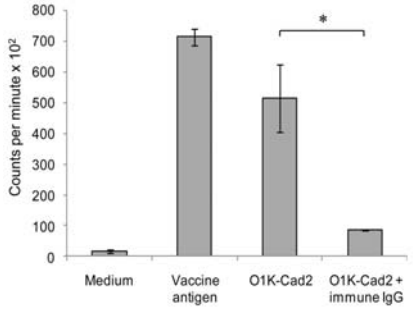
Depletion of CD4⁺ T cells during FMDV vaccination



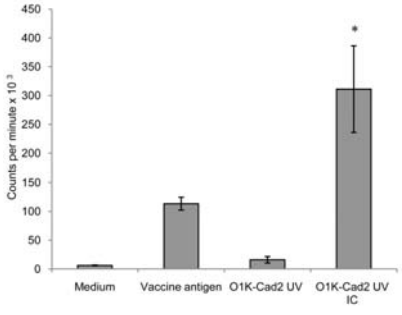
Antibody from immune cattle can form complexes with FMDV which allow the virus to enter, replicate in, and kill moDC



FMDV immune complexes significantly reduce T cell proliferative responses



Using IC to target inactivated FMDV to moDC results in significantly increased T cell stimulation

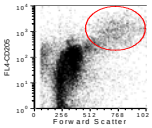


Antigen targeting



Infection of DCs with recombinant viruses *in-vitro*

(20 hours incubation)

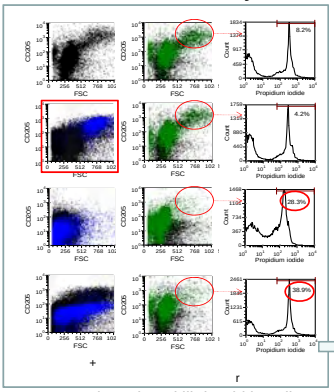


Vector efficiency at delivering antigen to DCs *in vitro*?

Recombinant virus	% GFP⁺ cells	Mean fluorescence (GFP)
No Virus	0.01	109
Adenovirus	**37.52**	**570**
MVA	31.60	172
Fowlpox	25.51	257

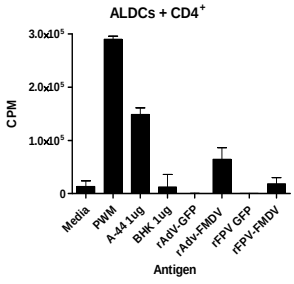
huAdV5 delivers antigen to more cells
Adenovirus-infected cells express antigen more efficiently

Viability of dendritic cells infected by recombinant viruses



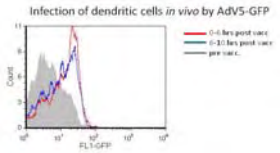
MVA and Fowlpox kill dendritic cells

T cell proliferation

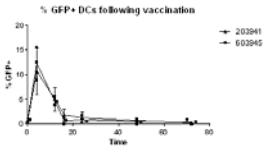




AdV5-GFP + Adjuvant – subcutaneously



% of DCs GFP+ post inoculation





Ryan Waters

INVESTIGATING FMDV MYOCARDITIS

Outline

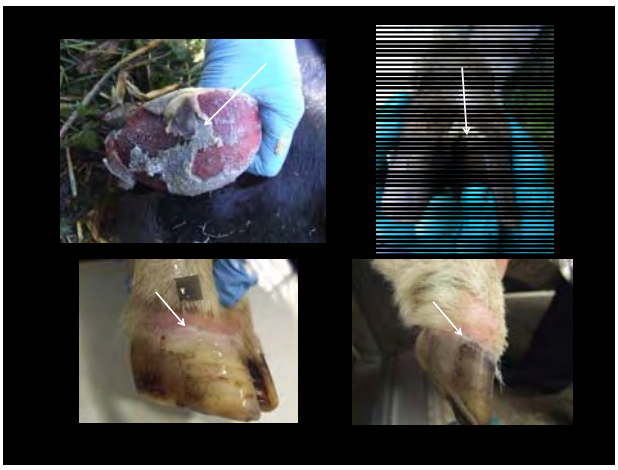
- Introduction
- Experimental design
- Results
- Conclusion

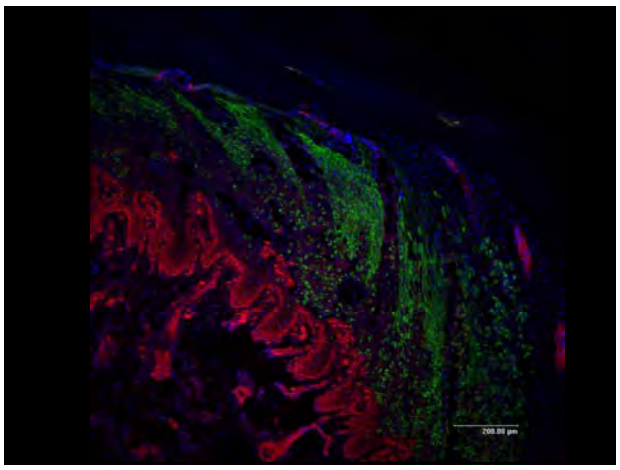
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- Introduction
- Experimental design
- Results
- Conclusion

Introduction

- Foot and mouth disease - **High morbidity**, Low mortality – Tiger heart…
- Epitheliotropic virus
- Acute morbidity associated with epitheliotropism





Low Mortality rate?

- Reported high mortality rates in neonatal animals - Myocarditis:
- Reported outbreaks with high mortality in non-neonates: Domestic and wild animals
- Associated with certain strains i.e O Iran 2006
- Need for understanding basic pathogenesis-
  - Is virus replicating?
  - Where?
  - any subclinical heart damage?

Outline

- Introduction
- Experimental design
- Results
- Conclusion

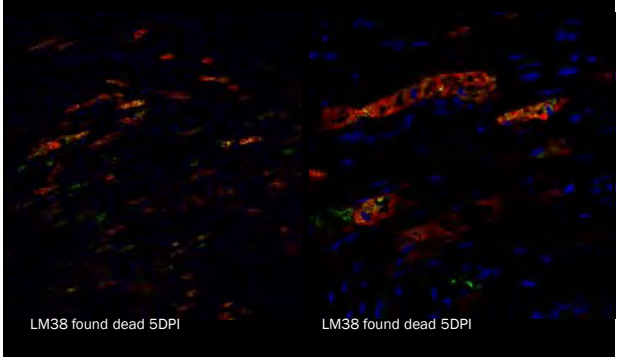
Experimental design

- 4 Neonatal lambs – 4 contact challenged, 1 control
- Euthanased 0,2,3,4,7
- 10 Neonatal lambs – 8 ICBI, 2 control
- Eutnanased 0,2,4,7,10

Outline

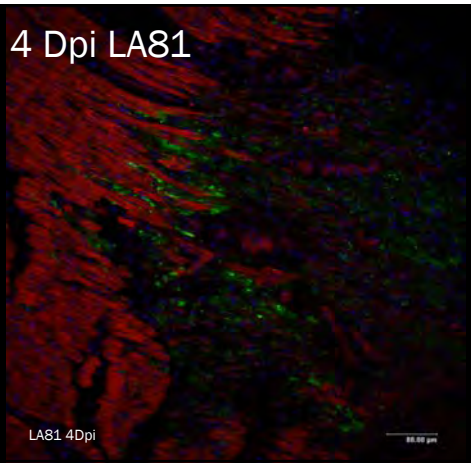
- Introduction
- Experimental design
- Results
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Is the Virus replicating?



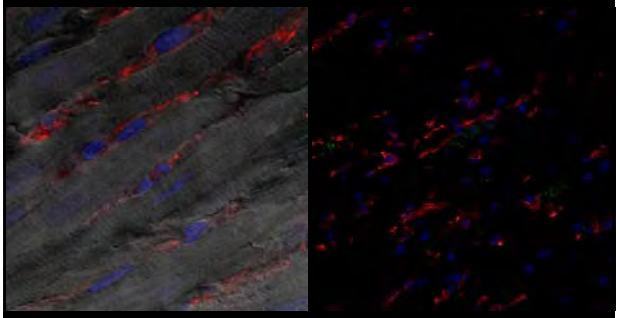
LM38 found dead 5DPI LM38 found dead 5DPI

4 Dpi LA81

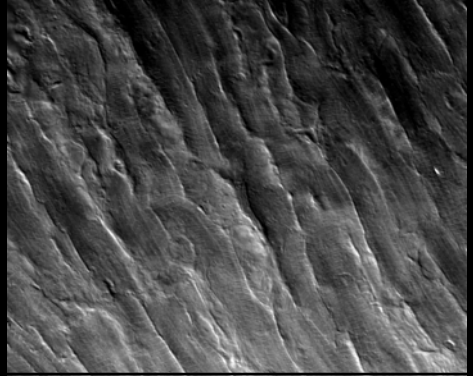


LA81 4Dpi

WHERE IS VIRUS REPLICATING?

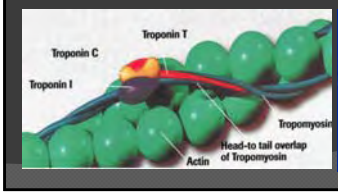


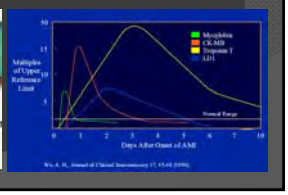
WHERE IS VIRUS REPLICATING?

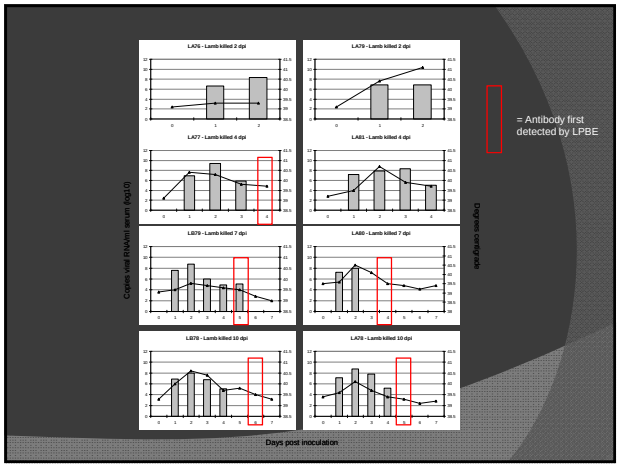


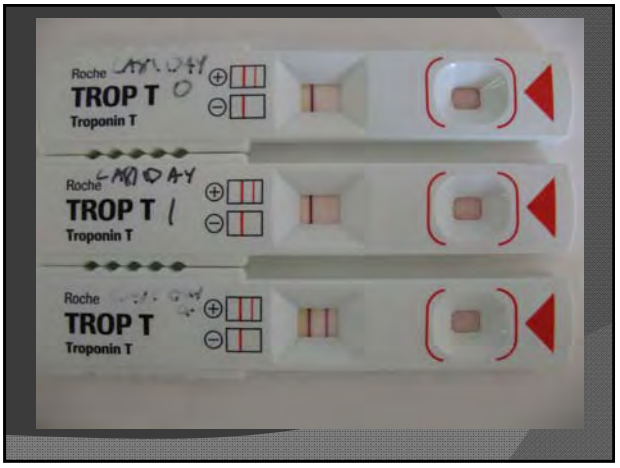
Subclinical heart damage

- ICBI lambs only considered as all were sub clinical
- Combination of immunofluorscence and Cardiac Troponin T assay





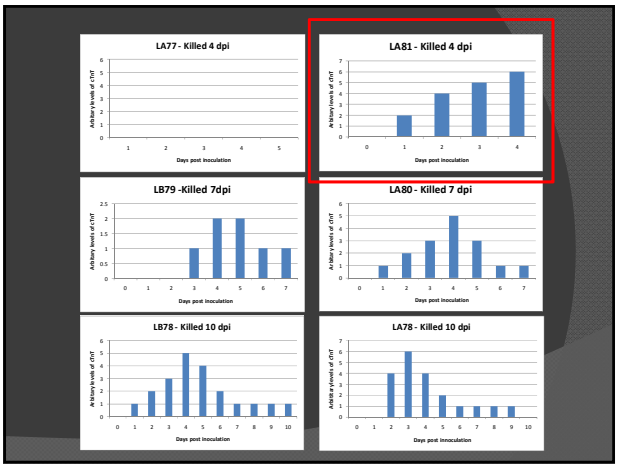


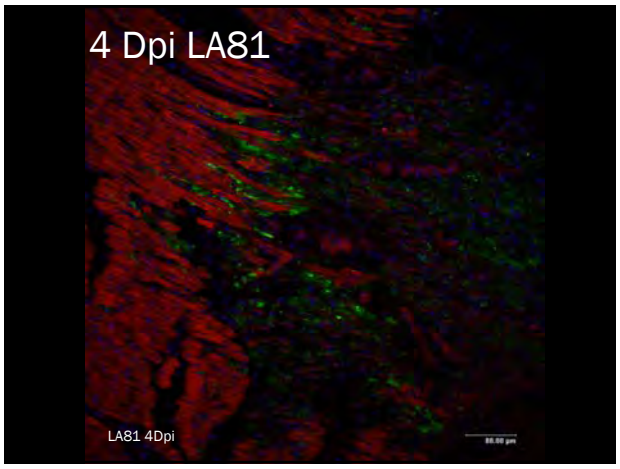


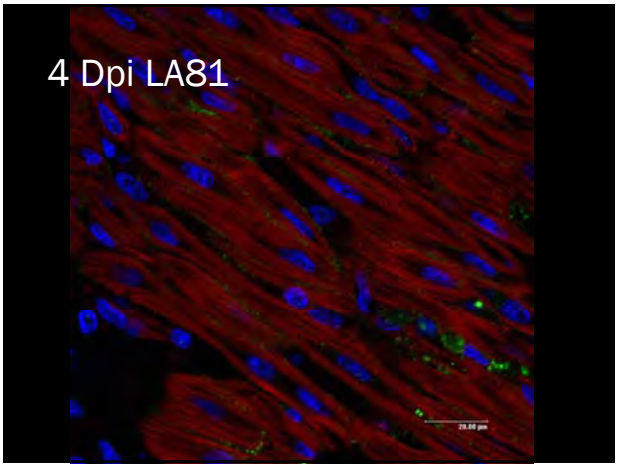
Lamb id	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	cTn heart LOG	immuno heart
LA82	Killed / Neg	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	NEG	NEG
LB82	Killed / Neg	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	NEG	NEG
LA76	NEG	NEG	Killed / Neg	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	8.8	NEG
LA79	NEG	NEG	Killed / Neg	Killed	Killed	Killed	Killed	Killed	Killed	Killed	Killed	8.6	NEG
LA77	NEG	NEG	NEG	NEG	Killed / Neg	Killed	Killed	Killed	Killed	Killed	Killed	7.17	NEG
LA81	NEG	POS 2+	POS 4+	POS 5+	Killed / POS	Killed	Killed	Killed	Killed	Killed	Killed	11.23	Pos
LB79	NEG	NEG	NEG	POS 1+	POS 2+	POS 2+	POS 1+	Killed / POS	Killed	Killed	Killed	7.39	Pos
LA80	NEG	POS 1+	POS 2+	POS 3+	POS 5+	POS 3+	POS 1+	Killed / POS	Killed	Killed	Killed	6.12	NEG
LA78	NEG	NEG	POS 4+	POS 6+	POS 4+	POS 2+	POS 1+	POS 1+	POS 1+	POS 1+	Killed / Neg		NEG
LB79	NEG	POS 1+	POS 2+	POS 3+	POS 5+	POS 4+	POS 2+	POS 1+	POS 1+	POS 1+	Killed / POS 1+	7.09	NEG

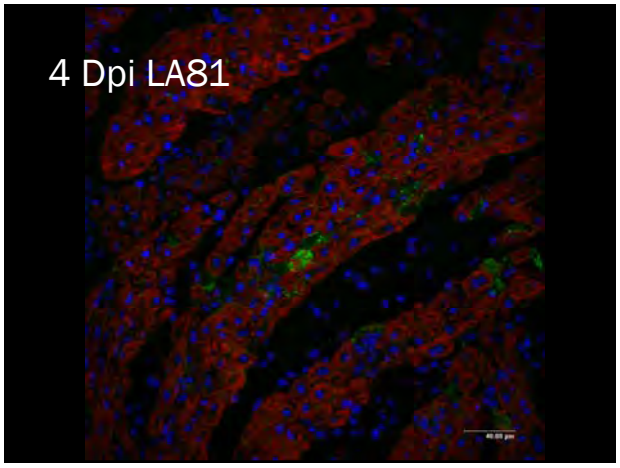
5 / 8 animals had detectable

cTnT = **63%**







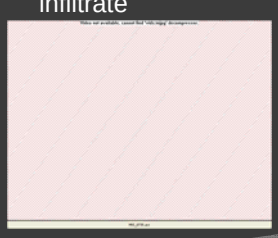


Conclusions

- FMDV replicates in the hearts of neonatal lambs, infecting only the cardiomyocytes
- Direct damage of cardiac myocytes, extensive-diffuse replication ➔ Death
- Subclinical heart damage relatively common

Other work

- In vitro culture of cardiac myocytes
- Characterisation of inflammatory cell infiltrate






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Eoin Ryan
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defra
Institute for Animal Health

FMD Week2010

INDUCTION OF EARLY SPECIFIC LOCAL IMMUNE RESPONSES IN THE RESPIRATORY TRACT OF FMD-INFECTED CATTLE

Mariano Pérez Filgueira

Institute of Virology, INTA- CONICET. Buenos Aires, ARGENTINA

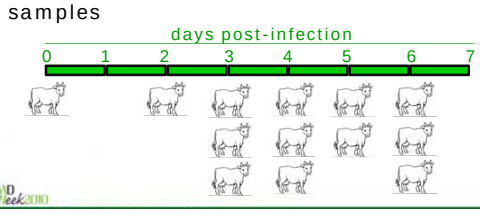
VIENNA. AUSTRIA • 27TH SEPTEMBER - 1ST OCTOBER

SUMMARY

- An ELISpot assay was set up to assess FMDV-specific antibody secreting cells (ASC) from lymphoid tissues in cattle
- This strategy was used to measure adaptive responses along the respiratory tract in cattle infected by the oronasal route
- The aim of this approach was to describe different parameters at the onset of the FMDV-specific local humoral response: tissues involved, magnitude, isotype profiles and time course of the response

EXPERIMENTAL DESIGN

- FMDV-seronegative Hereford cattle (~300 Kg) were infected by controlled aerosol exposure to FMDV O1 Campos (10^7 BTID) and sacrificed between 2 and 6 dpi to obtain lymphoid tissue samples



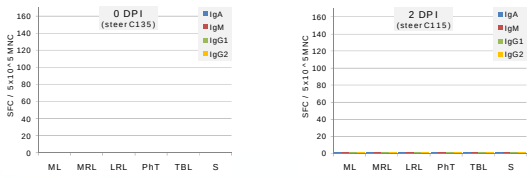
EXPERIMENTAL DESIGN

- FMDV-ASC ELISpot assay was developed to be applied to lymphocytes isolated from:
  - mandibular lymph nodes (ML)
  - lateral and medial retropharyngeal lymph nodes (LRL and MRL)
  - pharyngeal tonsil (PhT)
  - tracheo-bronchial lymph nodes (TBL)
  - spleen (S)
- Serum samples were also obtained for lpELISA

RESULTS

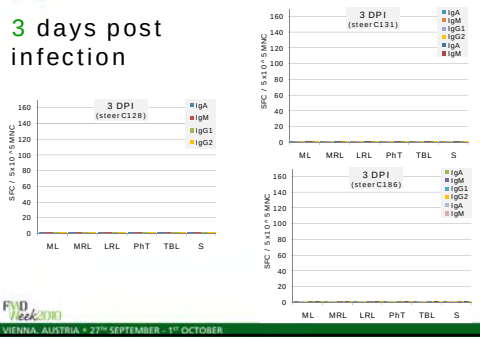
0 days post infection

2 days post infection



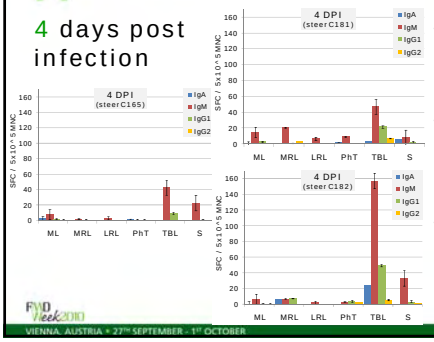
RESULTS

3 days post infection



RESULTS

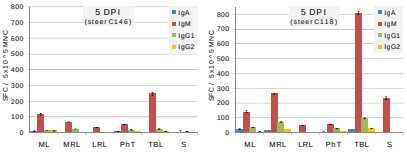
4 days post infection



- Tracheobronchial lymph nodes (TBL)> mandibular (ML) = medial retroph. lymph nodes (MRL)
- IgM (40-155 spc/5x10^5 cells in TBL) > IgG1 (up to 50 spc/5x10^5) > IgA
- Systemic responses detected in spleen are still low (15-60 spc/5x10^5 cells)

RESULTS

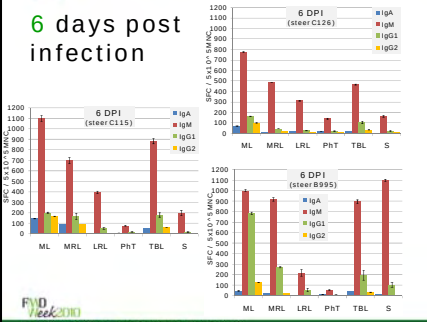
5 days post infection



- Tracheobronchial lymph nodes (TBL)> mandibular (ML) = medial retroph. lymph nodes (MRL)
- IgM (240-800 spc/5x10^5 cells in TBL) > IgG1 (~200 spc/10^6 cells in TBL)
- Systemic responses detected in spleen raised up to 230 spc/5x10^5 cells for IgM (animal C118)

RESULTS

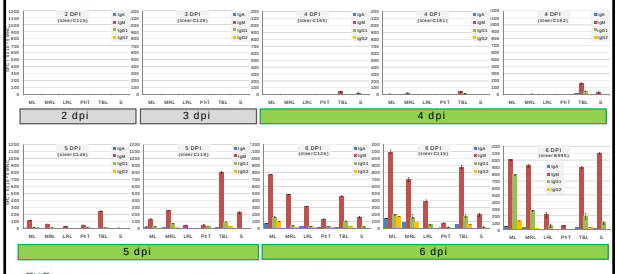
6 days post infection



- Mandibular lymph nodes (ML) > tracheobronc. (TBL) = medial retroph. lymph nodes (MRL)
- IgM (880-1100 spc/5x10^5 cells in TBL) > IgG1 (~200 spc/10^6 cells in TBL). Clear class switch in animal B995 (780 spc/5x10^5 cells in ML)
- Systemic responses (spleen) higher than mucosal responses in one animal (B995)

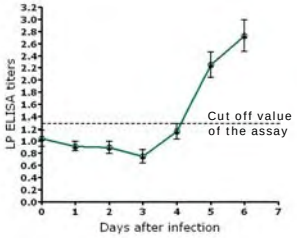
RESULTS

Time course of mucosal adaptive responses induced in FMDV infected cattle



RESULTS

Time course of systemic FMDV-specific humoral response in the infected animals



- Mean lpELISA titers for all animals at each time
- Systemic seroconversion was detected between 4 and 5 dpi

CONCLUSIONS

- FMDV-specific responses were detected at the respiratory tract of infected animals from 4 dpi
- Tracheobronchial LN showed the highest reactivity at 4 and 5 dpi

  This is consistent with a recent report describing the lungs as early viral replication sites from 24 hpi (Arzt et al. 2010)

- Mandibular and retropharyngeal LN responses were lower than TBL responses at 4 and 5 dpi but surpassed TBL at 6 dpi

CONCLUSIONS

- Isotype profiles were consistent with a primary response, although switch to IgG1 was already detected at 4 dpi
- FMDV-ASC were found in spleen at 4 dpi; accordingly systemic seroconversion was observed from 4 to 5 dpi. This leaves a narrow window of time between the onset of local and systemic adaptive responses
- Currently, we are studying the neutralizing activity of the antibodies induced and its correlation with the clearance of the viremia after infection

AUTHORS AND ACKNOWLEDGEMENTS

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¡Muchas gracias!



Imperial College London

EpiCollect

David Aanensen
Department of Infectious Disease Epidemiology
Imperial College London

Background

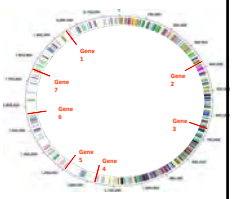
- Web Applications for global molecular epidemiology using open technologies.
- Multilocus Sequence Typing – www.mlst.net

The ability to identify and map incidence of strains of a pathogen is a crucial tool in understanding their spread and epidemiology.

- For most pathogens, many different strains exist in a population and we want to be able to distinguish them and map their distribution.
- E.g. strains of influenza, Antibiotic-resistant strains of bacterial pathogens

Methods

- Precise data generated by molecular methods (typically DNA sequences) can be stored (and queried), along with epidemiological data, in web-accessible databases.



Staphylococcus aureus



- MRSA – methicillin-resistant *Staphylococcus aureus*

- methicillin-sensitive *S. aureus* (MSSA)

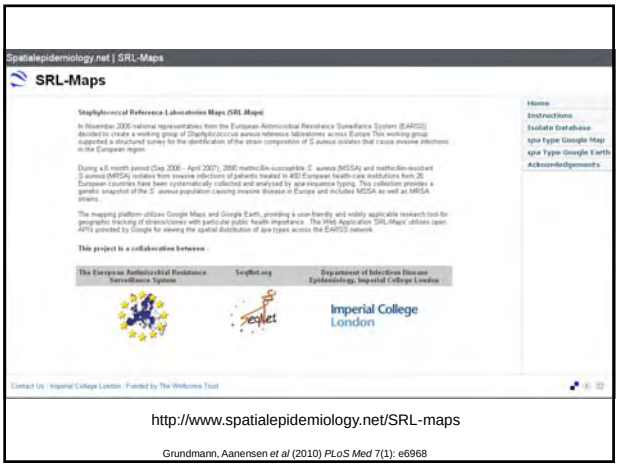
↓

- methicillin-resistant *S. aureus* (MRSA)

Identifying different strains can help understand pathogen evolution and the spread of infection

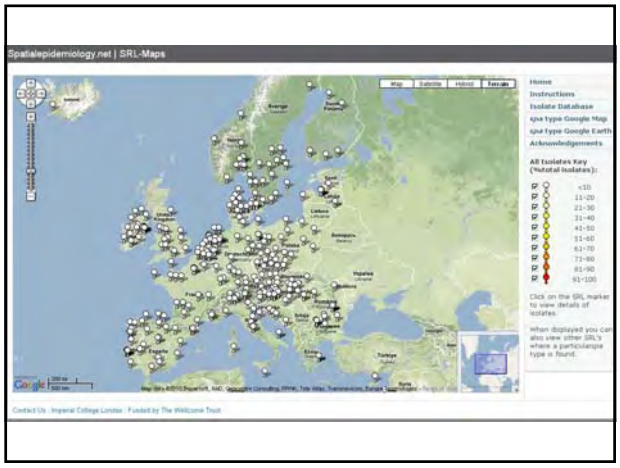
Questions?

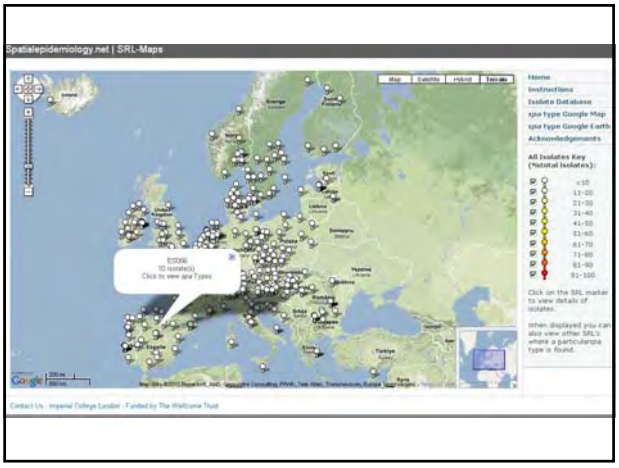
- Which strains are more likely to cause disease?
- Where are these strains found?
- How are they spread?
- For example, we would like to identify and track those strains causing disease between patients, between wards, between healthcare institutes and between countries.

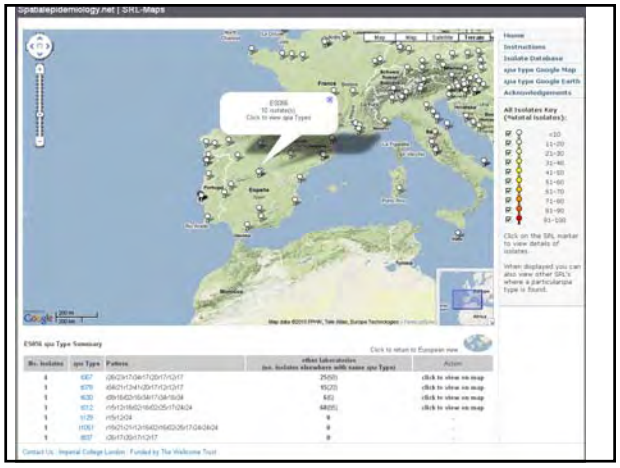


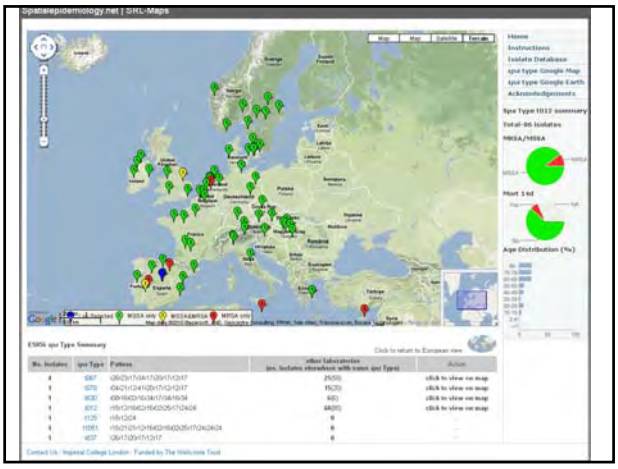
http://www.spatialepidemiology.net/SRL-maps

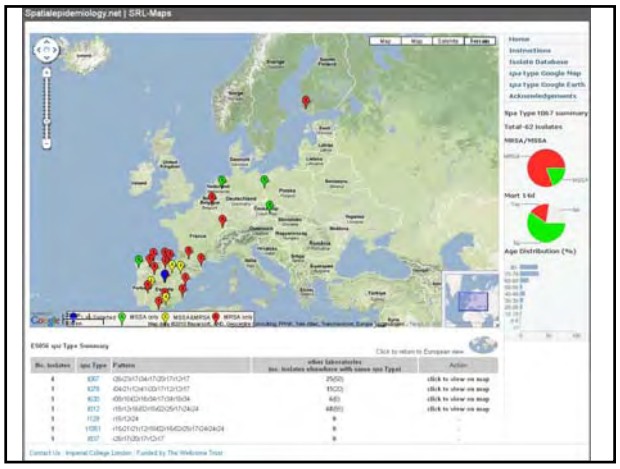
Grundmann, Aanensen *et al* (2010) *PLoS Med* 7(1): e6968











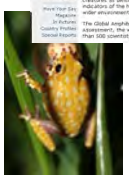
*Batrachochytrium dendrobatidis (*Bd*)*
(a fungus)









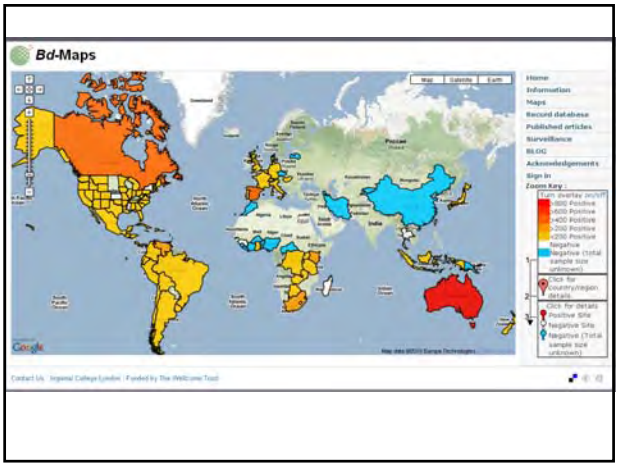


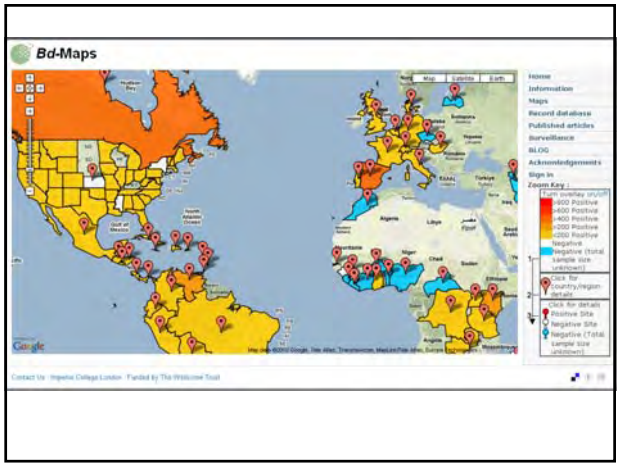


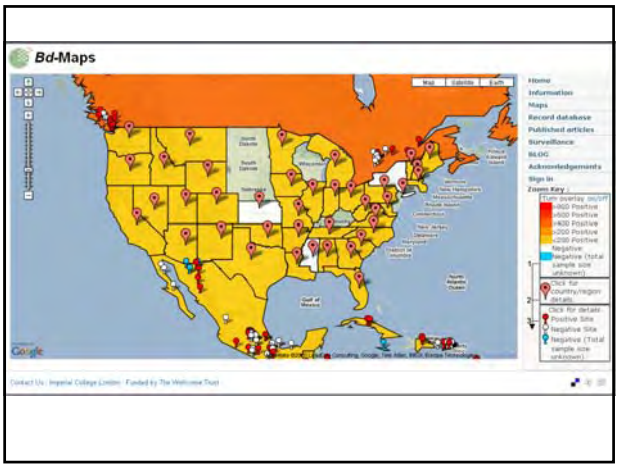
Bd-Maps

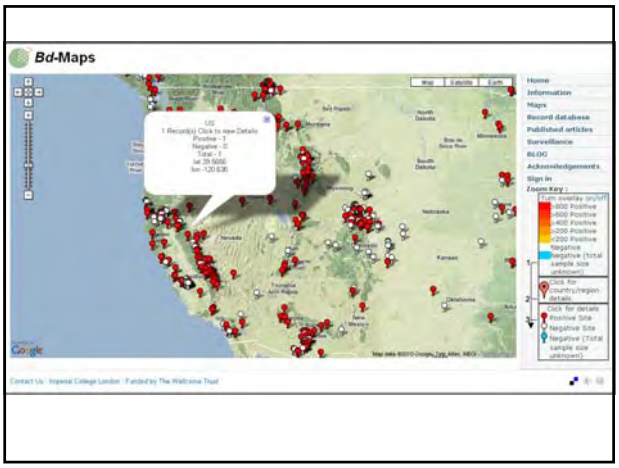
- **Globally, amphibians are declining faster than any other class of vertebrates**
- *Bd* causing wide-spread die-offs and species declines
- Demands logical collection of incidence, location and species distribution

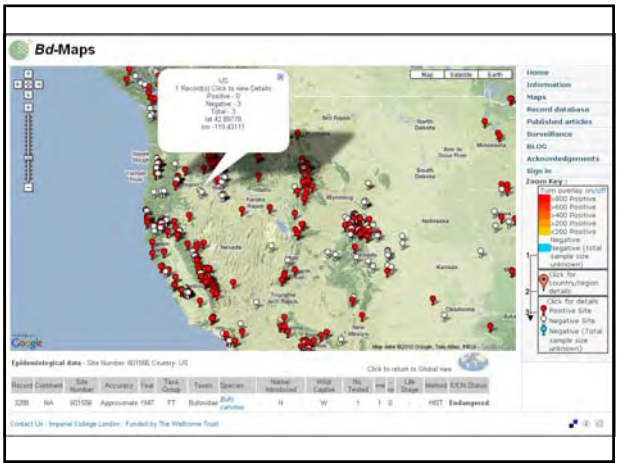
http://www.spatialepidemiology.net/bd-maps

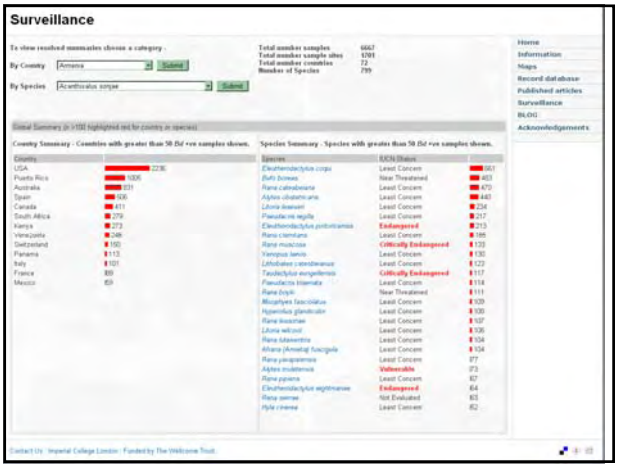












Smartphones for data collection - EpiCollect

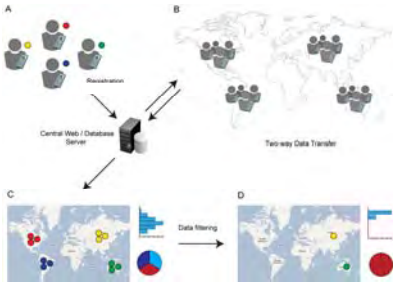
- GPS – 'location aware'
- Cameras for stills or videos
- Keyboards for text entry
- Data transfer to / from central web databases.



Aanensen *et al* (2009) *PLoS ONE* 4(9): e6968

EpiCollect

http://www.epicollect.net



Mobile phone app (Android and iPhone) and web application for data visualisation.

EpiCollect http://www.epicollect.net

- Questionnaires of any nature.
- Any projects where centralising textual data along with GPS and / or photos from many different people, from many different places, would be useful.

EpiCollect http://www.epicollect.net



Create a Project website at EpiCollect.net

Design a form for data collection

Load Project into the EpiCollect mobile app and collect data, including GPS position and photo

View data collected at your project website or on your phone (download, view on maps/charts, filter.)

EpiCollect http://www.epicollect.net

- Completely free and open, with project websites and data storage using Google AppEngine.
- demo

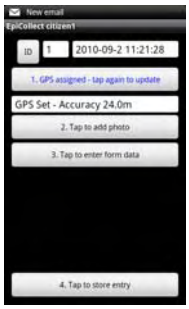







EpiCollect
Success
Project Loaded
OK
Project URL is:
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html?name=overtheair1

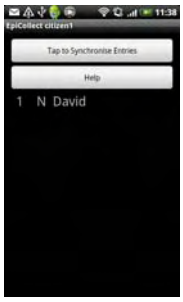

EpiCollect
New Entry
List Entries
Display Map
Select Project
eufmd
Project URL is:
http://www.epicollect.net/project.
html?name=eufmd
Help


EpiCollect citizen1
ID
1
2010-09-2 11:21:28
1. GPS assigned - tap again to update
GPS Set - Accuracy 24.0m
2. Tap to add photo
3. Tap to enter form data
4. Tap to store entry


EpiCollect citizen1
ID
1
2010-09-2 11:21:28
1. GPS assigned - tap again to update
GPS Set - Accuracy 24.0m
2. Photo added - tap to change
3. Tap to enter form data
4. Tap to store entry


EpiCollect citizen1
What is your name?
David
Male or Female?
male
Confirm


EpiCollect
New Entry
List Entries
Display Map
Select Project
eufmd
Project URL is:
http://www.epicollect.net/project.
html?name=eufmd
Help





Animal Health surveillance in Kenya / Tanzania



Ongoing monitoring of:

East Coast Fever; anthrax and rabies; PPR; FMD

Gabriel Turasha (Vetaid Tanzania) Nick Short(RVC) Niall Winters(IOE)

Resource mapping and further work with SACIDS

Some projects people have set up

- Archaeological dig sites – Europe
- Plant distribution Yellowstone National Park-USA
- Street art collection - UK
- Drug administration monitoring and evaluation across Africa (schistosomiasis and lymphatic filariasis). SCORE and Task force
- Malaria surveys

- No reliance on data networks for collection.
- Data can be sent to any / multiple servers
- Simple text (XML) description of project forms and for definition of server locations.
- Code open

XML project definition

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        </input>

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                        <item>
                                <label>male</label>
                                <value>male</value>
                        </item>
                        <item>
                                <label>female</label>
                                <value>female</value>
                        </item>
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```

XML project definition

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    <input ref="name" required="true" title="true">
        <label>What is your name?</label>
    </input>

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                <label>male</label>
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    </select1>
</xform>
```

XML project definition

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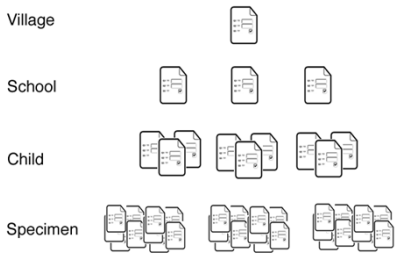
XML project definition

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    <input ref="name" required="true" title="true">
        <label>What is your name?</label>
    </input>

    <select1 ref="sex" required="true" chart="pie">
        <label>male or female?</label>
            <item>
                <label>male</label>
                <value>male</value>
            </item>
            <item>
                <label>female</label>
                <value>female</value>
            </item>
    </select1>
</xform>
```

Increased project complexity



All defined in XML
as well as text fields each form can also include one or more..

- <gps>
- <picture>
- <video>
- <sound>
- <barcode>
- <bluetooth>

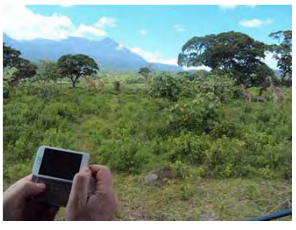
- Skip patterns and validation.

Acknowledgements

- Imperial –Dr Derek Huntley, Jon Evans, Chris Powell, Prof. Brian Spratt
- SRL-Maps – Prof Hajo Grundmann (RIVM) and EARSS
- Bd-Maps – Dr Dede Olson (US Forest Service) and Dr Matt Fisher

Funded by:
wellcome trust

d.aanensen@imperial.ac.uk



Applications of Replikins^{R} and BioRadar Global Surveillance System^{TM} in FMDV surveillance and vaccine production

S. Bogoch [1], E. Bogoch[1] and P. Willeberg[2]

[1] Replikins, Ltd., 38 the Fenway, Boston, MA 02215, USA

[2] Center for Animal Disease Modeling and Surveillance, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

Replikins the biochemistry of rapid replication

REPLIKINS

A biochemical key to rapid replication

- A new group of peptides in viruses and other infectious organisms and proteins related to rapid replication
- Named "Replikins" because of their close quantitative relationship to rapid replication and outbreaks
- Discovered in the disease organism's genome
- Small peptides strictly defined by the presence and concentration of certain amino acids, and the spaces between them
- Increase in the number of Replikin peptides per 100 amino acids (Replikin Count), in the overall genome correlates with rapid replication and disease outbreaks

Replikins the biochemistry of rapid replication

No structures of infectious organisms have been known previously which correlate quantitatively and temporally with epidemic outbreaks, course, and lethality, and which permit early or advance warning and rapid specific response.

Replikins the biochemistry of rapid replication

References

1. *Bogoch S and Bogoch ES. Replikins: the chemistry of rapid replication. Begell House, N.Y. 2005; Library of Congress*
2. *<replikins.com>, 'Replikins Press'-33 online reports, 2006-2009;*
3. *www.uspto.gov. 2001-2009;*

THESE REFERENCES PRESENT EVIDENCE THAT
-Replikins are specific epitopes in influenza viruses
-increase in concentration of Replikins (Replikin Count) signals influenza outbreaks
-conservation of Replikins occurs back to 1918
-cross-strain conservation of Replikins occurs
-all above make pan-flu vaccines possible,

Data from CDC-Harvard and Scripps-Crucell (4,5) confirms Replikins:

-inhibitory antibody lands on and binds to Replikins as the specific epitopes in influenza viruses
-conservation of Replikins back to 1918
-cross-strain conservation of Replikins
-all above make pan-flu vaccines possible, as Bogoch and Bogoch previously showed (1-3)

References

1. *Bogoch S and Bogoch ES. Replikins: the chemistry of rapid replication. Begell House, N.Y. 2005;*
2. *<replikins.com>, 'Replikins Press'-33 online reports, 2006-2009;*
3. *www.uspto.gov. 2001-2009;*
4. Sui, J. et al. "Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses.", Nature Structural and Molecular Biology, published online:22 February,2009, doi:10.1038-nsmb.1566.
5. Ekeirt, D.C. et al, "Antibody Recognition of a Highly Conserved Influenza Virus Epitope", Science DOI: 10.1126-science.1171491, Science Published online, Feb. 26 2009

Replikins the biochemistry of rapid replication

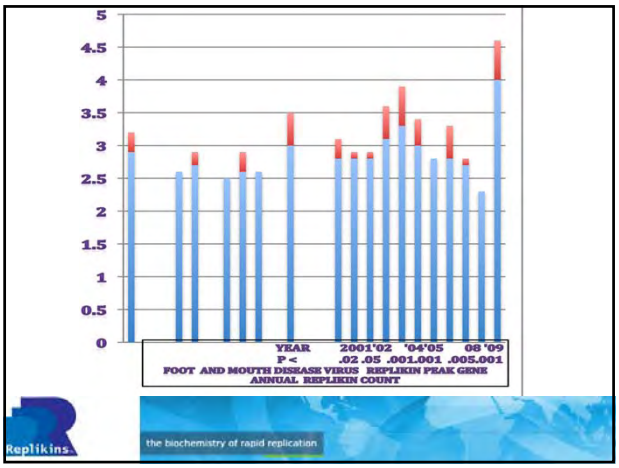
FMD

14/14 correct predictions in influenza 2001-2010
H1N1 Pandemic of 2009 was correctly predicted and published one year earlier, in April 2008 (see refs)
Foot and Mouth Disease Outbreaks in 2010 Were Predicted in 2009 by the Quantitative Increase of Replikins in Foot and Mouth Disease Virus Gene

2009: Highest Replikin Counts of FMD virus peak gene in 52 years;

Completely Synthetic Replikins FMDV Vaccine based on conserved replikins now available

Replikins the biochemistry of rapid replication



Conservation of Replikins in Foot and Mouth Disease Virus

- Each year from 1936 to the present
- Permits strain-specific and inter-strain vaccines

Replikins
the biochemistry of rapid replication

EARLY WARNING ➔ RAPID RESPONSE

By quantitatively analyzing Replikin sequences

BIORADAR™

Predicts and Tracks:	• ***outbreaks*** and ***epidemics*** • ***the geographical location*** of such outbreaks
Measures:	• ***infectivity*** ***TWO SEPARATE REPLIKINS GENES*** • ***lethality***
Applies to:	• ***multiple diseases and strains of diseases*** e.g. influenza, SARS, Foot and Mouth Dis., HIV, malaria
Found in:	• ***multiple disease hosts*** (animals and humans)

Replikins
the biochemistry of rapid replication

REPLIKINS VACCINES

Biological Vaccines –*Too Little, Too Late*

- There are over six billion people and countless animal species
- Current production methods cannot meet world demand
- Annual and *ad hoc* formulations are sub-optimal
- Production against the latest emerging strain takes too long
- Contaminants and side effects are common problems

Replikins Synthetic Vaccines – *a Solution*

- Non-biological, produced via solid-phase chemical synthesis
- No biological contaminants, no need for potentially toxic preservatives
- Produced in as little as seven days
- Can be rapidly mass produced to meet world pandemic needs
- More cost-effective to produce
- Readily transported without refrigeration

Replikins
the biochemistry of rapid replication

BioRadar™ services are available

To national governments and public health groups, for surveillance and early warning efforts

and

to pharmaceutical companies, for increasing specificity and the time available for vaccine production and testing

Replikins
the biochemistry of rapid replication

APPENDIX 93

Real-time training for improved FMD diagnostic and response in the field: Survey report

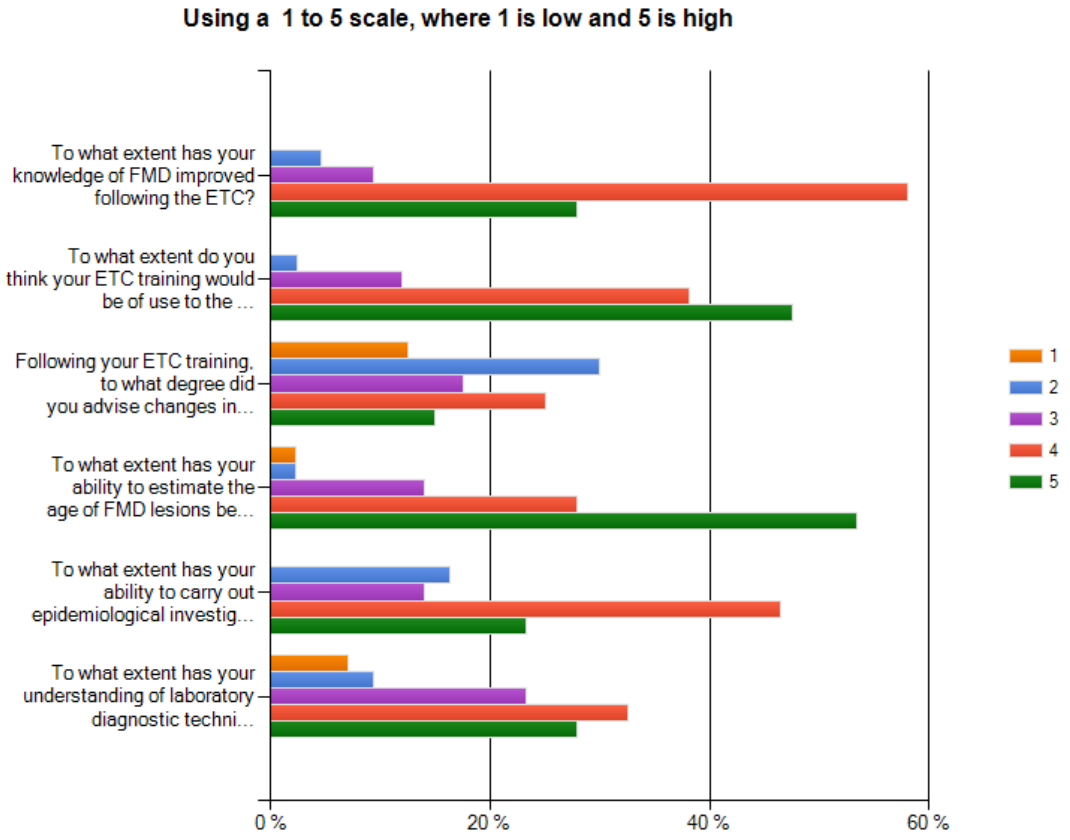
70 questionnaires sent 44 responses received: (15 trainers and 29 trainees) for the first two questions: 60% responses and 26 for the second part for the questionnaire on the proposals for improvement.

Main conclusions:

1. Excellent evaluation of the previous sessions and real added value: improvement of the knowledge of FMD, including lesions ageing , outbreak investigation and usefulness in the European countries for the countries preparedness and in case of outbreak. The duration and the program were highly appreciated.
2. Proposals for improvement of FMD Investigation:
- Refresher courses mixing technical, clinical and management problem solving
- Participation in FMD technical workshops or conferences

I/Overall assessment and added value of the previous sessions

Question 1-2: training assessment:


Using a 1 to 5 scale, where 1 is low and 5 is high
To what extent has your knowledge of FMD improved following the ETC?
To what extent do you think your ETC training would be of use to the ...
Following your ETC training, to what degree did you advise changes in...
To what extent has your ability to estimate the age of FMD lesions be...
To what extent has your ability to carry out epidemiological investig...
To what extent has your understanding of laboratory diagnostic techni...
0 %
20 %
40 %
60 %
1
2
3
4
5

II/ Proposals for improvement:

26 responses to the questions on the proposals/suggestions for improvement in the future:

Question 1: on "what improvements do you suggest for the documentation provided?"

- 11 no suggestions.
- suggestions to include biohazard poster from Ireland with pictures in the documentation.
- Adapt more the trainer's document to what he is expected to do
- Provide the documents in advance before the course
- Include in the material on outbreak investigation discussion on difference between herd investigation versus village investigation.
- Timely provision, harmonization of presentations with contents of manual, specified homework.

Question 2: "Did the talks cover all your theoretical needs/gaps (yes/no)? if no, which subjects should be added?":

Yes: 20/ No: 6

- Suggestion to add talks on lab methods, more EU legislation, on epidemiology investigation.

Question 3: "Which elements could be improved in or added to the outbreak investigations?"

- Purpose of OI to be more clear: (sources? Spread? what to do?)
- More knowledge of local situation, i.e. how many outbreaks with 20 km of investigation site in last 12 months or similar.
- More field investigation and work with local veterinarians.
- Investigate different animal species
- Talk more about lesion age and epidemiological investigation
- Clinical and epi teams could interact more to ensure that both aspects are covered by course participants.
- - OI should better focus on local conditions, more specific OI ppt on E.Anatolia
- - During OI interviews in villages interviewers should take turns, and have midterm reviews
- Investigation needs to have clear purpose: what questions should be answered by the end?: source? spread? what should be done next?
- Proposals for improvement of photos taking

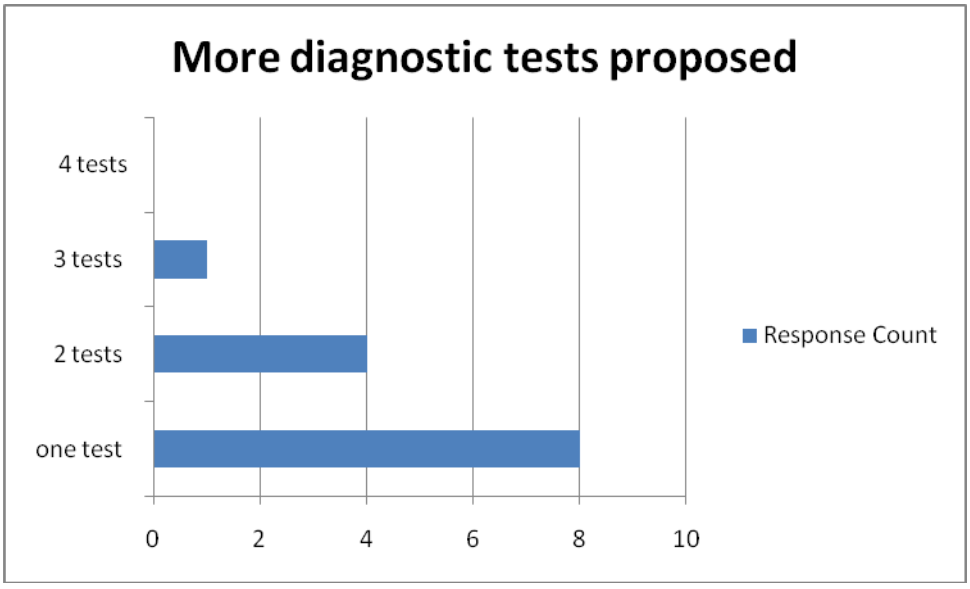
Question 2-4: "What is your opinion about the balance between field work and laboratory work?"

Found to be well balanced

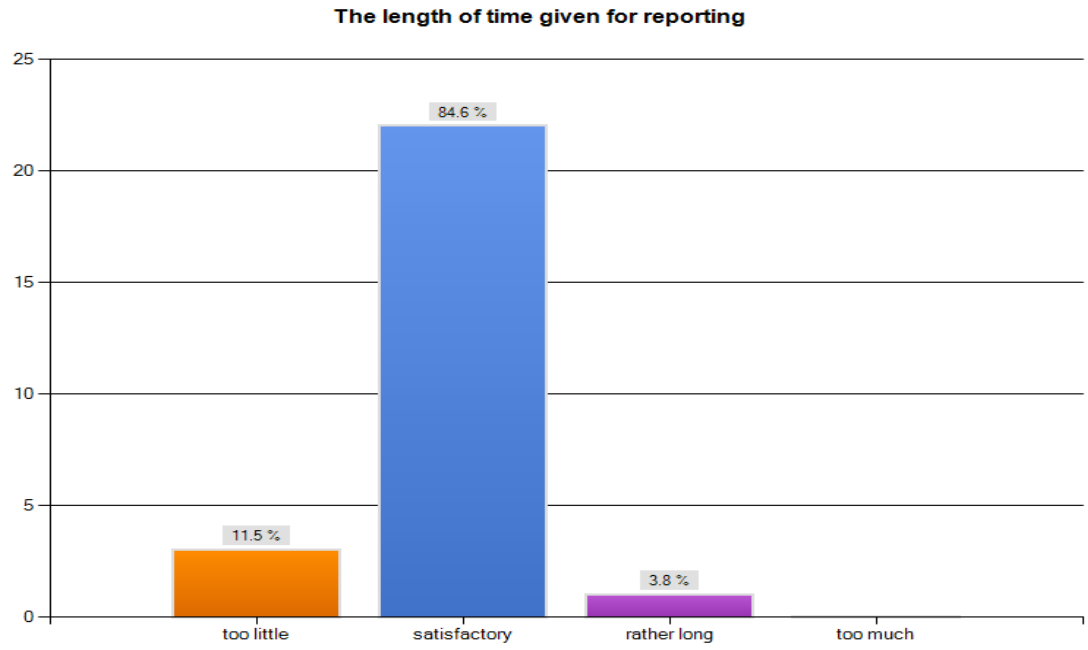
One proposal to add more field work.

Question 2-5 "Were the range of laboratory tests demonstrated and/or practiced sufficient? If not, which tests would you like to see or have discussed in addition?"

23 responses and 8 made proposals: Use more diagnostic tests, such as PCR and ELISA

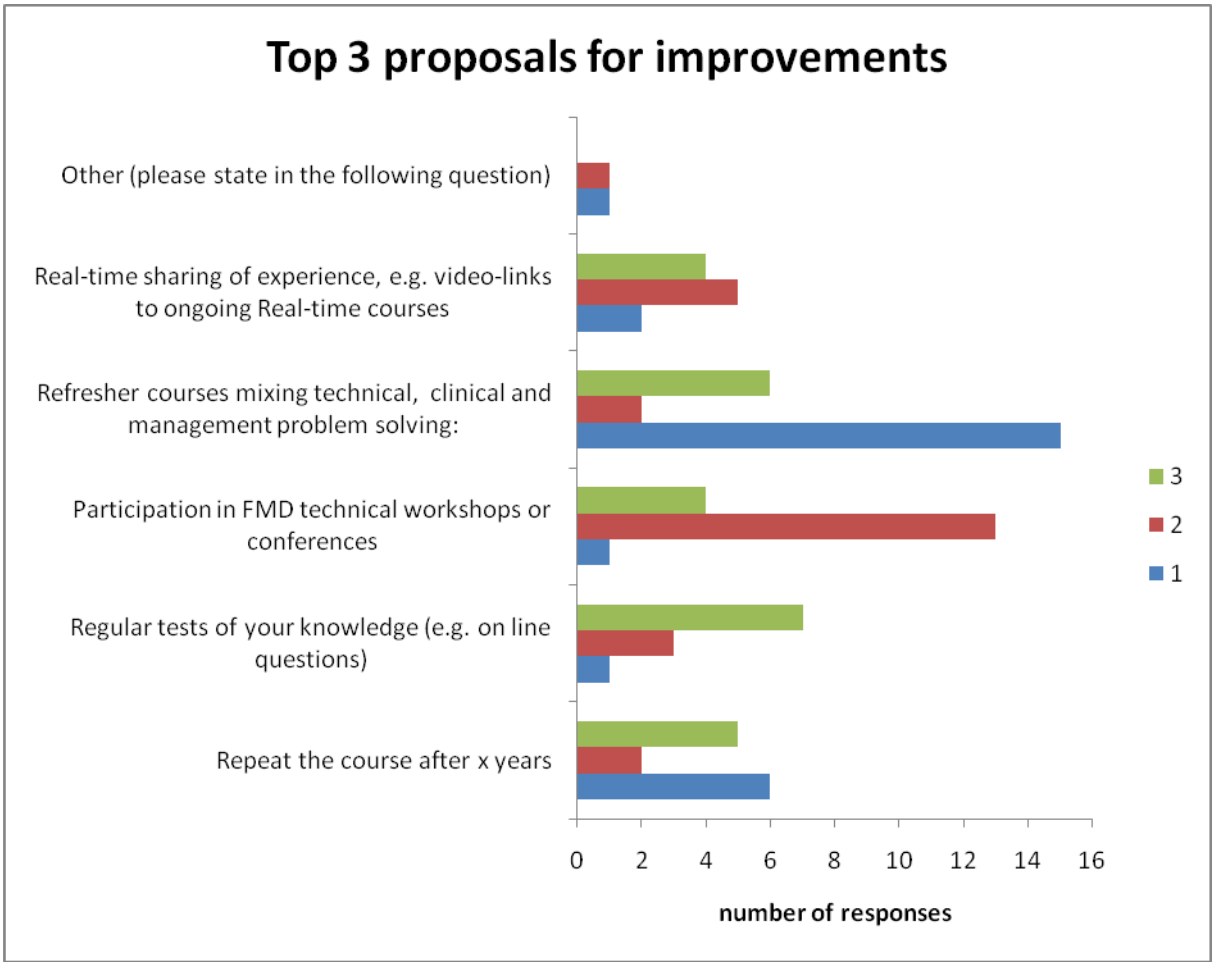


Question 2-6: “the length of time given for reporting



Question 2-7: In order to retain or further improve your level of experience in FMD investigation, which of the following do you think would be useful? Please rank your top 3 options below, where 1= HIGH and 2,3 are lower ranks: "top 3 for improvement proposals"

1. Refresher courses mixing technical, clinical and management problem solving
2. Participation in FMD technical workshops or conferences
3. Regular tests of your knowledge (e.g. on line questions)



Question 2-8: "In order to retain or further improve your level of experience in FMD investigation, can you suggest other ways?"

16 proposals / 10 no proposals

- An EuFMD occasional newsletter (email) highlighting particular issues that have been raised during current outbreaks would be very useful. The recent outbreaks in SE Asia especially in countries previously free of FMD should mean that valuable lessons can be learned and inform policy in other countries.
- discussion groups, review and critique of RT course reports
- Support the organization of FMD local conferences/ workshops in our countries in order to share the EuFMD Real Time course materials with our local colleagues.
- Organize office exercise between countries on the level of their administrations to improve rapid alert system and coordination of their activities in the case of real outbreak. It can be organized on line. Most important is to prepare realistic scenario of

outbreak to recognize weakness of communication and differences in contingency planing. It will be useful in real situation of outbreak, to recognize competent authority of neighboring country with its organization scheme and capacity.

FULL PAPERS

CONFIDENCE IN INDIRECT ASSESSMENT OF FOOT-AND-MOUTH DISEASE VACCINE POTENCY AND VACCINE MATCHING CARRIED OUT BY LIQUID PHASE ELISA AND VIRUS NEUTRALIZATION TESTS.

*Blanca Robiolo [a], José La Torre [a], Eduardo Maradei [b], Claudia Perez Beascoechea [b], Alejandro Perez [b], Cristina Seki [a], Eliana Smitsaart [c], Norberto Fondevila [d], Eduardo Palma [d], Nesya Goris [e,1], Kris De Clercq [e], Nora Mattion [a, *]*

[a] Centro de Virología Animal, Instituto de Ciencia y Tecnología Dr. César Milstein, CONICET, Saladillo 2468, (1440) Buenos Aires, Argentina. [b] Servicio Nacional de Sanidad Animal (SENASA), Fleming 1653, (1640) Martínez, Argentina. [c] Biogénesis-Bagó S.A., Ruta Panamericana Km 38.2, (1619) Garín, Argentina. [d] Instituto Nacional de Tecnología Agropecuaria, Centro de Investigación de Ciencias Veterinarias y Agronómicas (INTA-CICVyA), CC77, (1708) Morón, Argentina. [e] Veterinary and Agrochemical Research Centre, Virology Department, Section of Vesicular and Exotic Diseases, Groeselenberg 99, 1180 Brussels, Belgium. [1] Present address: Okapi Sciences, Ambachtenlaan 1, 3001 Heverlee, Belgium

ABSTRACT

The repeatability and reproducibility of the Expected Percentage of Protection (EPP) as a serological potency surrogate for A24 Cruzeiro FMDV strain was assessed, and compared with the results obtained with challenge in the Protection against Podal Generalization (PPG) test. Indirect serological assessment of vaccine matching between the serotype A FMDV strains A24 Cruzeiro and A/Argentina/01 was also carried out by lpELISA and VNT. The results obtained in this study strongly support the replacement of challenge tests for vaccine potency by indirect serological assays, at least for A24 Cruzeiro FMDV strain. While determination of EPPs by lpELISA titers showed an excellent repeatability, reproducibility and concordance with PPG for vaccine potency, assessments of cross-protection by VNT titers were more consistent with the PPG outcome.

1. INTRODUCTION

The gold standard test for foot-and-mouth disease (FMD) vaccine potency is the in vivo challenge procedure carried out in the target species to determine the efficacy of the vaccine [1]. However, the challenge tests have several drawbacks regarding standardization, cost, use of large animals and facilities with high biosecurity levels, among others, and its replacement by reliable indirect tests, if possible by in vitro tests, is a priority task from the perspective of the 3R (Refinement, Reduction, Replacement) concept [2].

According to the OIE Manual, indirect tests may be used to assess the potency of a vaccine provided that a statistical evaluation has established a satisfactory correlation between the results obtained by the in vivo potency test in cattle and the alternative test, using the relevant vaccine serotype [1]. A thorough evaluation is provided by the expected percentage of protection (EPP) method, which estimates the likelihood that cattle would be protected against a challenge of 10,000 infective doses after a single or boosted vaccination [4]. The clinical protection data is derived from previously performed experiments that have been carried out on a significant number of cattle immunized with the vaccine strain in question, and challenged with the homologous virus. Each animal is scored as protected or not protected, and tables of correlation based on logistic regression models are established between antibody titer and clinical protection [1, 3, 5, 6]. In Argentina, the validation of the lpELISA and the construction of correlation curves of lpELISA titers with PPG, have been reported for the four FMDV strains present in the vaccines (A24 Cruzeiro, A/Argentina/01, O1 Campos and C3 Indaial). The concordance between the in vivo and the serological outcomes was established considering 40 bovine PPG trials carried out with batches of commercial vaccines manufactured during the years 2001 to 2008 [6].

In the present study, the repeatability and reproducibility of the EPP determined by two ex vivo serological tests, lpELISA and virus neutralization (VNT) was carried out using the sera from animals involved in the ten PPG trials described by Goris et al. [3]. The suitability of serological measurements of cross protection for vaccine matching purposes was also assessed.

2. MATERIALS AND METHODS

2.1 Vaccine and animal trials

PPG trials were carried out as reported previously [3, 9]. Briefly, sixteen animals were vaccinated with 2 ml of a monovalent vaccine batch formulation of purified A24 Cruzeiro/Brazil/55 (A24 Cruzeiro) antigen in a water-in-oil emulsion and challenged at 30 days post vaccination (dpv) with homologous (A24 Cruzeiro) or heterologous [A/Argentina/2001 (A/Arg/01)] FMDV strains. From the number of vaccinated protected animals (i.e. absence of FMDV-induced lesions at the feet), the PPG percentage was determined. Ten independent PPG trials were conducted according to the protocol established by the Argentine Animal Health Service (SENASA) in Act No. 351/2006 [10], except that the animals were challenged at 30 days post vaccination (dpv) instead of at 90 dpv. All PPG trials were carried out using the same vaccine within an 11-month period (January-November 2006). The sera collected at 30 dpv were used in the present study. The 95% confidence intervals (95%CI) were calculated using Soft Graph Pad PRISM 4.

2.2. Serological tests

lpELISA was carried out and validated as described previously [6]. VNT was conducted following the protocol of the OIE Manual [1], using BHK-21 c13 cell monolayers. The virus titers and the titer of positive working control sera were charted, monitored and compared to their predetermined values [6].

It is important to emphasize that in potency tests, all the reagents (antigen and sera) were homologous, but when determining cross-protection, the sera used were raised against the vaccine strain A24 Cruzeiro, whilst the antigen used was the heterologous A/Arg/01.

2.3. Expected Percentage of Protection

The EPP was determined from the serological titers obtained for each individual serum, referenced to predetermined tables of correlation between serological titers and clinical protection against virus challenge, based on logistic regression models, as reported previously [6, 11]. The tables of correlation used in this study were: i) SENASA-CEVAN's correlation curves for lpELISA titers for A24 Cruzeiro and A/Arg/01 strains, and ii) the A24 Cruzeiro PANAFTOSA's curve for VNT titers. In each trial, the mean EPP (average of the 16 individual EPPs) and the 95% CI were calculated. An EPP <75% is an indication that the vaccine will give a low protection against homologous challenge [6]. For the calculation of the variability within each trial (intra-trial variability) and among the trials (inter-trial variability), the standard deviation (SD) and coefficient of variation (CV %) were calculated.

It is worth mentioning that in cross protection studies, A/Arg/01 was considered alternatively as an emerging strain or as a vaccine strain. This is because this strain, which represents the predominant isolate from the year 2001 outbreaks, was subsequently incorporated into the Argentine vaccine used in regular vaccination campaigns. In the first case, the A24 Cruzeiro logit regression curve was used for the determination of cross protection by EPP, while in the second case, the homologous A/Arg/01 logit regression curve [6] was used for comparison purposes.

3. RESULTS

3.1. Vaccine potency assessment by EPP for FMDV strain A24 Cruzeiro

The A24 Cruzeiro monovalent vaccine exceeded a potency of 75%, determined by EPP using lpELISA titers in ten replicate trials (Table 1), and by PPG after homologous challenge, in six of those trials [3]. The vaccine also achieved an EPP >75% in 9 out of 10 replicate trials, when the potency was calculated with VNT titers and the PANAFTOSA's logit regression curve. In one trial (trial 9), the vaccine was rejected by VNT EPP (EPP=73.5%), while it was approved by PPG and lpELISA.

The mean EPP of the 10 trials, calculated from lpELISA titers (87.4 %) or VNT (85.8%), was very close to the mean outcome of the 6 challenge trials (PPG= 88.5%). The 95% CI of the mean lpELISA EPP or VNT EPP of the 10 trials was well above the approval limit of 75%. However, in some individual trials, the lower limit of the 95% CI was slightly below the approval value (trial 7 for lpELISA EPP, and trials 7, 9 and 10 for VNT EPP). The lower limit of the 95% CI for the PPG test was below 75% in five out of six trials (Table 1).

VNT and lpELISA titers were subsequently grouped in fixed intervals (Fig. 1). The protection levels corresponding to each group of titers (shown by the hatched bars), matched closely with the VNT and lpELISA logit regression curves for A24 Cruzeiro.**Table 1.** Intra-trial repeatability and inter-trial reproducibility of vaccine potency assessment by PPG or EPP for FMDV strain A24 Cruzeiro.

	A24 Cruzeiro									
Trial [a]	**PPG (%)**	**95%CI**[b]	**lpELISA**				**VNT**			
			Mean titer	**EPP**[c] **(%)**	**95% CI**	**CV%**	**Mean titer**	**EPP (%)**	**95% CI**	**CV%**
1	100	[80.6 - 100.0]	2.50	89.9	[85.6 - 94.2]	8.90	1.76	93.0	[89.3 - 96.7]	7.50
2	93.8	[71.8 - 98.6]	2.66	91	[85.2 - 96.8]	11.98	1.81	91.9	[85.5 - 98.4]	13.25
3	ND	--	2.50	89.5	[86.1 - 92.9]	7.16	1.84	93.9	[89.6 - 98.2]	8.57
4	ND	--	2.27	83.7	[75.9 - 91.5]	17.56	1.74	92.7	[88.6 - 96.9]	8.35
5	ND	--	2.50	88.3	[83.5 - 93.1]	10.19	1.71	87.7	[81.3 - 94.1]	13.68
6	ND	--	2.67	91.8	[88.0 - 95.6]	7.74	1.57	84.0	[76.9 - 91.1]	15.87
7	93.8	[71.1 - 98.5]	2.15	80.4	[73.4 - 87.4]	16.31	1.38	75.4	[67.6 - 83.2]	19.37
8	81.3	[56.2 - 93.0]	2.21	83	[77.3 - 88.7]	12.80	1.53	83.6	[75.0 - 92.1]	19.20
9	87.5	[64.2 - 96.3]	2.38	87.3	[82.5 - 92.2]	10.41	1.41	73.5	[61.5 - 85.4]	30.47
10	75.0	[50.2 - 89.4]	2.49	88.9	[82.4 - 95.4]	13.74	1.49	82.1	[73.9 - 90.4]	18.85
Mean	**88.5**	**[80.7 - 93.5]**	**2.43**	**87.4**	**[84.7 – 90.1]**	**4.32**	**1.63**	**85.8**	**[80.5 - 91.0]**	**8.58**

[a] 16 animals per trial; [b] from Goris et al., 2008. [c] mean of 16 EPPs from individual animals; ND: not determined.

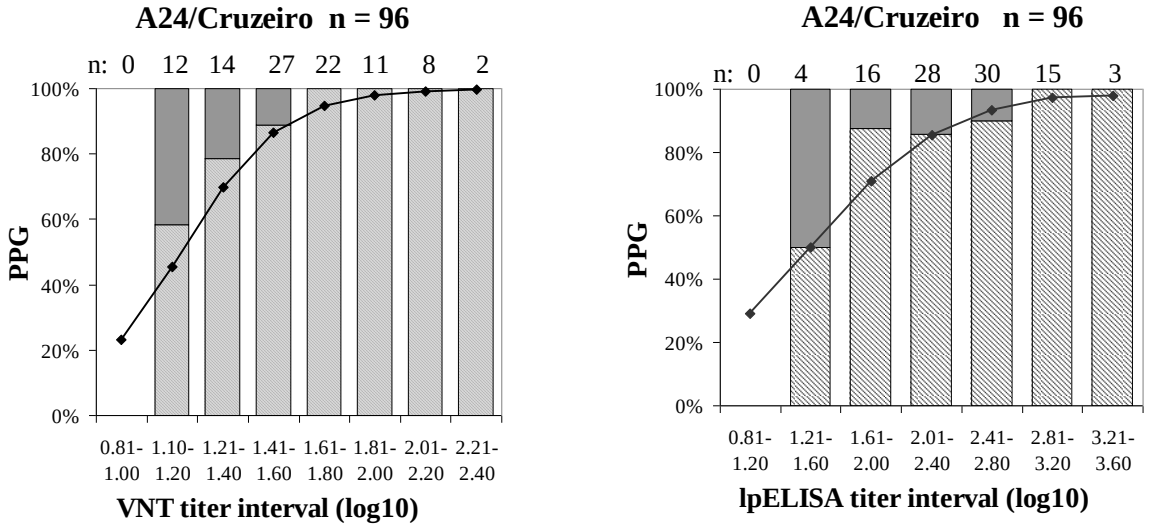


Figure 1. Potency trials. Distribution of VNT and lpELISA titers for A24 Cruzeiro FMDV strain in six challenge trials. Histograms showing the data of PPG for each VNT or lpELISA titer interval for FMDV strain A24 Cruzeiro. The VNT PANAFTOSA's logit transformation curve for A24 Cruzeiro is shown superimposed in the left panel. The SENASA-

CEVAN´s logit transformation curve for A24 Cruzeiro is shown superimposed in the right panel. The number of animal sera included in each titer interval is shown at the top of each bar. (n): number of animal sera tested.

3.2. Intra-trial repeatability and inter-trial reproducibility of vaccine potency assessment by EPP

The vaccine used was the same in the 10 trials, and although it showed a slight decrease in potency over time [3], it always protected over 75% of the animals by PPG. Some variation was found in the individual animal titers determined by both serological methods and consequently the EPPs showed a considerable dispersion (not shown). The CVs% for intra-indirect potency repeatability (variations of the sixteen EPPs calculated from the titer of individual animals within each trial) were ≤20% in the 10 trials for lpELISA and in 9 out of 10 trials of VNT, except trial 9 (CV%=30.47) in which the vaccine was rejected by VNT EPP (Table 1). The CV% for inter-indirect potency test reproducibility (variations of the ten EPP replicates) was 4.32 % for lpELISA EPP and 8.58 % for VNT EPPs

3.3. Cross protection trials

As it was reported previously, animals vaccinated with the A24 Cruzeiro vaccine and challenged with A/Arg/01 strain in four cross protection trials (trials 3-6), were not protected by PPG [3]. In the current study, cross protection was evaluated indirectly in the ten trials by EPP using lpELISA or VNT titers and relating them to the SENASA-CEVAN's or the PANAFTOSA logit regression curve for A24 Cruzeiro, respectively. In this case, A/Arg/01 was considered as a hypothetical emerging strain. The rationale of this procedure was based on the suggestion of the OIE Manual to determine vaccine matching through measuring the reactivity of post-vaccination sera raised against relevant vaccine strains (in this case A24 Cruzeiro), with the emerging strain (in this case A/Arg/01). The probability of protection was then assessed using the correlation curves that associate antibody titers (VNT or lpELISA) with protection from viral challenge (logit regression curves for A24 Cruzeiro).

In addition, we used those antibody titers to determine the EPPs using the homologous lpELISA logit regression curve for A/Arg/01, as it was available from previous work [6]. It has to be pointed out that this is not the case for most emerging FMDV strains, where the homologous logit regression curve would not be available. Moreover, this was not done for VNT because such curve for A/Arg/01 is unavailable.

The animals were not cross protected by the A24 Cruzeiro vaccine in any trial, when measured through VNT EPP and the PANAFTOSA A24 Cruzeiro curve (Table 2). The mean EPP of ten trials obtained by VNT (31.6%) was close to the mean PPG value of the four challenge trials (26.6%).

In the case of lpELISA EPP, the results varied according to the regression curve used (Table 2). When the reference vaccine strain (A24 Cruzeiro) curve was used, the mean EPP (74.3%) was very close to the approval value. However, using the homologous curve for the challenge strain (A/Arg/01), the mean EPP (55.7%) was more consistent with the PPG outcome.

The upper limit of the 95% CI for lpELISA EPPs was above the approval value in 8 of the trials. In contrast the upper limit of the 95% CI for VNT EPPs was always below 75%, while for PPG exceeded 75% in one out of four A/Arg/01 trials (trial 3, Table 2).

At the individual animal level, the 47 animals unprotected after heterologous challenge with A/Arg/01 had VNT titers below 1.04, and 5 animals with titers below the detection limit (VNT <0.85) were protected. There were no unprotected animals with high VNT titers, although this number was quite small (not shown).

The distribution of A/Arg/01 VNT titers is shown in the histograms of Fig. 2 (left panel), where the number of animals included in each titer interval is displayed at the top of the bars. Most VNT titers (84%) were found at the test detection limit (≤ 0.85), which corresponded to an EPP = 19.8%. The histogram of lpELISA titers grouped in fixed intervals showed a different distribution, with protection values distant from both type A logit regression curves (Fig. 2, right panel; full line A24 Cruzeiro; dotted line, A/Arg/01).

Table 2. Comparison of cross-protection assessment between FMDV strains A24/Cruzeiro and A/Arg/01 by PPG or EPP.

	A/Arg/01										
	PPG		lpELISA					VNT			
Trial [a]	(%)	95%CI[b]	Mean titer	EPP[c] (%)	95%CI	EPP[c] (%)	95%CI	Mean titer	EPP[c] (%)	95%CI	
1	ND	--	1.73	41.7	[31.7 – 51.7]	66.2	[59.1 – 73.3]	0.98	32.5	[21.3 - 43.6]	
2	ND	--	2.01	59.6	[47.9 - 71.4]	76.8	[70.0 – 83.7]	0.98	38.6	[27.6 - 49.5]	
3	56.3	[33.2 - 76.6]	2.01	57.9	[46.7 – 69.1]	76.4	[70.3 – 82.6]	1.17	50.6	[34.9 - 66.2]	
4	25.0	[10.5 - 50.0]	1.74	41.9	[30.8 – 53.0]	66.0	[58.3 – 73.8]	0.97	29.1	[18.5 - 39.7]	
5	12.5	[3.8 - 36.4]	2.15	61.9	[48.0 – 75.8]	78.3	[70.5 – 86.0]	0.85	19.8	[19.8 - 19.8]	
6	12.5	[3.9 - 36.6]	2.09	63.3	[50.7 – 75.9]	78.9	[72.1 – 85.7]	0.85	19.8	[19.8 - 19.8]	
7	ND	--	1.83	47.3	[33.6 - 60.9]	69.1	[60.5 – 77.6]	0.89	23.6	[15.5 - 31.8]	
8	ND	--	1.83	47.7	[35.6 – 59.7]	69.8	[62.4 – 77.2]	1.18	56.7	[48.6 - 64.8]	
9	ND	--	2.09	66.2	[54.5 - 77.9]	79.9	[72.5 –87.2]	0.91	25	[21.7 - 28.4]	
10	ND	--	2.22	69.5	[55.8 – 83.2]	81.7	[73.6 – 89.7]	0.85	19.8	[19.8 - 19.8]	
Mean	**26.6**	**[17.4 - 38.5]**	**1.97**	**55.7**	**[48.4 - 63.0]**	**74.3**	**[70.1 - 78.5]**	**0.96**	**31.6**	**[22.1 - 41.0]**	
				SENASA's curve for A/Arg/01		SENASA's curve for A24			PANAFTOSA's curve for A24		

[a] 16 animals per trial; [b] from Goris et al., 2008; [c] mean of 16 EPPs from individual animals; ND: not determined

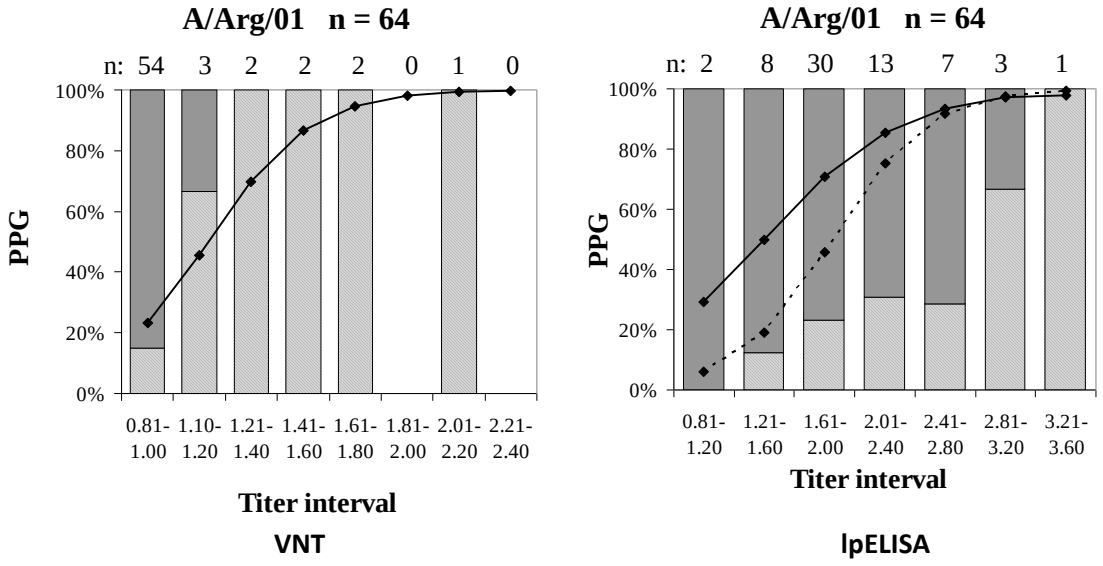


Figure 2: Cross protection trials. Distribution of VNT (left panel) and lpELISA (right panel) titers for A/Arg/01 FMDV strain in four challenge trials. Histograms showing the data of PPG for each VNT or lpELISA titer interval for FMDV strain A/Arg/01. In the right panel, the lpELISA SENASA-CEVAN´s logit transformation curves for A24

Cruzeiro (full line) and A/Arg/01 (dotted line) are shown superimposed. The number of animal sera in each titer interval is shown at the top of each bar. (n), number of animal sera tested.

3.4. Concordance of EPP and PPG

EPPs determined by serology were highly concordant with challenge results, except for one trial, where the A24 Cruzeiro vaccine was rejected by VNT EPP, but approved by PPG and lpELISA. The EPPs used in these tables were obtained from the correlation curve corresponding to the challenge strain.

The lpELISA EPPs were highly reliable for determination of vaccine potency, while they were not so useful in cross protection trials, when the heterologous strain A/Arg/01 was treated as an emerging strain. Considering the difference found in the results using different curves for determination of vaccine matching by lpELISA titers, the bovines were further categorized as "protected" or "unprotected" according to the individual serum titer needed to reach 75% protection in each correlation curve (Table 3). The number and the percent protected animals were consistent with the previous observation that PPG and VNT seemed more reliable than lpELISA for vaccine matching assessment, provided an lpELISA correlation curve for the emerging strain is unavailable. Interestingly, when using the homologous A/Arg/01 curve, the lpELISA results matched exactly the PPG results (26.6%). On the other hand, lpELISA results for vaccine potency purposes showed a better match with PPG than VNT.

Table 3. Number of animals with an EPP≥ 75% compared to the individuals protected in PPG, in six replicates of protection or four replicates of cross-protection trials.

Challenge strain	Animal status	PPG	EPP		
			lpELISA A24	lpELISA A/Arg/01	VNT
	Potency trials				
	Protected	85	84	--	74
A24/Cruz	Unprotected	11	12	--	22
	% Protected	**88.5**	**87.5**		**77.1**
	Cross-protection trials				
	Protected	17	33	17	6
A/Arg/01	Unprotected	47	31	47	58
	% Protected	**26.6**	**51.6**	**26.6**	**9.4**

*Challenge strain. [a] Nº of animals protected from challenge, [b]: SENASA-CEVAN's curve for A24 Cruzeiro; [c]: SENASA-CEVAN's curve for A/Arg/01; [d]: PANAFTOSA's curve for A24 Cruzeiro. Protected: EPP ≥ 75%; unprotected: EPP < 75%.

4. DISCUSSION

In this study the repeatability and reproducibility of the EPP was determined in ten replicates of PPG trials with the same A24 Cruzeiro vaccine. Although potency trials are intrinsically very variable and the influence of many factors must be considered, the ten in vitro trials behaved similarly and the inter-trial variation was within acceptable limits of reproducibility, especially for homologous EPPs determined by lpELISA titers (CV 4.32 %, Table 1). This variation was lower than the variation obtained in PPG (CV 10.4%) for the same A24 Cruzeiro vaccine.

On average, the mean EPP values obtained from VNT and lpELISA titers for the A24 Cruzeiro vaccine were highly comparable between each other and with PPG. One of the conclusions arising from this study is that the EPP determined by lpELISA titers is an indirect method statistically reliable to be used as a surrogate of protection for vaccine potency purposes, with an excellent concordance with the PPG outcome. Based on EPP results, the VNT falsely rejected the vaccine batch in one out of ten occasions (trial 9, Table 1). As the CV% variation obtained

in this particular VNT EPP determination was high (CV 30.47%), it should be necessary to introduce in the future more appropriate validation criteria for the VNT, similarly to what has been done for the lpELISA test in use [6]. Based on the limited data available, the results indicated that the VNT correlation curve obtained in this study for A24 Cruzeiro strain had a good fitting with the one developed by the Cuenca del Plata project [11] (Fig. 1).

When a new FMDV strain emerges in the field, there is no statistical correlation curve readily available for that isolate. It has been suggested that in this case, besides the challenge method, a post vaccinal serum panel derived from existent vaccines strains may be used for indirect assessment of the degree of matching by *r* values or by EPP. In view of the results obtained in this study, the use of an indirect assay more related with protection, such as VNT, may reflect better the outcome of PPG trials for vaccine matching. It has been reported previously that the same is true for *r* values determination [8]. Although non-neutralizing antibodies may also be protective, in vitro neutralization may be more relevant to in vivo protection than other measures of virus-antibody interaction [12].

In cross protection trials, it is worth mentioning, that the results obtained using the lpELISA logit regression homologous curve for A/Arg/01 were consistent with those of PPG in heterologous challenge tests. However, in most outbreaks, a curve for the new emerging strain would not be available. In this regard, the results obtained with the lpELISA curve of A24 Cruzeiro are misleading; although consistent with the fact that protection against A/Arg/01 requires higher lpELISA titers than for A24 Cruzeiro [6]. The lpELISA EPPs (homologous A/Arg/01 curve) indicated in all cases a lack of cross protection, although the data appeared to overestimate the degree of protection observed in vivo, and there were four trials in which it was not possible to state with 95% confidence that less than 75% of the animals would be protected. On the other hand, the analysis of VNT EPPs for A/Arg/01 was limited by the fact that a validated correlation homologous curve of VNT titers with PPG is unavailable for this strain.

The possibility of including and monitoring standard reagents and internal control sera increases the level of confidence in the results from serological tests and in the overall potency test system, a procedure which is not feasible for the in vivo challenge tests. It is known that indirect methods need to be standardized in each country or laboratory for the particular conditions (reagents, vaccines, serological techniques), and that serum titers obtained by different groups with different test systems cannot be directly compared [7, 13]. Nevertheless, they are less variable, more easily standardized and in addition save the lives of many animals, contributing to the 3R rule.

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IN VITRO ALTERNATIVES FOR FOOT-AND-MOUTH DISEASE VIRUS CHALLENGE IN THE PD_{50} VACCINE POTENCY TEST ARE SEROTYPE-DEPENDENT

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1. INTRODUCTION

The *in vivo* 50% Protective Dose (PD_{50}) test is the standard European procedure for foot-and-mouth disease (FMD) vaccine potency testing. Due to ethical reasons, current research alliances focus on the replacement of the *in vivo* viral challenge by *in vitro* alternatives. Previously, Goris et al. (2008) have validated an *in vitro* model for the FMD virus (FMDV) reference strain O_1 Manisa. The present study aims at developing a comparable model for serotype A and to test both models for serotype-independence.

2. MATERIALS AND METHODS

A group of 85 cattle were used for five replicates of *in vivo* PD_{50} vaccine potency tests. This group was tested using a single FMDV A Iran 1996 (A96) vaccine batch in a homologous A96 challenge test. Serum samples were collected from all animals at 21 days post vaccination and their A96 antibody (Ab) titres were determined in a Virus Neutralisation Test (VNT) and a Liquid Phase Blocking ELISA (LPBE) by two different laboratories, VAR and ARRIAH. The serological data were analysed with logistic regression and models for an alternative *in vitro* vaccine potency test were constructed as described previously (Goris et al., 2008).

3. RESULTS AND DISCUSSION

The challenge of the A96 vaccine resulted in an overall PD_{50} of 20.5 with 95% confidence intervals (CI) between 13.1 and 23.5 PD_{50}. For the *in vitro* alternatives, the VNT Ab titres and the LPBE Ab titres from the two laboratories were used to build 4 different A96 models. Each model has its own cut-off titre with a corresponding sensitivity and specificity for this cut-off point. The Ab titres of the individual sera were then evaluated with the specific model cut-off and a serum with an Ab titre greater than or equal to this cut-off was classified as originating from an animal that is protected against the virus challenge. Likewise sera with a lower Ab titre were classified as originating from unprotected animals. With these results, the PD_{50} of the vaccine was determined.
Two models were made for the VNT A96 assay: one for ARRIAH and one for the VAR laboratory. The resulting overall PD_{50} ranged from 11.1 for ARRIAH and 10.2 for the VAR as can be seen in Table 1. Both *in vitro* models thus seem to predict a lower PD_{50} than the *in vivo* test. A possible reason lies in the number of protected animals that did not have a detectable Ab titre in the VNT. However, for both models the 95%CI overlapped with the 95%CI interval of the *in vivo* test.
Likewise two models were made for the LPBE A96 assay of ARRIAH and the VAR laboratory. Here the resulting overall PD_{50} ranged from 6.0 for ARRIAH to 14.7 for the VAR (Table 1). The LPBE A96 model from ARRIAH predicted a significant lower PD_{50} whereas the LPBE A96 model from the VAR estimated a PD_{50} within the range of the confidence interval of the *in vivo* test. A possible reason for this difference can be the larger number of protected animals that did not have a detectable Ab titre in the LPBE of ARRIAH where they did have detectable Ab in the LPBE of the VAR.
When the serological data of the present study were entered into the previously described O_1 Manisa (O_1) potency model of Goris et al. (2008), the overall PD_{50} varied between 5.8 and 8.7 PD_{50} for the VNT and between 2.1 and 13.1 PD_{50} for the LPBE, demonstrating a difference between the PD_{50} predicted by the different A96 models and the different O1 Manisa models. Only the LPBE O_1 model and the LPBE A96 model from the VAR predicted a similar PD_{50} close to the *in vivo* test result.

Table 1.
Overall *in vi*vo and alternative PD_{50} results of an FMDV A96 vaccine with 95% confidence intervals.

Lab	Test	Model	Overall PD_{50}	95% CI
-	-	In Vivo	20.5	14.9 - 27.4
ARRIAH	VNT A96	VNT A96	11.1	9.2 - 19.1
VAR	VNT A96	VNT A96	10.2	8.4 - 19.1
ARRIAH	VNT A96	VNT O_1	5.8	5.0 - 14.0
VAR	VNT A96	VNT O_1	8.7	6.9 - 14.6
ARRIAH	LPBE A96	LPBE A96	6.0	5.0 - 11.2
VAR	LPBE A96	LPBE A96	14.7	12.5 - 21.1
ARRIAH	LPBE A96	LPBE O_1	2.1	1.7 - 3.1
VAR	LPBE A96	LPBE O_1	13.1	11.2 - 20.5

In conclusion, for each serological assay the corresponding alternative *in vitro* potency test generates a PD_{50} estimate that can be interpreted in a similar way as the *in vivo* PD_{50} result. The differences between the *in vivo* and *in vitro* PD_{50} are most likely caused by clinically protected animals that did not have detectable Ab titres, an observation previously made by several authors (McCullough et al., 1992; Doel et al., 1999; Brehm et al., 2008). The LPBE models of the VAR were less affected by this and showed a smaller difference between the *in vivo* and *in vitro* PD_{50} results.
When the A96 Ab titres where evaluated with the FMDV O_1 models, the differences between the *in vivo* and *in vitro* PD_{50} values were even greater, suggesting a serotype dependency for 3 of the 4 A96 models. Similar findings stating that each lab should have its own serological database for estimating protection and slope values of serological assays were published previously by several authors (McCullough et al., 1992; Pay and Hingley, 1992; Smitsaart et al., 1998).
This strengthens the conclusion that for each serological assay the corresponding alternative *in vitro* potency test has to be validated for every individual laboratory and its serotype independency has to be investigated.

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IDENTIFYING EPITOPES AND QUANTIFYING THEIR ROLE IN LOSS OF CROSS-NEUTRALIZATION

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ABSTRACT

Introduction

The prediction of viral cross-protection remains an important unsolved problem. Improved methods of prediction are critical for predicting the severity of FMD outbreaks within endemic settings where multiple serotypes and subtypes commonly co-circulate, as well as for deciding whether appropriate vaccine(s) exist and how much they could mitigate the effects of any outbreak.

Materials and Methods

To find the substitutions in surface-exposed structural proteins that are correlates of loss of cross-neutralization, we used linear mixed effects models to account for variation in pairwise cross-neutralization titres. To attempt to identify causation, however, we must also control for the phylogenetic relationships between virus strains. We identified those branches in the phylogenetic tree that are associated with significant drops in cross-neutralization. A model containing these terms controls for the phylogeny, so if changes to surface-exposed structural proteins significantly improve model fit, they must be capturing another feature of the substitutions. Since changes to the epitopes found in these proteins cause the loss of cross-neutralization, and do so independently of phylogeny, this method will identify them.

Results

The method described above is able to identify epitopes using cross-neutralization titres, viral sequences and structural data. It is further able to quantify the specific effects of some individual residues in determining cross-neutralization.

Discussion

Identifying and quantifying the importance of sites that predict viral strain cross-neutralization not just for single viruses but across entire serotypes can help in the design of vaccines with better targeting and broader coverage. These techniques can be generalized to any infectious agents where cross-neutralization assays have been carried out. As the parameterization uses pre-existing datasets, this approach quickly and cheaply increases both our understanding of antigenic relationships and our power to control disease.

1. INTRODUCTION

The emergence of antigenically novel viruses, against which existing vaccines do not provide adequate protection, may require the selection of new vaccine seed strains. Currently, where no appropriate vaccine exists, field isolates are, when possible, adapted for vaccine production, amplified and then processed into vaccines (*e.g.* [8]). Only at this stage, often after several months, can the new vaccines be inoculated into animals and tested for efficacy *in vivo* and subsequently *in vitro*. Due to the time and expense required, there is a limit to the number of isolates that can be submitted to undergo this procedure, and a sub-optimal choice of vaccine strain may therefore be made. An *in silico* predictor that identifies those strains likely to provide the broadest cross-protection could therefore substantially enhance capacity to develop appropriate vaccines rapidly and effectively, whilst minimising the cost and the need for animal experimentation. The aim of the current study is to develop an *in silico* tool to predict vaccine efficacy using sequence data, neutralising titres and structural information.

2. MATERIALS AND METHODS

In previous work, we described the data collected and the analysis to accurately estimate r_1-values from serological data[7]. Here we look instead at the predictive modelling. There were three stages:

1. First, a linear mixed-effects model[14] was built with log titre of the VNTs as the response variable (which are normally distributed – Lilliefors normality test, $p>0.5$) – using raw titres gave us neither normally-distributed ($p<10^{-15}$) nor homoscedastic residuals[12] – using the R statistical software[10] and the modelling package lme4[1]. Sequence-based predictors (a selection of counts in candidate areas and the count of total amino acid substitutions were used to test the model) were used as the explanatory variables, and the model selection approach outlined below used to generate a model that predicted cross-reactivity directly from sequence data (Equation 1).
2. The fixed effects in this model were then replaced with phylogeny-based effects (see below) to control for the phylogenetic structure of the data (Equation 2).
3. Individual areas and residues were added to this model to identify epitopes (Equation 3).

2.1 Model selection

The predictive models (Equation 1) were generated by sequentially adding the count of non-synonymous changes in each of the candidate antigenic areas as a fixed effect in a standard stepwise regression which continued for a variable number of steps until no further terms could be added. Specifically, the probability that each model was a significantly better predictor than its precursor was assessed by a likelihood ratio test since the models were nested. For multiple tests each with p-values p_i, the statistic $-2 \Sigma \log p_i$ is expected to be χ^2 distributed with twice as many degrees of freedom as tests under the null hypotheses[4]. When this was not the case ($p<0.05$), then the best predictors that were individually significant (after a Holm-Bonferroni correction for the number of terms[5]) were used as bases for the next step of the regression. The stepwise regression technique was repeated until no more terms could be added to form a small set of candidate models. The best of the final models were then cross-validated.

2.2 Controlling for phylogeny

Amino acid substitutions on the capsid (including those identified above) are correlated with antigenic distance; this could be a direct relationship or may arise indirectly via relationships between substitutions, phylogenetic history and antigenic drift, as is found in influenza A[6]. Neglecting to control for evolutionary history has caused false positive rates of between 20 and 40% in similar analyses[9]; these arise because substitutions that constitute the shared history of virus pairs have only occurred once and therefore constitute only a single independent piece of evidence that these substitutions are important. To account for these repeated measures it is necessary for us to implement phylogenetic control.

However, existing mechanisms for controlling for phylogeny focus on properties (or traits) of the leaves of tree and not the relationships between them[3]. Indeed it is these relationships (the *contrasts*) that are used to control for the evolutionary history, whereas for us these are the signal – the cross-reactivity. We wish instead to identify the causes of the changes in cross-reactivity while controlling for the common evolutionary history.

In practice, each branch on the phylogenetic tree (see above) represents a set of common substitutions by which any pair of viruses either side of the branch differ (unless multiple and/or convergent substitutions have occurred). Any comparison of antigenicity between two viruses either side of the branch will be affected by those changes. A fixed effect is therefore added to the model for each branch (δ_i, Equation 2); this is non-zero if the branch is travelled (and thus these changes have occurred) in the traversal of the tree between the protective strain from which the serum is derived and the virus isolate in a cross-reactivity test. Including these terms in the analysis controls for repeated measures of this traversal.

Because of the necessarily limited number of protective strains we do not explore every path through the tree, and so there is some ambiguity in the allocation of weights to branches (essentially we have more unknowns than equations). These ambiguities mean that the models cannot be used predictively, but this does not prevent their use for phylogenetic control.

Model development begins by constructing a model with all possible fixed effects, which are removed stepwise until all of those left significantly improve the model fit ($p<0.05$). In this manner we have controlled for repeated measures of every significant piece of shared phylogenetic history. Because the phylogenetic trees are different for the two serotypes the serotypes are modelled separately.

2.3 Identifying epitopes

The phylogenetic control terms account for repeated measurement of all significant shared phylogenetic history. However, in doing so they remove all significant direct effects of substitutions at individual branches of the tree. Consequently, the substitution count in any area can only significantly improve the model if it corresponds to multiple and/or convergent substitutions at the same sites in different branches. Modelling phylogenetic control in this way therefore provides a conservative estimate of the number of areas that directly affect antigenicity.

Sequence-based predictors were added to the models – again, substitution counts for each of the candidate areas identified in the structural analysis – to determine which sub-sequences were the best predictors after controlling for phylogeny (d_1, Equation 3), and these were then compared to bootstrapped samples from the remaining capsid surface (randomly assembled sub-sequences of the same length as each candidate). Since changes to the capsid proteins must be responsible for loss of cross-reactivity, comparing specified sub-sequences to the rest of the capsid after controlling for phylogenetic structure directly determines whether these areas contain true predictors or whether they contain correlates, with the true epitopes being found elsewhere. To identify the individual constituent residues of epitopes, exactly the same mechanism is used on individual residues instead of areas.

3. RESULTS

3.1 Relating antigenic differences to sequence variation

Structural data were used to identify candidate areas of the capsid that might be antigenically significant (29 and 28 areas for SAT1 and SAT2 respectively – see [11] for details). These provide the starting point for a linear mixed-effects approach used to predict r_1-values from the sequence data and, ultimately, to identify antigenically significant areas of the capsid. The final model took the form:

$$\log(t_{p,c}) = k_0 + \sum_{i=1}^{N} k_i \times d_i(p,c) + \varepsilon_S + \varepsilon_E + \varepsilon_C + \varepsilon_R \qquad (1)$$

$$\text{where } \varepsilon_i \sim N(0, \sigma_i^2)$$

where $t_{p,c}$ is the titre for a neutralisation test for protective strain p and challenge virus c, ε_S and σ_S^2 are the best linear unbiased predictor and associated variance for the random effect of serum, ε_E and σ_E^2 are the equivalent measures for experiment, ε_C and σ_C^2 for challenge virus, ε_R and σ_R^2 are the model residuals and associated variance, k_0 is the average titre and d_i is a raw count of the number of amino acid changes between the protective strain and challenge virus in a single candidate area identified from the structural modelling (the i^{th} out of a total of N areas identified as potentially antigenically significant), with k_i the regression coefficients.

3.2 Controlling for phylogenetic structure

To identify those areas that are directly responsible for antigenic variability it is necessary to develop models that additionally control for the phylogenetic relationships between virus strains. The phylogenetic control is analogous to the predictive model (Equation 1):

$$\log(t_{p,c}) = k_0 + \sum_{i=1}^{N} m_i \times \delta_i(p,c) + \varepsilon_S + \varepsilon_E + \varepsilon_R \qquad (2)$$

where δ_i is a delta function which is 1 if p and c are separated by branch *i* of the phylogenetic tree and 0 otherwise. Loss of cross-reactivity is caused by amino acid substitutions in the capsid proteins, and any individual substitution must occur in a specific branch of the phylogenetic tree (though we may not be able to determine

which). Each branch partitions the tree into two groups, and where a branch effect represents changes that impact significantly on cross-reactivities, they will be higher between viruses within the groups than those between groups (after controlling for other effects). For instance, where a terminal branch is identified, the fixed effect of that branch specifies an amount by which the virus to which it leads (the first group) is antigenically distant from all other viruses (the second group). A significant internal branch, similarly, identifies a clade that is antigenically distant from the rest of the tree. By building a model containing all of the branches in the tree, and then using a stepwise elimination procedure to remove branches which do not significantly improve the model fit ($p>0.05$), we are left with the set of branches that, when traversed, significantly account for reductions in antigenic cross-reactivity.

Twelve phylogenetic branches are significant in SAT1 and twenty-one in SAT2 (black lines, Figure 1). For SAT1 these are six branches that each partition individual topotypes from the rest of the tree, and five terminal branches that lead to viruses for which large numbers of titres have been obtained (including the three protective strains) as well as one that is antigenically very distant from the protective strains (ZAM/2/93, which has no r_1-value above 0.2). For SAT2, there are six internal branches throughout the tree and fifteen terminal branches leading to ten of the thirteen viruses that are antigenically distinct (again, all r_1-values are below 0.2) and five other viruses (including the four protective strains).

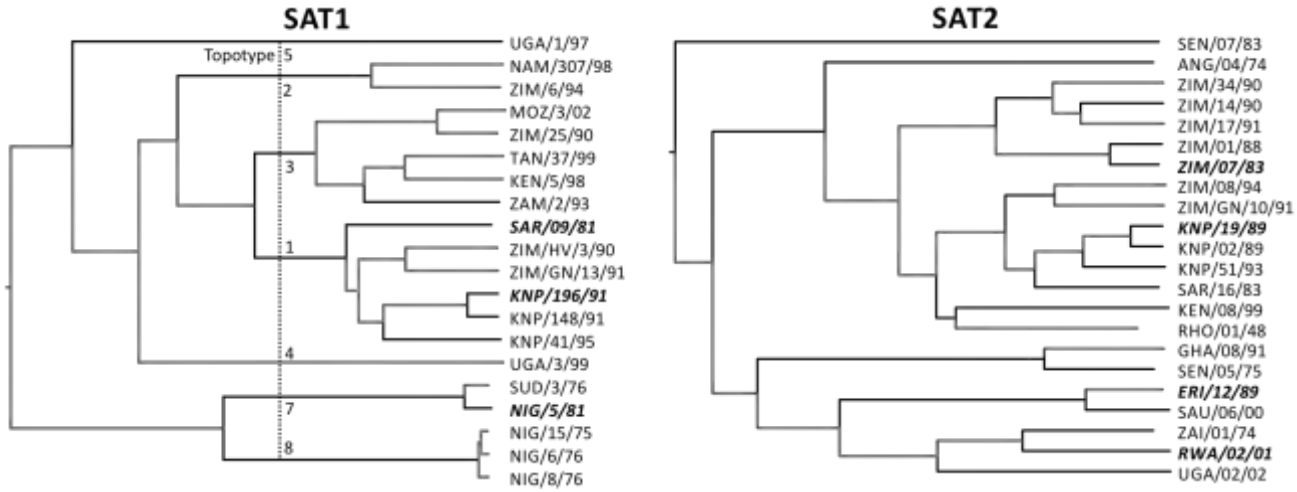


Figure 1. Phylogenetic trees indicating the branches controlled for in the analysis. SAT1 and SAT2 phylogenetic trees, showing protective strains (bold) and branches associated with significant drops in antigenic cross-reactivity (black lines, $p<0.05$). Topotypes are shown for SAT1.

3.3 Identifying epitopes

Any model containing these terms controls as completely for the phylogeny as is possible with the data available, and should a model have a significantly better fit than the phylogenetic model on its own, it must achieve this by some mechanism other than phylogenetic correlation. A simple combination of Equations 1 and 2 provides a potential model, with an additional term for the raw count of the number of amino acid changes between the protective strain and challenge virus in a single candidate area:

$$\log(t_{p,c}) = k_0 + k_1 \times d_1(p,c) + \sum_{i=1}^{N} m_i \times \delta_i(p,c) + \varepsilon_S + \varepsilon_E + \varepsilon_R \qquad (3)$$

where d_1 is the count of substitutions at a specific site, and k_1 the associated regression coefficient. The phylogenetic control terms account for repeated measurement of all significant shared phylogenetic history. However, in doing so, they remove all significant direct effects of substitutions at individual branches of the tree, but are not designed to capture the interactions involved in multiple and/or convergent substitutions at the same sites in different branches. Consequently, the substitution count in any area significantly improves the model if it corresponds to this substitution structure. Parallel and/or back-mutations, relatively frequent in such highly variable viruses, are therefore strong signals used by the model to determine antigenically significant areas. The phylogenetic control is therefore conservative in that significant sites with substitutions at only one branch in the tree will not be identified, as the different substitutions in that branch cannot be readily disambiguated.

Nevertheless, after controlling for phylogeny, the twenty-nine SAT1 areas tested with the model were collectively significant predictors ($p<0.05$[4]), but the twenty-eight areas for SAT2 were not significant (collectively or individually).

Because substitutions are ultimately responsible for the loss of cross-reactivity, the substitutions contributing to counts in these SAT1 areas must be responsible for this loss unless they are co-occurring with causative substitutions. Any such causative substitutions should, however, be identifiable because substitution counts in areas containing them will improve the model fit. Comparing the individually best SAT1 areas from above with bootstrapped random sequences of the same length from other parts of the capsid, however, fails to identify other causative substitutions, and eight areas were instead found to be significant after a Holm-Bonferroni correction for the number of terms[5] ($p<10^{-12}$ collectively). Of these eight terms, seven were individually significant in the previous test ($p<0.05$). These seven terms consisted of five that corresponded exactly to the five areas identified as the constituent parts of the Site 3 conformational epitope for A10[13] ($p<10^{-8}$ collectively), one was the VP1 G-H loop ($p<0.001$), and the last was the VP3 G-H loop, previously identified as Site 3 on A12[2] ($p<0.01$).

To identify the specific residues responsible for these drops in antigenic cross-reactivity, Equation 3 is trivially modified to test substitutions to the 62 individually variable residues in the seven areas identified (instead of the 29 candidate areas). Again, this is a conservative test, as it will only identify residues where multiple/convergent substitutions occur at different branches in the phylogeny. Collectively, changes to the residues are significant after controlling for phylogeny ($p<0.005$), but only two residues are individually significant ($p<0.05$). Bootstrap comparisons with other residues showed these to be the two most significant predictors of loss of cross-reactivity out of all the residues in the capsid, and both are adjacent to residues identified by MAb escape mutant studies on A10 as part of Site 3[13]. These were residue 138 on the VP3 E-F loop and residue 198 on the VP2 H-I loop – see [11] for alignment – and the expected effects of substitutions at those residues are a reduction in cross-reactivity of 25% (95% CI 8% – 40%) and 16% (95% CI 0% – 30%) respectively.

4. DISCUSSION

The identification of antigenic sites on individual FMDV isolates is time consuming, with the consequence that data are not available for all serotypes, much less for all isolates. Indeed, very little is known about the important epitopes for SAT1 and SAT2 viruses, impacting on the potential to both design vaccines with broader or better targeted antigenic cover and predict the efficacy of a particular vaccine strain against circulating viruses in the field. We have identified seven areas containing what we believe to be three epitopes for SAT1, and we provide evidence that these are conserved across our whole sample. We have further quantified the effect of substitutions at two specific residues in one of these epitopes. The conservative phylogenetic control employed throughout the analysis means that this may not be an exhaustive list of antigenically significant areas of the capsid, and is almost certainly not for residues, as it will only identify ones where multiple/convergent substitutions occur at different branches in the phylogeny. The areas that are identified do, however, correspond to epitopes identified by MAb escape mutants for other serotypes, and both of the specific residues found are (after alignment) adjacent to ones which are part of Site 3 on A10[13]. Confirmatory evidence that the phylogenetic control is acting as expected is provided by the fact that for SAT1 all of the internal branches that are identified as antigenically significant correspond to previously identified antigenically important events, that is to say branches that partition individual topotypes from the rest of the tree.

5. CONCLUSIONS

The technique developed here can be used directly for any FMDV serotype and potentially for any similar virus where cross-reactivity, sequencing and structural studies have been carried out, both to identify epitopes, and to predict vaccine match for new isolates and estimate efficacy of new candidate seed strains. This can be done by exploiting historical datasets, and is therefore a quick, low cost and valuable method for better understanding antigenic relationships. In summary, the use of sequence data to predict antigenic relationships is a powerful tool that has the potential to be applied to a variety of different infectious agents.

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PREDICTING SITES OF ANTIGENIC IMPORTANCE OF SEROTYPE O FMD VIRUSES USING SEROGICAL AND CAPSID SEQUENCE DATA

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INTRODUCTION

Current methods for vaccine matching of FMDV field strains include using both VNT and ELISA methods to determine antigenic relationships using r_1 values (Paton et al., 2005). These methods are time consuming, not always reproducible and there is a certain level of uncertainty over their predictive value. Previous studies (Reeve et al., 2010) have demonstrated the value of linear mixed effects modelling as a potential in silico method of determining antigenic relationships between South African Territory viruses (SAT 1 and 2) using sequence data alone.

This linear mixed effects model uses neutralisation data from a number of heterologous viruses against several homologous sera, along with capsid sequence data to determine which surface exposed regions of the virus capsid are the best correlates of loss of cross reactivity within the serological data. The aim of this study is determine if a similar model can be developed for serotype O using sequence and serology data. It is also anticipated that the model will highlight further potentially antigenic regions located across the surface of the serotype O in addition to the five known antigenic sites already described (Crowther et al., 1993; Kitson et al., 1990; Mahapatra et al., 2008). These regions can then be taken forward for analysis using our in house reverse genetics system.

MATERIALS AND METHODS

Selection of test isolates

The 70 serotype O viruses included in this analysis were obtained from the World Reference Laboratory (WRL) for FMD at the Institute for Animal Health, Pirbright (United Kingdom). These viruses were selected to give the broadest geographical distribution and therefore to represent a wide range of antigenic relationships.

Serum selection and generation of serological data

A total of three different bovine vaccinate sera (either day 21 or day 28 post vaccination) were selected for use in this study; these were O Kaufbeuren, O BFS 1860 and O UKG 2001. Serum neutralistion values for each of these viruses were generated using previously described methods (Rweyemamu, 1984; Rweyemamu et al., 1978; Rweyemamu and Hingley, 1984); all the tests were carried out at least twice. This generated a final dataset comprising 496 replicates of individual titres and r_1 values against the 3 homologous sera utilised in this study.

Generating 'best estimate' r_1 values

The current procedure for vaccine matching is by determining the relationships of heterologous viruses to vaccine strains by virus neutralisation test (VNT), with the r_1 value of the heterologous virus determined by comparing the titre generated against the titre of the homologous strain on the day of test. There are inherently a large number of variables with the VNT and in order to give confidence in the results the test is repeated at least twice by the same user. The vaccine strain titre generated on the day of test is also anticipated to help account for any variation within a given test. The mean of the two values are used for further analysis

For this analysis, as with the work conducted on the SAT viruses, it was decided that a more statistical approach for generating r_1 values should be developed using a linear mixed effects model. This enables the comparison of data generated on different days by different users, taking into account both the fixed and random effects in order to give a set of "best estimates" for the r_1 values of an heterologous virus to a given vaccine strain. This was performed by estimating the true underlying titres of these viruses using the mean serum titres of both the heterologous and homologous viruses throughout the tests as the fixed effect, with the date of test and the homologous serum included as random effects. Once the true titres had been determined then the best estimate r_1 values could be calculated. These best estimate r_1 values represent a more precise way of estimating the true relationships between viruses and will be taken forward as the gold standard dataset for the subsequent data analysis.

Determining the surface accessibility of type O residues

In order to determine the surface exposure of the residues that make up a serotype O capsid the previously published O BFS structure ((Acharya et al., 1989), PDB ID: 1BBT) was selected and a multimeric structure was prepared to account for interfaces between protomers. A visual inspection was then carried out on the molecular surface using the Pymol molecular graphics system v1.2r0 (DeLano Scientific LLC). Additionally the structure was analysed using the PISA software (Krissinel and Henrick, 2007) in order to determine the surface area of amino acids already involved in interactions and therefore not available for interaction with antibody.

Generation of the sequence data and selection of surface exposed regions

Once the surface exposure of the residues was determined this data was applied to the sequences of the viruses selected for this study. Firstly the P1 sequence, flanked by parts of the L protein and 2B was generated, yielding a sequence around 2.2kb in length. The sequences were aligned using the Bioedit program (Hall, T. A. 1999). The four virion polypeptides (VP1-4) that make up the capsids were further divided into linear sub-sequences, starting from the first exposed residue and stopping at a residue just prior to the next buried residue. In applying this method the sequences were divided in 48 discrete regions, made up of between 1-40 residues. The VP1 GH loop was the longest (antigenic site 1a of serotype O-(Kitson et al., 1990)), with most linear regions being between 4-10 amino acids long. It is these linear regions (and the changes occurring within them) that were used as the candidate regions when building the predictive model, with their antigenic significance and their correlation to r_1 values assessed.

RESULTS

Development of a predictive linear mixed effects model

In order to develop a predictive model for generating r_1 values the equation used to generate best estimate r_1 values was adapted. The fixed effect (mean serum titres of both the heterologous and homologous viruses) was removed and replaced with a predictive term based on non-synonymous changes in capsid-coding sequence between the homologous and heterologous viruses within the candidate regions selected.

Essentially each region was assessed within the model to determine the significance of changes within each region on improving the fit of the predictive model to the true titres of viruses. The region that most improved the predictive model was added first, followed by other regions in a standard stepwise regression that sequentially added all regions deemed to have a significant impact in improving the predictive model.

The probability that including a region yielded a significantly better predictor than if it was not included was assessed by a likelihood ratio test, since the models were nested. For multiple tests, each with p-values p_i the statistic $-2 \Sigma \log p_i$ is expected to be χ^2 distributed with twice as many degrees of freedom as tests under the null hypotheses (Fisher, 1970). When this was not the case ($p<0.05$), then the best predictors that were individually significant (after a Holm-Bonferroni correction for the number of terms (Holm, 1979)) were used as the basis for the next step of the regression. This regression was repeated until no more candidate regions could be added, thus forming a predictive model with only a small number of the candidate regions.

In the present study inclusion of just four candidate sequence regions yielded the best predictive model. The region determined to have the greatest predictive power was the C terminus of VP1 (already described as antigenic site 1b on type O), followed by residues 98-102 on VP1, residues 70-82 on VP2 (part of antigenic site 2) and finally residues 84-85 on VP3. These are numbered as regions 1-4 respectively.

The location of regions 1-4 on the molecular surface of the O BFS capsid and a comparison of these locations with the known antigenic sites is shown in Fig 1. Interestingly two of the regions included in the predictive model are already located on antigenic sites, with the other two regions in close proximity with the antigenic sites, potentially interacting with these sites. The only substitution that occurs within region 4 is on the O Kaufbeuren homologous virus, against which all heterologous viruses yielded low r_1 values. The inclusion of this region within the model appears to indicate that this single amino acid residue could be an important antigenic determinant.

This model was then applied and used to generate a set of predicted r_1 values from just sequence data alone. When these predicted r_1 values and the individual r_1 values generated on the day of test are compared to the best estimate set of r_1 values it can be seen that the predicted r_1 values are more accurate at determining antigenic relationships than the individual r_1 values calculated on the day of test (see Fig 2). The predicted r_1 values fall within the 95% confidence intervals for the best estimate r_1 values 88.5% of the time, compared to only 77.9% of the individual r_1 values from the day of test.

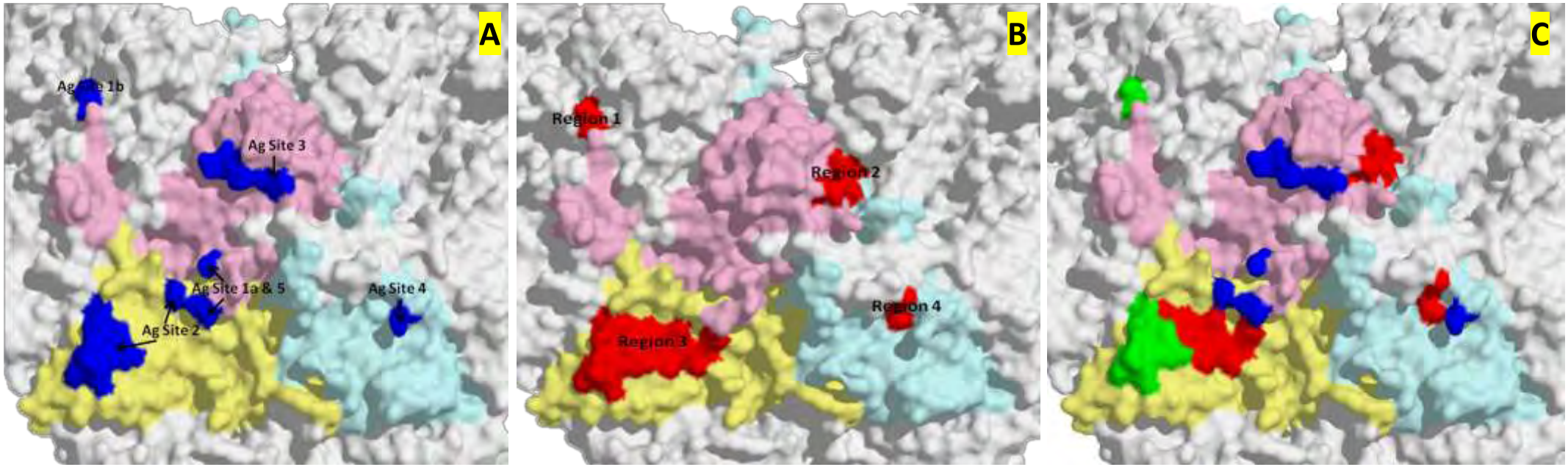


Fig 1: A comparison of the location of the known antigenic sites of serotype O (A) with those regions included within the predictive model (B). Image C is an overlapped image of A and B. As can be seen two of the regions in the predictive model overlapped. The Antigenic sites are coloured blue; the predictive model regions are red with the regions where the two overlap coloured in green. VP1 is coloured light pink, VP2 light yellow and VP3 light blue.

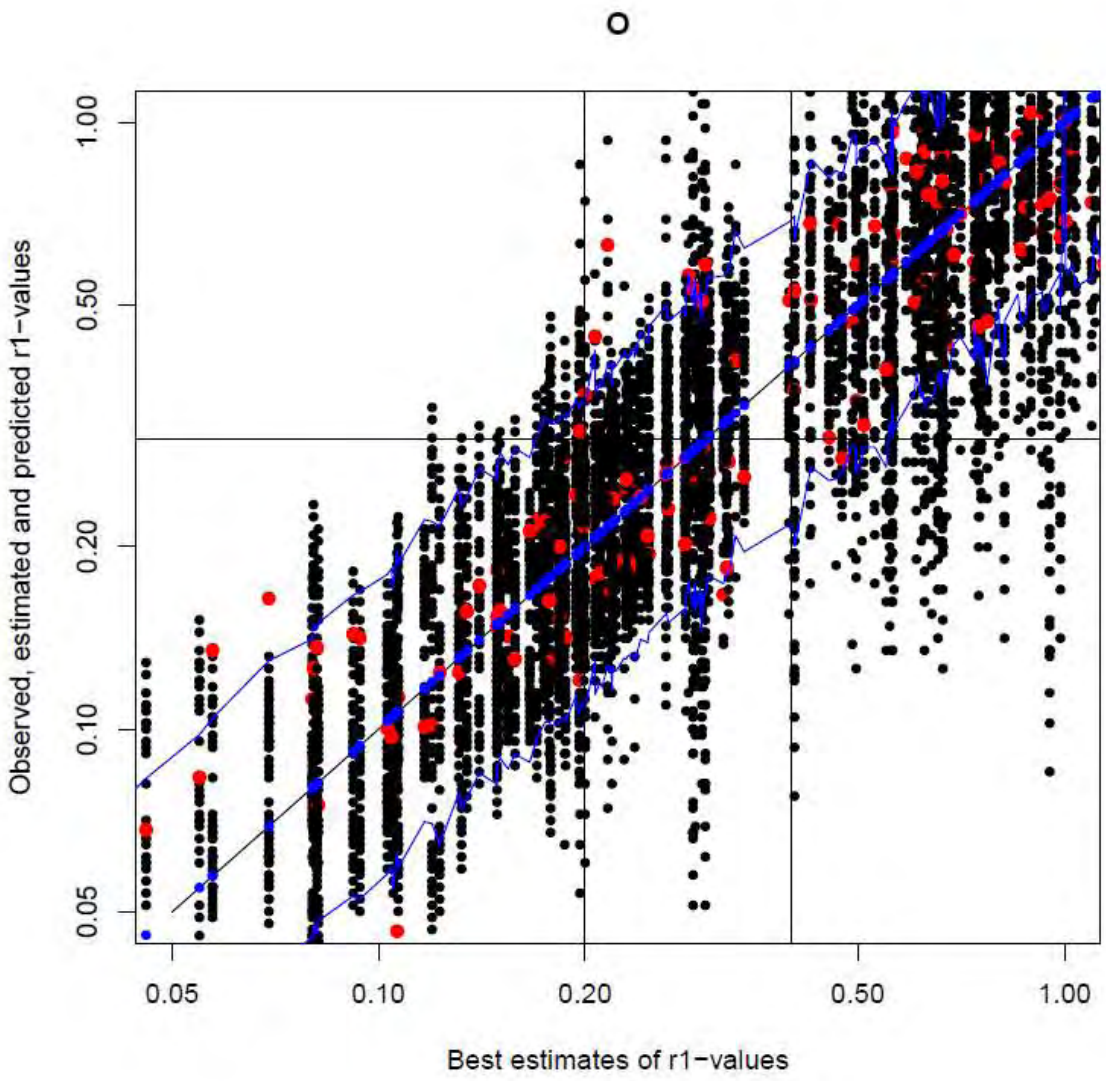


Fig 1: Bootstrap samples of individual serological r1-values (black dots), predictions (red dots) and matching best estimates and their confidence limits (Blue dots and blue line respectively) against best estimates for type O r1-values. Because of the log-normally distributed variance structure of the r1-values, data are plotted on a log scale. N=142.

Discussion

This study was carried out to determine if the linear mixed effects modelling successfully applied for predicting antigenic relationships of SAT FMDV viruses (Reeve et al., 2010) can be adapted to Foot and Mouth disease serotype O FMDV. The initial results from this study suggest that this is indeed the case, with the r_1 values predicted using our model falling within the 95% confidence intervals for the best estimate r_1 values 88.5% of the time, compared to the individual r_1 values generated the day of test which fell within the 95% confidence intervals 77.9% of the time.

The predictive model selected four discrete linear regions on the surface of serotype O FMDV that correlated with the antigenic relationships between the viruses employed in this study. Two of these regions are already identified neutralising antigenic sites, with the remaining two regions being located adjacent to other identified antigenic sites. It would be interesting to investigate if these two regions make up part of a larger conformational epitope with those residues making up the antigenic sites. Region 4 is the most interesting of the areas selected as the substitution occurs only on the O Kaufbeuren virus. The model appears to be predicting that this single residue change may be responsible for the low r_1 values generated using this sera and it will be interesting to test this finding using a reverse genetics system. Another interesting observation is that changes within the GH loop of VP1 (antigenic site 1a) are not predicted to correlate with the changes in antigenicity of these viruses.

As mentioned above the O Kaufbeuren sera generated low r_1 values for almost all heterologous viruses tested. It is thought that this serum may therefore be introducing a bias into the predictive model, for example in selecting region 4 where the only substitution between all viruses occurred only on the homologous virus. In order to take this into account a further homologous serum system needs to be included into the model and it is anticipated that the predictive power of the model could be improved. This will also make it possible to further validate the predictions made by removing an entire set of r_1 values. These removed r_1 values can then be predicted by the model and compared to the actual results. Work is ongoing within our lab to further refine the model.

In addition, the work conducted on the SAT serotype viruses went on to develop a model to predict the percentage drop in r_1 values of two individual residues. It is hoped that by using a similar model predictions can be made about the impact of individual residues on viral antigenicity of serotype O viruses. Once accomplished, these predictions can then also be tested using the reverse genetics system.

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ANTIGENIC VARIATION OF FOOT AND MOUTH DISEASE VIRUS SEROTYPE A

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ABSTRACT

One of the difficulties in controlling and finally eradicating foot and mouth disease (FMD) comes from the large diversity that exists among the seven different Serotypes and the strains that exist within these. Vaccination against one of these Serotypes will not cross-protect against all strains within that Serotype or the other Serotypes. Vaccine selection is done through serological testing and then calculating a relationship coefficient (r_1) between the field virus in question and the vaccine virus considered to be an antigenic match. How the field viruses are antigenically related to each other and have evolved is not easily identified by this method and the serological relationships may alter depending on what serum is used. In this study the antigenic relationships among 48 viruses were quantified using antigenic cartography; a method that uses serological data to resolve paradoxes, increase resolution and visualize the relationship between all of the viruses. We have shown that antigenic cartography can be used to assess the antigenic variation within foot and mouth disease virus (FMDV) and that it may be used to ascertain whether new vaccine strains are needed and which viruses would be potentially good candidates for such work. This method also allows for comparison between antigenic and genetic data.

INTRODUCTION

Currently, inactivated FMDV vaccines will not protect against all strains within a particular Serotype (1). Virus neutralisation tests (VNT) and liquid phase blocking ELISA (LPBE) serological tests are used to suggest which vaccine strain may protect against a field virus by calculating a relationship coefficient (r_1) (2) (3) (4) (5). While these tests provide an estimation of the antigenic relationship between a field virus and a vaccine virus, they do not show the relationship among FMDV strains. Studying and understanding the antigenic relationship among viruses gives insight into how the virus might evolve antigenically. Also, the resulting landscape can be used as a tool to further identify new field strains that are good vaccine candidates and may show gaps where no vaccine virus exists.

Antigenic cartography is a computational technique that enables quantitative and visual interpretation of neutralisation data. It was first applied to human influenza A (H3N2) viruses (12) and has since been applied to other viruses including swine influenza (6), equine influenza (Lewis, N. personal communication), the lyssaviruses (7) and enterovirus (8). This technique should be appropriate to FMDV because the principles are applicable to all binding assay data and VNT is used as a gold standard in FMDV diagnostic laboratories worldwide. Also it is felt that this type of analysis allows for improved resolution of the data as the relationship between viruses is based on all other relationships present.

Currently for routine diagnostic the viral capsid protein, VP1, is sequenced. This is thought to be the most diverse and also the most antigenetic of all capsid proteins. However, for this study the full capsid, including VP1, VP2, VP3 and VP4, was sequenced because it is known that antigenic sites exist on all capsid proteins (9). Analysis will be carried out on the full capsid sequence and compared to the antigenic data obtained, with the thought that the full capsid will give a more complete picture of the antigenic relationship.

MATERIALS AND METHOD

Sera

Seven different bovine sera, harvested 21 days post vaccination were used (supplied by EURL for FMD). The vaccines were inactivated and all are oil adjuvant-based except for A/IRQ/24/64, which is aqueous based. Each serum used is from an individual animal and has been inactivated at 56°C for 30 minutes prior to use. All serum was then stored at -20ºC.

Virus Propagation

Viruses used for neutralisation were obtained from the World Reference Laboratory (WRL) for FMD at the Institute for Animal Health (IAH), Pirbright UK. These viruses were then either propagated once or twice in renal swine (RS) cells in order to obtain a virus titre above 10^3 $TCID_{50}$. Tissue culture supernatant was harvested when greater than 80% CPE was noted and the viruses were stored in glycerol at either -20ºC or -80ºC.

Virus Neutralisation Test (VNT)

A 2 dimensional virus neutralisation test, the gold standard for FMDV vaccine strain selection, was used to obtain neutralisation titres. This assay is performed at the World Reference Laboratory at the Institute for Animal Health for diagnostic purposes. This method determines a relationship coefficient, "r_1-value", by using five virus doses and linear regression to obtain the serum neutralisation titre at a virus dose of a $100TCID_{50}$. The OIE also suggests using one pre-aliquoted virus dose of a $100TCID_{50}$ to determine the neutralisation titres (10). Due to this second method being quicker, saving resources and giving similar results to the gold standard (data not shown), this test was used for the majority of VNTs carried out. For this study each of the 48 viruses (table 1) was tested against seven different sera by VNT. Each test was repeated for robustness.

Antigenic Cartography

Antigenic cartography is based on the concept of space shape and multidimensional scaling (11), (12). Space shape is a tool used to represent antigenic relationships between antibody and antigen by calculating coordinates in multiple dimensions relating to different physio-chem properties of the antigen/antibody bond. Lapedes and Farber (2001) used this concept of space shape with titres obtained from hemaggluttination assays to illustrate antigenic differences between viruses and sera (11). A key finding was that these antigenic relationships relate linearly to the logarithm of the hemagglutin inhibition measurement. Smith, et al (2004) used this key concept with a modification combining metric and ordinal multidimensional scaling to construct an antigenic map (12). To make such an antigenic map each neutralisation value is converted to a target distance by taking the difference between the $\log_2$ reciprocal neutralisation titre against a virus and the maximum $\log_2$ reciprocal titre achieved by that serum against any other virus. Thus a virus having the highest neutralisation titre against a serum will be placed closest to that serum. Calculating the distances in this way allow one to view the titre differences in two-fold dilutions (denoted as one antigenic unit, AU) no matter what the magnitude of the actual titre is. Three criteria are then examined to assess the reliability of the antigenic maps: 1) assessing the error between target distance and map distance for each serum titre, 2) self consistency of the map in multiple repeated optimizations and 3) randomly omitting titres and assessing the precision of predicting those titres using the antigenic maps. This method was applied to all 48 viruses in which neutralisation results were obtained.

Sequence Data

Of the 48 viruses used for antigenic interpretation, 36 viral capsid sequences were obtained for phylogenetic analysis. Six viruses were unable to be sequenced due to agreements with the pharmaceutical companies, which supplied these viruses. 11 of the viruses were previously sequenced by the Molecular Characterisation and Diagnostics Group (MCD) at the Institute of Animal Health, Pirbright, UK and were kindly shared for use in this project. The other, 25 viruses, were sequenced from the same stock viruses used for VNT. The RT-PCR was carried out using random hexamers and KOD polymerase. The primers were designed by the MCD group.

Phylogenetic Analysis

Full capsid (P1) sequences were used for the phylogenetic analysis. It was determined that the best molecular model is TN93 using Tree-Puzzle (13). The phylogenetic trees were constructed using maximum likelihood methods in both PhylML (14). Similar phylogenetic outcomes were seen using multiple evolutionary models (including GTR) as well as different phylogenetic methods (neighbourhood adjoining methods, maximum likelihood and bootstrapping). An outgroup from Serotype O (OManisa) was used to root each of the phylogenetic trees.

RESULTS

The full capsid (P1) sequences cluster according to the geographic origin of the virus, as can be seen by figure 1. This concurs with routine VP1 sequencing carried out for diagnostics in the MCD group.

The antigenic cartography maps appear stable with a predictability of 0.96 +/- 0.17 antigenic units. The position of each virus is less than one antigenic unit (one two-fold dilution) error from the neutralisation values obtained in the laboratory. As can be seen by figure 2 the picture drawn for the antigenic relationship of FMDV Serotype A doesn't follow the same pattern as the phylogenetic results. There are clear discrepancies between genetic and antigenic relationships. In blue and yellow are viruses to highlight this point. On the phylogenetic tree A/IRN/23/2009 and BAR/02/09 (coloured blue) appear to be very close; however, antigenetically they are distant. For the field viruses A/IRQ/24/2009, A/PAK/02/2009 and A/LIB/14/2009 (coloured in yellow) also appear closely related phylogenetically however they appear antigenically similar.

DISCUSSION

We have quantified the antigenic relationships among Serotype A FMDV using antigenic cartography. The maps are robust and repeatable; however, there are large discrepancies between the antigenic and genetic relationships. Various reasons could account for what is seen, including the hypothesis that one amino acid can cause a significant antigenic change, and these will be considered. More work will also be undertaken to look at the raw sequence data, specifically looking at the already identified antigenic sites, to identify if a relationship can be drawn between the antigenic and genetic data. Additional sera will also be added in order to improve the robustness of the map. Lastly, all remaining viruses will be sequenced and added to the phylogenetic tree.

Table 1: Viruses and Sera used for Virus Neutralisation Tests

Below are the viruses and sera used to construct the matrix that was then used for antigenic cartography. All of these are Serotype A FMD viruses.

Viruses:

A/ARG/2001	A/IRN/31/2005	A/PAK/02/2009
A/Alem/ARG/81	A/IRN/32/2001	A/PAK/23/2009
A/BAR/02/2009	A/IRN/36/2007	A/SAU/15/2005
A_{24}/Cruzeiro/BRA/55	A/IRN/41/2003	A/SUD/03/77
A/EGY/01/72	A_{24}/IRQ/24/64	A/SUD/01/2006
A/EGY/01/2006	IRQ24/09	A_{15}/Bangkok/TAI/60
A/ERI/98	A_{21}/Lumbwa/KEN/3/64	A/TAI/118/87
A/ETH/09/2008	A_{23}/Kitale/KEN/64 (A/KEN/64/65)	A/TAI/01/2006
A_{11}/Germany/c.29 (AGB)	A/KEN/42/66	A/TOG/09/2005
A/GHA/16/73	A/KEN/01/2003	A/TUR/04/2006
A/IRN/01/2005	A/KEN/08/2008	A/TUR/07/2008
A/IRN/33/2004	A/KEN/22/2009	A/TUR/20/2006
A/IRN/2/87	A/LAO/07/2006	A/TUR/24/2007
A/IRN/1/96	A/LIB/14/2009	A/UGA/13/66
A/IRN/07/2004	A/MAI/97	A_{12}/119/Kent/UK/32
A/IRN/10/2003	A/MAI/12/2006	A/VIT/04/2004
A/IRN/22/99	A/MAI/16/2006	
A/IRN/23/2009	A/NGR/02/73	

Sera:

A/IRQ/24/64	A/ERI/03/98	A/IRN/22/99
A/IRN/02/87	A/ARG/01	
A/IRN/01/86	A/MAI/97	

Figure 1: Phylogenetic Tree

The phylogenetic tree shown below is a maximum likelihood PhylML tree using evolutionary model TN93. The viruses are represented according to the three prototypes within Serotype A:

Africa (purple), Asia (red), Euro-South America (green). The viruses highlighted in yellow and blue are viruses that appear closely related phylogenetically but differ in their antigenicity (see figure 2)

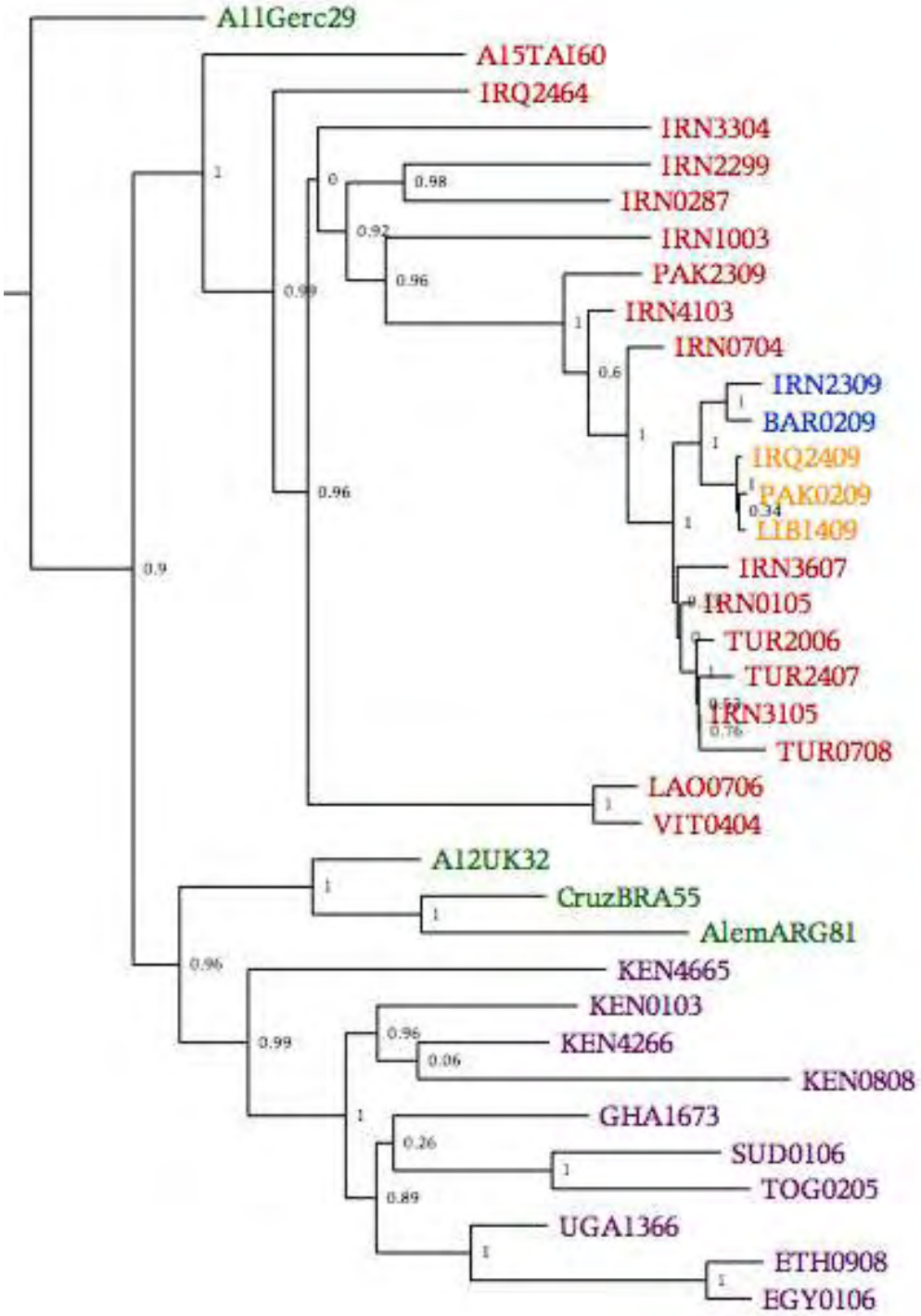
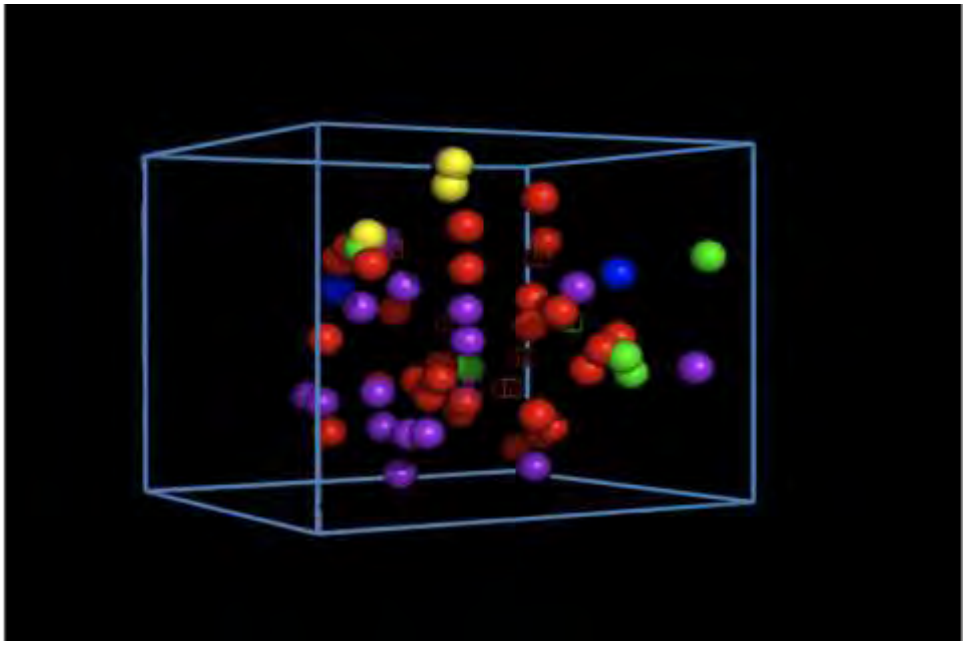


Figure 2: Antigenic map

Below is a 3 dimensional antigenic map that is derived from the neutralisation results obtained from the viruses and sera listed in table 1. The circles represent the viruses and the cubes show the seven sera. They have been coloured coded according to the 3 prototypes within Serotype A. The prototypes are as following Africa (purple), Asia (red) and Euro-South America (green). The yellow circles highlight Asian viruses that are closely related phylogenetically and antigenically. The blue circles shows Asian viruses that are closely related phylogenetically and distantly related antigenically.



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QUANTITATIVE SINGLE SERUM DILUTION ELISA FOR THE ASSESSMENT OF HERD IMMUNITY AND EXPECTANCY OF PROTECTION AGAINST FMDV IN VACCINATED CATTLE

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ABSTRACT

A single serum-dilution liquid phase ELISA (slpELISA) was standardized to be used for serological evaluation of herd immunity against foot-and-mouth disease. The absorbance value at a dilution 1:64 of each serum sample was interpolated in a standard curve by plotting the antibody titers of six control sera determined by end point dilution liquid phase ELISA (lpELISA), against the absorbance values for the same control sera at 1:64 dilutions, in the titer range of 1.40 to 2.40. The reliability of the antibody titers was confirmed by the simultaneous titration of 60 cattle sera by slpELISA and lpELISA, which showed an acceptable correlation (R^2 >0.87) for viral strains A24/Cruzeiro, A/Argentina/01, O1/Campos and C3/Indaial. Titers obtained by both methods were not significantly different ($p>0.05$), thus confirming that slpELISA could be used successfully to replace the conventional serial dilution ELISA for the assessment of protection status of cattle in epidemiological studies.

1. INTRODUCTION

Foot-and-mouth disease (FMD) has been the most important disease affecting animal production in Argentina since first recognized in 1870. Although the slaughter policy has been considered and applied in specific cases, the main strategy adopted by Argentina against FMD was compulsory massive vaccination of cattle [Mattion et al., 2004].

Cattle are vaccinated systematically twice a year, and before they are moved to other premises. Presently, the tetravalent vaccine is composed of inactivated oil adjuvanted suspensions of viral particles belonging to the strains A24/Cruzeiro, A/Argentina/01, O1/Campos and C3/Indaial.

Due to the size and territorial heterogeneity of Argentina and the magnitude of the cattle population, FMD vaccination campaigns have encountered a number of operational challenges. For this reason, the evaluation of the effectiveness of the vaccination campaigns are of crucial importance.

The level of antibodies against different serotypes of FMDV is measured using a liquid phase blocking competitive ELISA (lpELISA) [Hamblin et al., 1986] whose development and validation have been published [Maradei et al., 2008; Periolo et al., 1993; Robiolo et al, 1995]. However, for the large number of samples to be tested in epidemiological studies, the use of this lpELISA is labor-intensive and expensive. Instead, herd immunity can be studied more readily using a single serum dilution liquid phase ELISA (slpELISA), provided it is validated against an end point dilution lpELISA correlated with protection from challenge in vivo in the target species [Maradei et al., 2008].

In the present study, a slpELISA was modified, from the end point dilution lpELISA, and validated for the specific purpose of field studies. Assessment of the protection status of cattle after vaccination campaigns in the Buenos Aires province in the year 2004 is described in this work as an example.

2. MATERIALS AND METHODS

2.1. Sera

Serum samples from animals vaccinated with FMDV strains A24/Cruzeiro, A/Argentina/01, O1/Campos and C3/Indaial were provided by Sanitary Units of Buenos Aires Province. A total of 20,742 bovine sera were used in this study for assessment of herd immunity. Sera from 60 vaccinated animals of different ages were used in concordance studies between lpELISA and slpELISA. Sera from another 10 animals vaccinated with the four FMDV strains were used in studies of intra-laboratory repeatability (intermediate precision). Samples were selected

from animals of different ages, in order to have low, medium and high titer sera, covering the 1.4 to 2.40 titer interval.

2.2. Single dilution liquid phase blocking ELISA

The assay was carried out as described previously [Maradei et al., 2008)] except that only one dilution of each serum was tested in 96-well plates. Each plate allowed the testing of 68 samples. Serum samples at a final dilution of 1:64 were incubated overnight at 4ºC with a pre-titrated dose of the corresponding virus strain in a saline buffered liquid phase. The mixtures were then incubated for 1 h at 37ºC on wells coated with FMDV strain specific rabbit polyclonal antibody, in order to capture the viral particles which did not react with the bovine serum in the previous step. After a washing step, a second incubation was carried out with a saturating concentration of a pool of monoclonal antibodies (MAbs) specific for each of the virus strains under test [Seki et al., 2009]. Goat anti-mouse IgG conjugated with horseradish peroxidase (Jackson InmunoResearch) was added, and colour was developed after the addition of the substrate/chromophore mixture (H_2O_2/ABTS, Sigma, USA). The optical density (OD) readings were measured at a wavelength of 415 nm.

Six wells were used for the control of antigen concentration (100% reactivity) and two wells were used as reaction blanks, without viral antigen and without serum. Six positive control sera of known titers (high, medium and low) were assayed simultaneously as internal standards in each ELISA plate in three twofold dilutions (1:32, 1:64 and 1:128). Control sera antibody titers were expressed as the reciprocal $\log_{10}$ of serum dilutions giving 50% of the absorbance recorded in the antigen control wells ($OD_{50\%}$). One negative control sera was tested at a 1:64 dilution in duplicate wells.

The conditions of acceptance of each plate (listed below) were established as described previously [Maradei et al., 2008], with minor modifications. The blank OD was calculated as the average absorbance of two blank wells and should be <0.300. This value was subtracted from the OD value of every well. The OD of the control antigen was calculated as the average of the six wells. The OD of each of the six individual replicates should be >0.750 and <1.950, not differing from each other in more than 0.300. At least five out of six replicates must comply with this condition. The titer of the negative control serum, calculated as the average of two wells, should be ≤ 1.40 [Maradei et al, 2008)].

The dilutions used for the positive control sera corresponded to the linear part of the sigmoid curve obtained representing the absorbance values versus the $\log_{10}$ of the reciprocal dilutions used in the assay (1.50, 1.80 and 2.10). The coefficients of correlation must be ≥0.90 for each control serum. In addition, in order to validate each plate, titers of control sera should not differ more than ± 0.20 from the reference values.

2.3. Standard curve

Standard curves were generated in each slpELISA plate using the absorbance values of the six positive control sera at 1:64 dilutions, versus the reference titers of the same controls, determined by end point dilution. Titers of the control sera were selected in order to have at least six points in the linear region of this curve, between 1.40 and 2.40. These standard curves were validated with an $r > 0.90$, and were used to evaluate samples titers by linear regression, by interpolation of their absorbance values at a 1:64 dilution.

2.4. Repeatability and intermediate precision of slpELISA

The intra-laboratory repeatability (intermediate precision) was estimated with data obtained by two different analysts, using 6 replicates of each of ten sera reactive to A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indail FMDV strains, titrated in six different days, using two different lots of reagents, over a two months period. Sera were selected from animals of different age categories, in order to have low, medium and high titer sera, covering the 1.40 to 2.40 titer interval. Standard deviations (SD) and coefficients of variation (CV %) were calculated [Jacobson, R. H., 1998].

2.5. Correlation of slpELISA with lpELISA

In order to assess the reliability of the serum titers obtained in the slpELISA, a comparison was carried out between end point dilution lpELISA and single dilution ELISA. For this purpose, 60 serum samples from vaccinated animals were titrated simultaneously by both methods. Sera were selected from animals of different age

categories, in order to have low, medium and high titer sera, covering the 1.40 to 2.40 titer interval. Statistical analysis was performed using the Student test for paired samples and the Graph Pad PRISM software, version 4 [Motulsky, H. J, 2003].

2.6. Expectancy of protection

The available correlation curves between lpELISA and in vivo protection by the Protection against Generalization (PPG) method for each FMD virus strain were used for assessment of the Percentage of Expected Protection (EPP). The validation of these correlations has been described [Goris et al., 2007; Maradei et al., 2008: Mattion et al., 2004; Robiolo et al., 1995].

2.7. Analysis of herd immunity

The immunity level of cattle population was assessed using slpELISA and the EPP correlation tables. The target population was comprised of cattle from Buenos Aires Province (22 million cattle distributed in 55,000 farms), where 105 sanitary organizations are in charge of the administration and implementation of the vaccination campaigns. The study population was limited to 39 sanitary organizations which agreed to participate in the study.

A random two-stage sampling design was implemented to estimate the proportion of cattle protected against FMDV virus. At the first stage, farms (primary sampling units) were selected with a probability proportionate to the number of animals. At the second stage, an equal number of bovines (secondary sampling units) were selected form each farm.

For the study, cattle population was divided into three categories: category 1, animals of 6 to 12 months of age; category 2, animals of 12 to 24 months; and category 3, animals older than 24 months. The sampling size was calculated based on the following parameters: confidence level: 95% for each category; maximum relative acceptable error: 10%; rate of homogeneity: low; expected proportion of protected animals: in category 1, 65%; category 2, 86%; and category 3, 90%. Based on these parameters, 42 farms from each sanitary organization and 10 animals from category 1, 3 animals from category 2, and 2 animals from category 3 were selected.

Samples were collected during the first vaccination campaign of the year 2004 (February-March) and serum samples were taken immediately before vaccination.

The sera were tested by slpELISA for FMDV serotypes A/Arg/01 and O1/Campos. For practical and epidemiological reasons, C3/Indaial and A24/Cruzeiro, which are regional strains present in the vaccines, where not included in the surveillance. Serum samples with titers which correlated with an EPP ≥75% for each virus serotype were considered as a high immunity level [Maradei et al., 2008]. In this case, 75% EPP corresponded to titers ≥ 2.10 for both A/Arg/01 and O1/Campos.

3. RESULTS

3.1. Standardization of slpELISA for testing of serum samples in a single dilution

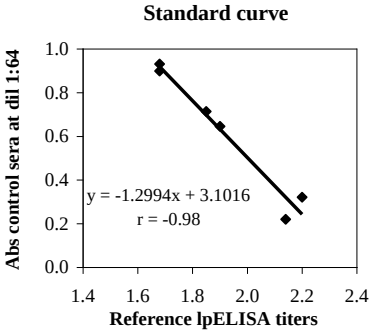
Antibody titers against FMDV strains A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial, of 68 serum samples were measured at a single dilution, using one microplate per strain. The validation of a standard curve per each plate was carried out simultaneously.

Six reference positive control sera were titrated in three serial twofold dilutions in the same plate, and the absorbance values were plotted against the $\log_{10}$ of the reciprocal dilutions. Linear curves were obtained for the six positive control sera of each strain, showing a similar slope. The end point titers of each set of six control sera were obtained at the mean 50% absorbance of the antigen control. The value of the correlation coefficient (r) of the regression line was higher than 0.90 for each serum and for the four virus strains (Fig. 1). The difference between the calculated lpELISA titer and the reference titer for each serum was ≤ 0.20.

The linear standard curve for the plate was obtained plotting the titers of the set of six control sera, against the absorbance value of each serum at a dilution of 1:64. The r value of the standard curves was higher than 0.90 (Fig. 1). The linear titer range of the regression curve (1.40 - 2.40) was appropriate for the purpose of this test, considering that it includes titers representing from non-protected (1.40) to almost fully protected animals (2.40), varying with the virus serotype.

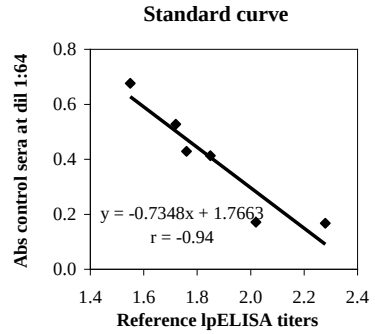
A24/Cruzeiro

Inv. dilution	Absorbance 32	64	128	r	Calculated lpELISA titer	Reference lpELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	0.028	0.321	0.702	1.00	2.14	2.20
C2	0.251	0.714	1.104	1.00	1.83	1.85
C3	0.447	0.899	1.140	0.98	1.72	1.68
C4	0.020	0.220	0.769	0.97	2.12	2.14
C5	0.188	0.646	0.986	1.00	1.90	1.90
C6	0.461	0.931	1.269	1.00	1.69	1.68



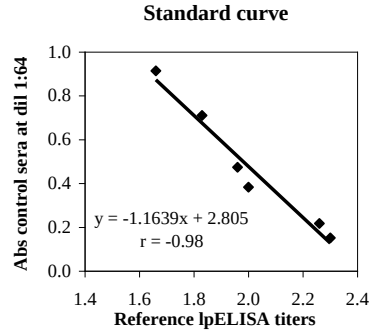
A/Arg/01

Inv. dilution	Absorbance 32	64	128	r	Calculated lp ELISA titer	Reference lp ELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	-0.003	0.167	0.523	0.98	2.13	2.28
C2	0.110	0.429	0.658	1.00	1.93	1.76
C3	0.331	0.676	0.818	0.97	1.69	1.55
C4	0.007	0.171	0.450	0.99	2.22	2.02
C5	0.087	0.413	0.681	1.00	1.92	1.85
C6	0.173	0.527	0.737	0.99	1.84	1.72



O1/Campos

Inv. dilution	Absorbance 32	64	128	r	Calculated lp ELISA titer	Reference lp ELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	0.030	0.218	0.574	0.98	2.18	2.26
C2	0.155	0.475	0.786	1.00	1.94	1.96
C3	0.399	0.711	0.958	1.00	1.72	1.83
C4	0.019	0.152	0.435	0.98	2.39	2.30
C5	0.100	0.384	0.634	1.00	2.07	2.00
C6	0.603	0.915	1.003	0.95	1.46	1.66



C3/Indaial

Inv. dilution	Absorbance 32	64	128	r	Calculated lp ELISA titer	Reference lp ELISA titer
Log_{10} inv.dil.	1.50	1.80	2.10			
C1	0.046	0.158	0.426	1.00	2.26	2.30
C2	0.068	0.267	0.572	0.99	2.04	2.12
C3	0.341	0.665	0.832	0.98	1.67	1.73
C4	0.105	0.364	0.617	1.00	1.97	2.04
C5	0.277	0.606	0.770	0.98	1.74	1.83
C6	0.453	0.709	0.818	0.97	1.54	1.60

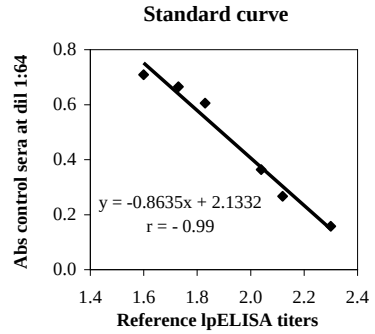


Figure 1*:* Validation of control sera and construction of the standard curves for A24/Cruzeiro, A/Arg/01, O1/Campos, and C3/Indaial FMDV strains for the titration of antibodies by single serum dilution liquid phase blocking ELISA. C1-C6: control sera.

The CV % for intra-plate and intra-day repeatability of the slpELISA for the FMDV strains A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial, complied with the acceptance criteria established (CV≤ 10%, data not shown).

The intra-laboratory repeatability precision of the lpELISA was assessed for the same sets of control sera during the validation of this test, as described previously [Maradei et al. 2008]. In the slpELISA, these control sera were used in the same dilutions and processed following the same procedures described previously. In the present study, the reproducibility of the slpELISA was examined for individual serum samples to be used in a

single 1:64 dilution. Data on intermediate precision of the test was obtained in the titer range 1.40 to 2.40. CV % values were in all cases <11% when carried out by different analysts on different days, complying with the acceptance criteria (CV % <15%).

3.2. Comparison of single and end point dilution tests

Sigmoid correlation curves were obtained when lpELISA and slpELISA antibody titers of 60 vaccinated cattle were compared using the program Graph Pad PRISM, version 4 [Motulsky, 2003]. The R^2 values obtained in this study were: 0.9526, 0.9384, 0.8793 and 0.9086 for A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial, respectively (Figure 2). Differences between slpELISA and lpELISA titers of paired serum samples were not significant (p>0.05) by Student's test.

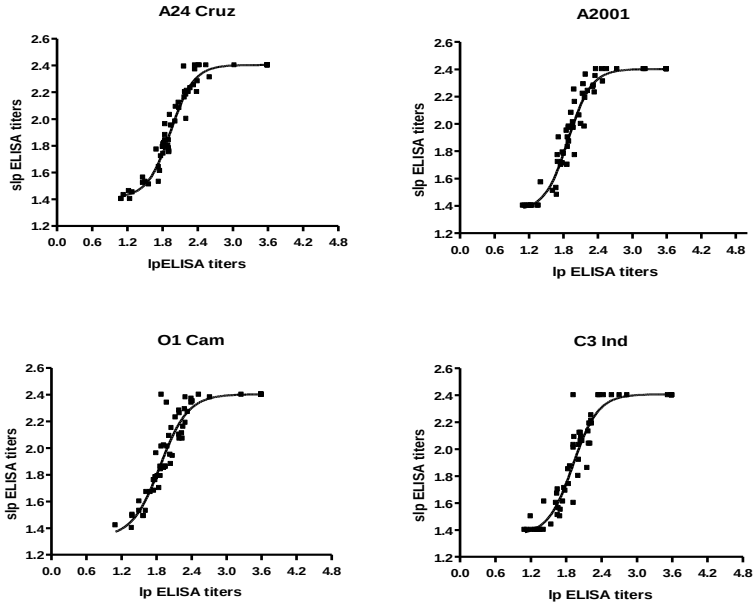


Figure 2: Correlation between antibody titers of 60 cattle sera determined by end point dilution lpELISA and single-serum dilution slpELISA, for A24/Cruzeiro, A/Arg/01, O1/Campos and C3/Indaial FMD virus strains. The R^2 coefficient is shown for each virus strain.

3.*3. Protection status of cattle population after regular and systematic vaccination campaigns.*

It was found that the higher the age of the animals, the higher the proportion of bovines with high immunity level (EPP ≥75%) for both serotypes tested. This is expected in a country where regular vaccination is practiced [Mattion et al., 2004]. On the other hand, the proportion of animals with high immunity levels was significantly higher (P < 0.05, 95% CI) for O1/Campos than for A/Arg/01 strain, for the three age categories (Table 1).

Table 1: Distribution of cattle with high immunity level (EPP ≥75%), by age category and FMDV serotype in Buenos Aires Province in the year 2004.

Age category	Nº of animals per category	High immunity level					
		A/Arg/01			O1/Campos		
		n	%	95% CI	n	%	95% CI
1	13 832	8 230	59.5	58.1-60.1	8 686	62.8	61.4-64.2
2	4 118	3 616	87.8	86.6-89.0	3 786	91.9	90.1-93.0
3	2 792	2 668	95.6	94.7-96.4	2 704	96.8	96.1-97.6

n: Number of animals with high immunity level; CI: Confidence interval.

Category 1: 6-12 months of age; category 2: 12-24 months; category 3: older than 24 months.

Immunity levels varied in different counties. For animals corresponding to category 2, the proportion of individuals with high immunity level for FMDV strain O1/Campos ranged from 75.4% to 99.2%, with a median value of 93.8%. Similar variability was observed for the other age categories and for FMDV strain A/Arg/01 (data not shown).

4. DISCUSSION

Using slpELISA, and taking advantage of the high correlation with the end point dilution lpELISA which has been correlated with the PPG test in Argentina [Maradei et al., 2008], it was possible to carry out the control of the vaccination campaigns of a large number of animals in extended geographical regions, at a considerable lower cost and speed. This technique also offers considerable time and money savings over more labor-intensive tests, such as virus neutralization.

The method described in this study represents a reproducible and practical epidemiological tool for assessment of the immune status of a vaccinated population, so that early management decisions regarding vaccination can be made. The procedure is also useful for specific and sensitive determination of seroconversion in non vaccinated populations, and in combination with the assessment of the presence of antibodies to non structural proteins [Robiolo et al., 2006], constitutes an early warning for eventual introduction of FMDV in naive susceptible populations.

This technique provides economy, considering that the number of samples that can be evaluated in a single plate with sufficient accuracy can be increased from 17 samples, in the final dilution lpELISA, to 68 samples, in the case of the single dilution slpELISA. This favours the use of the slpELISA for large epidemiological studies.

Although the response function is a sigmoid curve, only the linear region was used. The adjustment of the assay was of crucial importance in order to obtain a sufficient number of points in this region. The titers of the samples were calculated interpolating its absorbance in the standard curve, whose reliability was evaluated by the correlation coefficient. The linear relationship between the absorbance and the reciprocal of the serum titer for the fixed dilution chosen (1:64) was found to be between 1.40 and 2.40, with an r value above 0.90.

According to the lpELISA validated previously [Maradei et al., 2008], a negative serum is defined when it has a titer below 1.30, whereas a protective titer might be assigned for a value of 2.0 or higher, depending on the FMDV serotype. Thus, the linear titer range obtained may be considered appropriate for the specific purpose of this work.

Using the regression equation developed, and testing the sera at a single dilution of 1:64, the predicted titers were found very close to the titers obtained using full dilution ranges, which allowed the assessment of potential protective status of the animals for each antibody titer.

In the present study, a direct association was found between the proportion of individuals with high immunity level and age. This was expected since the older an animal, the higher the number of times it has been vaccinated against FMD. Assuming that the participating counties belonged to a region with no major differences regarding operational difficulties to implement the vaccination campaigns, the analysis of distribution of results allowed the observation of the levels of protection achieved by different sanitary organizations (not shown). On the other hand, the analysis of field data, along with data arising from controlled trials, may help to establish reference levels of protection for each animal age category.

The quantitative single serum-dilution liquid phase ELISA provides an adequate method for monitoring the effectiveness of vaccination campaigns and helps to identify failures in the compliance of the vaccination strategy. However, identification of specific causes of failure in achieving reference levels of protection requires investigation of other factors at the local level, such as vaccine coverage, time of vaccination, duration of the vaccination campaign, among others.

ACKNOWLEDGEMENTS

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CAN NEXT-GENERATION SEQUENCING BE USED TO UNRAVEL FINE SCALE FMDV POPULATION DYNAMICS?

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ABSTRACT

The sequence diversity of viral populations within individual hosts is the starting material for selection and subsequent evolution of RNA viruses such as foot-and-mouth disease virus (FMDV). Using next-generation sequencing (NGS) performed on a Genome Analyzer platform (Illumina), this study compared the viral populations within two bovine epithelial samples (foot lesions) from a single animal with the Inoculum used to initiate experimental infection. Genomic sequences were determined in duplicate sequencing runs, and the consensus sequence determined by NGS, for the Inoculum, was identical to that previously determined using the Sanger method. However, NGS reveals the fine polymorphic sub-structure of the viral population, from nucleotide variants present at just below 50% frequency to those present at fractions of 1%. Some of the higher frequency polymorphisms identified encoded changes within codons associated with heparan sulphate binding and were present in both feet lesions revealing intermediate stages in the evolution of a tissue-culture adapted virus replicating within a mammalian host. We identified 2,622, 1,434 and 1,703 polymorphisms in the Inoculum, and in the two foot lesions respectively: most of the substitutions occurred only in a small fraction of the population and represent the progeny from recent cellular replication prior to onset of any selective pressures. We estimated an upper limit for the genome-wide mutation rate of the virus within a cell to be 7.8×10^{-4} per nt. The greater depth of detection, achieved by NGS, demonstrates that this method is a powerful and valuable tool for the dissection of FMDV populations within hosts.

INTRODUCTION

RNA viruses evolve rapidly due to their large population size, high replication rate and poor proof-reading ability of their RNA-dependent RNA polymerase. These viruses are thought to exist as complex, heterogeneous populations comprising similar but non-identical genomes, but the evolutionary importance of this phenomenon remains unclear [1-3]. Consensus (Sanger) sequencing identifies the predominant or major viral sequence present in a sample, but is uninformative about minority variants that are present. Evidence for population heterogeneity, where individual sequences differ from the consensus sequence, has been routinely obtained using cloning approaches [4, 5], providing insights into the evolutionary processes that shape viral populations. Unfortunately, these cloning processes are laborious and typically provide only a limited resolution of the mutant spectrum within a sample.

Next-Generation Sequencing (NGS) techniques offer an unprecedented 'step-change' increase in the amount of sequence data that can be generated from a sample. Albeit mostly used for de-novo sequencing of large genomes, NGS can be applied to re-sequence short viral genomes to obtain an ultra-deep coverage. Therefore, NGS has the potential to provide information beyond the consensus for a viral sample by revealing nucleotide substitutions present in only a small fraction of the population. Several studies have previously used the 454 pyrosequencing platform (Roche Applied Science) to detect minority sequence variants for human viruses such as HIV-1 [6-12], hepatitis B [13, 14], hepatitis C [15] and attenuated virus [16]. A promising alternative to 454, is reversible terminator-based sequencing chemistry utilized by the Illumina sequencing platform (Genome Analyzer II). The lower costs of the runs and the higher throughput of this NGS approach are likely to make it

widely used for deep-sequencing genomic investigations in the future [17]. Illumina sequencing was recently used to obtain sequences of West Nile Virus, through virus-derived siRNA [18], mutant viruses of severe acute respiratory syndrome [19], and human rhinovirus [20].

The aim of this study was to explore the extent to which the Illumina sequencing platform can be used to characterize changes in viral sequence diversity generated during replication of foot-and-mouth disease virus (FMDV) within-host at a depth unobtainable by previous cloning techniques. Belonging to the *Picornaviridae* family, FMDV is highly infectious causing vesicular lesions in the mouth and on the feet of cloven-hoofed animals. The samples analysed here were collected during an infection experiment, in which a bovine host was inoculated with FMDV. We developed a protocol that enabled identification of artifacts introduced during amplification and sequencing which was used to validate and quantify the minority sequence variants that were detected. In particular, we expected to see evidence for the reversion of capsid amino acid residues responsible for heparan sulphate (HS) binding associated with replication of a cell culture adapted strain of FMDV in a mammalian host [21, 22]. Although this study was conducted using FMDV, we anticipate that the features we observe may be broadly representative of populations found in samples obtained from other positive-stranded RNA viruses.

MATERIALS AND METHODS

The samples analysed were collected during an infection experiment, in which a single bovine host was inoculated intradermolingually with a dose of $10^{5.7}$ 50% tissue culture infective doses ($TCID_{50}$) of FMDV (O_1BFS 1860). The full-length FMDV genome sequence of this sample had been previously determined using Sanger sequencing (EU448369) and was used as a reference genome in this study. The Inoculum was derived from a bovine tongue vesicle specimen that had been passaged extensively in cell culture (Cottam et al., 2008).

Total RNA (TRIzol, Invitrogen, Paisley, UK) was extracted from a sample of the Inoculum as well as two 10% tissue suspensions prepared from epithelial lesions (front left foot [FLF] and back right foot [BRF]) collected from the animal at 2 days post inoculation. Post RNA extraction, RT-PCR was carried out on each sample before sequencing on the Genome Analyzer II platform (Illumina). We repeated the PCR of the original reverse-transcribed sample in order to obtain an independent replica of the amplified sample. The number of viral RNA copies put into the initial PCR reaction was established by quantitative PCR for each of the samples [23]. Full details of genome amplification and the Illumina next generation sequencing platform can be found within Wright et al, 2011 (online access - doi: 10.1128/JVI.01396-10).

RESULTS

Description and filtering of Illumina data

Sequences from the Illumina Genome Analyzer platform consist of a collection of several million short reads. Each nt of each read is characterized by a quality score, which quantifies the reliability of the base-calling process during the sequencing. Only reads whose average error per nt was below 0.2% (66% for the first run and 95% for the second run) were considered for this analysis.

Coverage and consensus genomes

Reads that passed the quality test were aligned to the reference Inoculum genome. The mean coverage of the reference genome in the first run was 4863x for the Inoculum, 8665x for the FLF sample and 6594x for the BRF respectively, while for the second run it was 16827x for the Inoculum, 11924x for FLF and 15945x for BRF (Figure 1A, B).

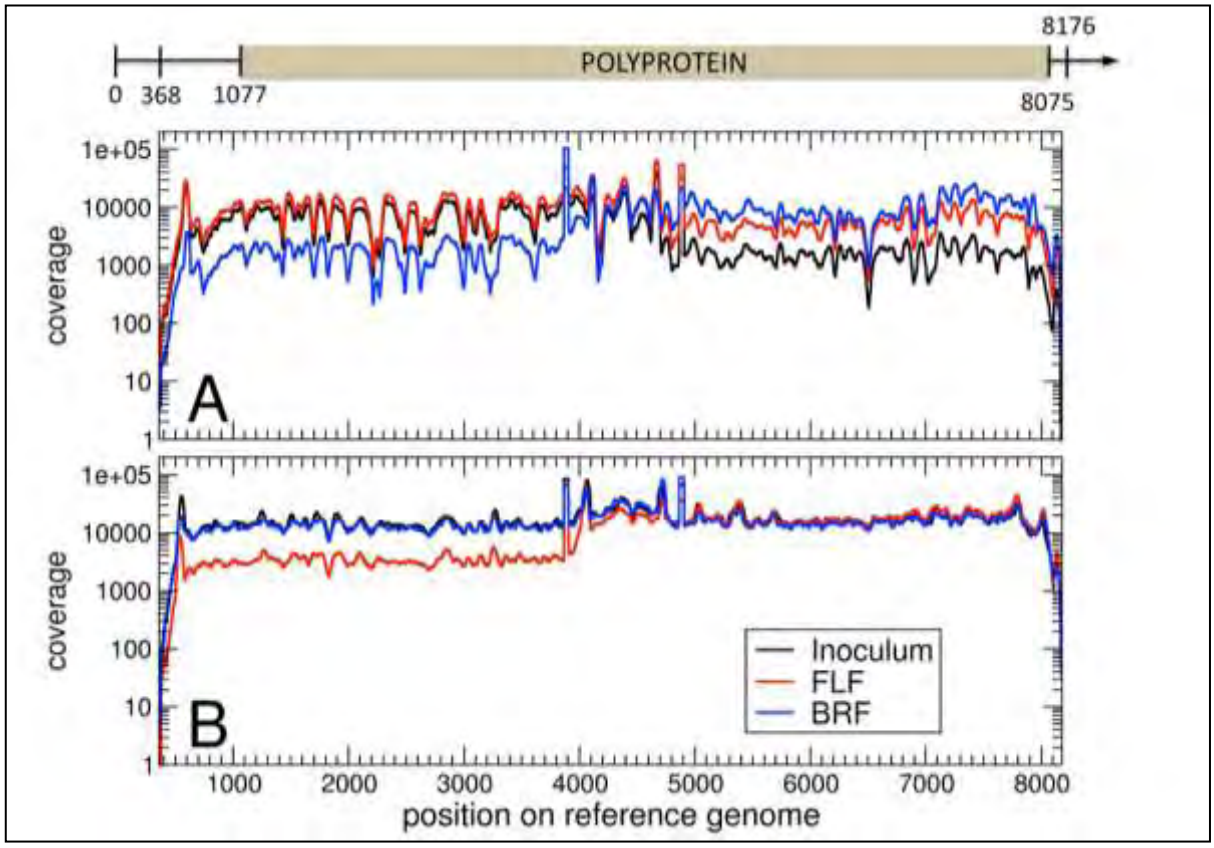


Figure 1: Coverage of the reference genome, obtained with the filtered, trimmed reads. Panel A: first dataset, panel B: second dataset. The three samples (Inoculum, Front Left Foot and Back Right Foot) receive a generous coverage from both runs, while fluctuations are higher on the first dataset. Average coverage is 4873x (Inoculum), 8665x (FLF) and 6594x (BRF) for the first dataset, and 16827x (Inoculum), 11924x (FLF) and 15945x (BRF) for the second dataset. On top of the figure, the sequenced fraction of the genome (nt 368-8176) is represented, together with the position of the polyprotein.

For some samples (Inoculum and BRF, first run and FLF, second run), the coverage for the two PCR fragments composing the viral genome was not equal.

We obtained consensus genomes for each sample, by identifying the most abundant nucleotide at each site in the aligned reads. As expected, the consensus for the Inoculum exactly matched the reference genome. For the FLF, both runs indicated two consensus level substitutions (nt 2767, G→A, and nt 8140, G→T). For the BRF sample, the two runs suggested slightly different consensus sequences: the first run revealed five consensus level substitutions (nt 2767, G→A, nt 3138, G→A, nt 5138, T→C, nt 7354, C→T, nt 8134, C→T), whereas the second run had none. However, at position 8134 about 30% of the reads in the second run showed a T in place of a C, and at position 2767 5% of the reads had an A in place of a T. At the remaining 3 sites, the second run had a small number of reads confirming the polymorphism found in the first run. This result indicates that the same pattern of variation is present in both runs for the BRF, although the frequency of mutation is not in quantitative agreement. Finally, the second run showed an almost consensus level substitution in 49.9% of the reads (nt 2754 C→T), which was present at a 10% frequency in the first run for the BRF.

Validation of polymorphic sites

Site by site mismatch frequencies are shown in Figure 2 (first run) and Figure 3 (second run). Using these raw data, and considering only sites receiving coverage of ≥ 100x, we found polymorphisms at 7,755 sites in the Inoculum, 7,730 in FLF and 7,710 in BRF, out of the 7,825 nt sequenced. While a few sites exhibited higher levels of polymorphism, the vast majority of sites displayed a mismatch frequency around 0.1%.

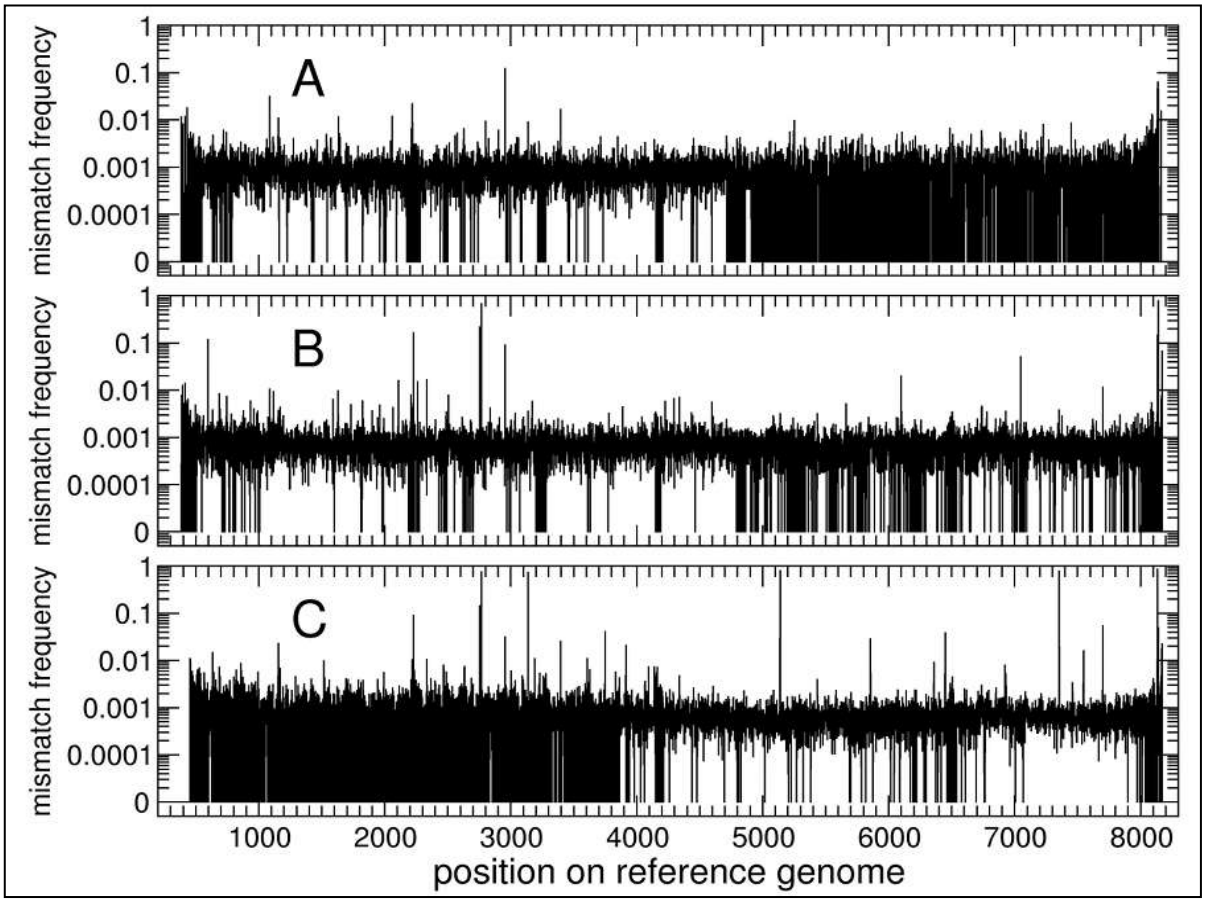


Figure 2: Frequency of mismatches (first dataset). Obtained by aligning the reads to the reference genome. Panel A: Inoculum, panel B: FLF, panel C: BRF. The average mismatch frequency lies around 0.1% for all the three samples. At few sites, the mismatch frequency is higher; as expected, the number of these peaks is larger in the FLF and BRF than in the Inoculum. A small fraction of sites show perfect agreement of all the reads with the reference genome (mismatch frequency = 0).

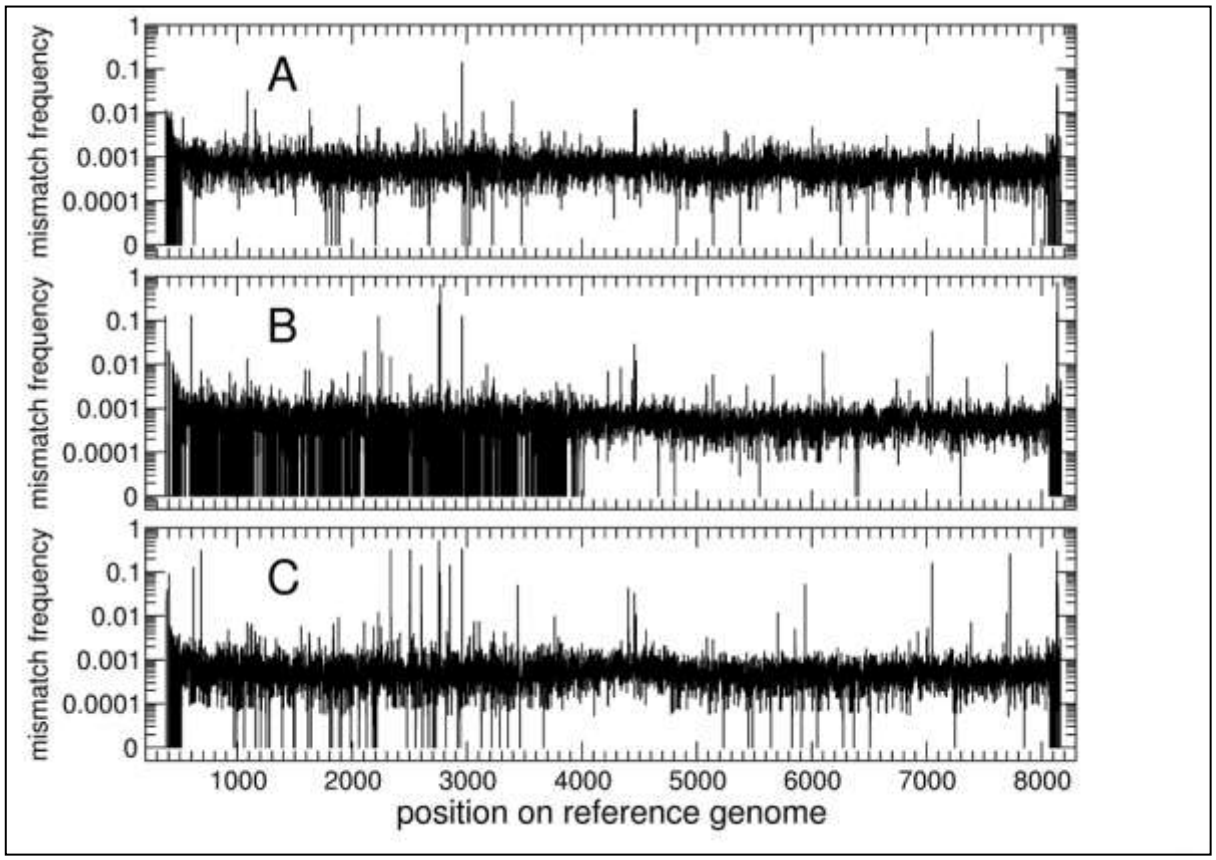


Figure 3: Frequency of mismatches (second dataset). Obtained by aligning the reads to the reference genome. Panel A: Inoculum, panel B: FLF, panel C: BRF. This second dataset has higher coverage than the first one, and a lower fraction of sites with no mismatches. The average mismatch frequency is very similar to that of the first dataset.

After screening for possible PCR and sequencing artefacts, we found that qualitatively validated polymorphisms were present at 2,622 sites for the Inoculum, 1,434 in FLF and 1,703 for BRF. The different consensus genomes obtained for BRF in the two runs can be in part reconciled by noting that all five substitutions observed (nt 2767, 3138, 5138, 7354, 8134) are qualitatively validated in each run. We observed 2,469 quantitatively validated sites in the Inoculum (94% of qualitatively validated sites), 1,303 sites from the FLF (91% of qualitatively validated sites) and 1,528 sites (90% of qualitatively validated sites) from the BRF.

Site-specific polymorphism (SSP) frequency at qualitatively validated sites was correlated between the two runs for each of the three samples (Figure 4). However, the correlation was slightly lower between runs for the BRF compared to the Inoculum and FLF (Spearman Rank correlation: 0.34 vs 0.44 and 0.50 respectively).

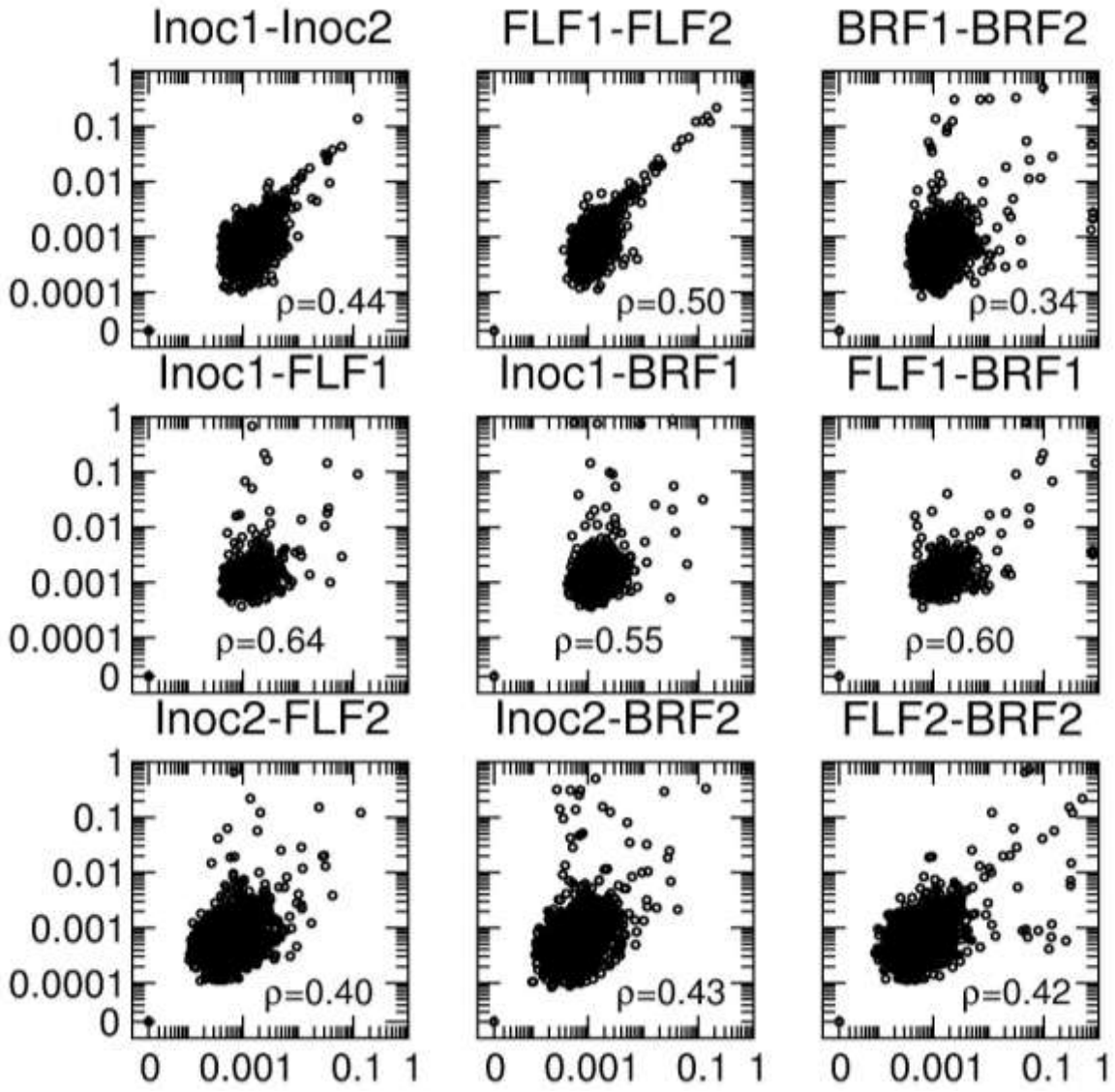


Figure 4: Correlations of polymorphism frequencies in the viral populations. Correlations were computed between the two runs (first row) and within each run (second and third row). The Spearman rank correlation ρ is indicated for each pair of datasets. Only qualitatively validated SSPs receiving coverage above 100x in both runs are shown. The correlation coefficients between the two runs in the Inoculum and FLF are similar, while they are lower for BRF. The remaining panels show that the first run is more correlated than the second.

The intra-run correlation for run 1 (Spearman Rank correlation: 0.64 [Inoc-FLF], 0.55 [Inoc-BRF] and 0.60 [FLF-BRF]) was higher than run 2 (Spearman Rank correlation: 0.40 [Inoc-FLF], 0.43 [Inoc-BRF] and 0.42 [FLF-BRF]). The reason for the poor intra-run correlation for run 2 is unclear. The number of viral RNA copies put into the initial PCR reactions was found to be large ($3.2x10^9$ for the Inoculum, $6.4x10^8$ for FLF and $2.4x10^8$ for BRF): assuming that the PCR process amplifies all genomes with the same probability, the probability of resequencing the same genome is exceedingly low ($<10^{-5}$), thus excluding the possibility of biases due to low viral load in the RNA. However, the second run in comparison to run 1 yielded lower amounts DNA library concentrations per sample prior to sequencing (3.4 vs 4.9 ng/µl, 3.7 vs 10.6 and 3.4 vs 9.5 ng/µl ng/µl for the inoculum, FLF and BRF respectively): factors that may have lead to the introduction of bias into the representative nature of the reads. The intra-run correlation, together with the high fraction of quantitative validation among the qualitatively validates SSPs provides sound evidence that nt changes are linked between the different samples. Inter-run correlation between the samples (Spearman Rank correlation: 0.34 vs 0.44 and 0.50) indicates that validated polymorphisms are unlikely to be artifacts.

Distribution of polymorphisms across the genome

There were 12 SSPs, whose average frequency between the two runs is above 1% in the Inoculum, 19 in FLF, and 25 in BRF (see Supporting Table S1 within Wright et al, 2011). Some of these were clustered in the capsid protein region (beginning of protein VP3) (1 in the Inoculum, 4 in FLF and 5 in BRF) and in the 3' untranslated region (6 in the Inoculum, 5 in FLF and 6 in BRF). Where single reads spanning these sites within the VP3 or 3'UTR were available, there was no evidence that these mutations were linked together on individual FMDV genomes. In particular, the first cluster was shared between the two foot samples and corresponded to changes encoding amino acid residues associated with heparan sulphate (HS) binding. The Inoculum used in this experiment had undergone extensive cell culture passage and, in common with other in-vitro adapted viruses, utilizes HS as a cellular receptor [24]. Subsequent replication in mammalian hosts drives the reversion of positively charged amino acid residues at specific sites in the viral capsid [21, 22]. A consensus level reversion (>50%) exists within both feet samples of run 1 compared to the reference Inoculum sequence. This reversion corresponded to a change from a glycine to an aspartic acid within the 60^{th} codon of viral protein 3 ($VP3^{60}$). Although below the level of the consensus sequence, additional qualitatively validated SSPs that were present in both feet samples were detected at four further sites (VP2 134, two codon positions within VP3 56 and VP3 59) that impact on the ability of FMDV to bind HS. All but one of the mutations clustered within the 3' UTR of the three samples were located within the first four RNA-RNA pairings either side of the apex of a conserved stem-loop. This structure, one of two stem-loops previously predicted for FMDV and other picornaviruses [25, 26] is thought to generate long-distance RNA-RNA interactions that may impact upon viral replication [27]. The presence of shared mutations between the two foot samples suggests a common history for the viruses. This may result from a shared route of intra-host transmission from initial replication sites in the tongue to epithelial sites in the feet via the blood. However an alternative explanation is that the virus is subject to a common selective pressure in both lesion sites.

Frequency of site-specific polymorphisms

Some variability was present almost everywhere on the genome. Only 61 sites exhibited no polymorphism (0.79%) in the Inoculum, 59 (0.76%) in FLF and 49 (0.64%) in BRF. These sites received relatively low coverage, suggesting the absence of observed genetic variability may be due to a lack of power to detect it.

Figure 5 provides a comprehensive picture of the heterogeneity in the viral populations by examining the proportion of sites experiencing different polymorphic frequencies.

Across the three samples, most sites exhibit a range of low-frequency SSPs between 0.01% - 1%. Only a few sites showed higher frequency polymorphism, and these sites were more numerous for the feet samples than the Inoculum, indicating the generation of new high-frequency substitutions during host passage, an observation confirmed by an entropy-like measure of diversity described in the full paper (see spendix). The dashed lines in Figure 5 correspond to the same analysis restricted to qualitatively validated sites and reveal a similar pattern.

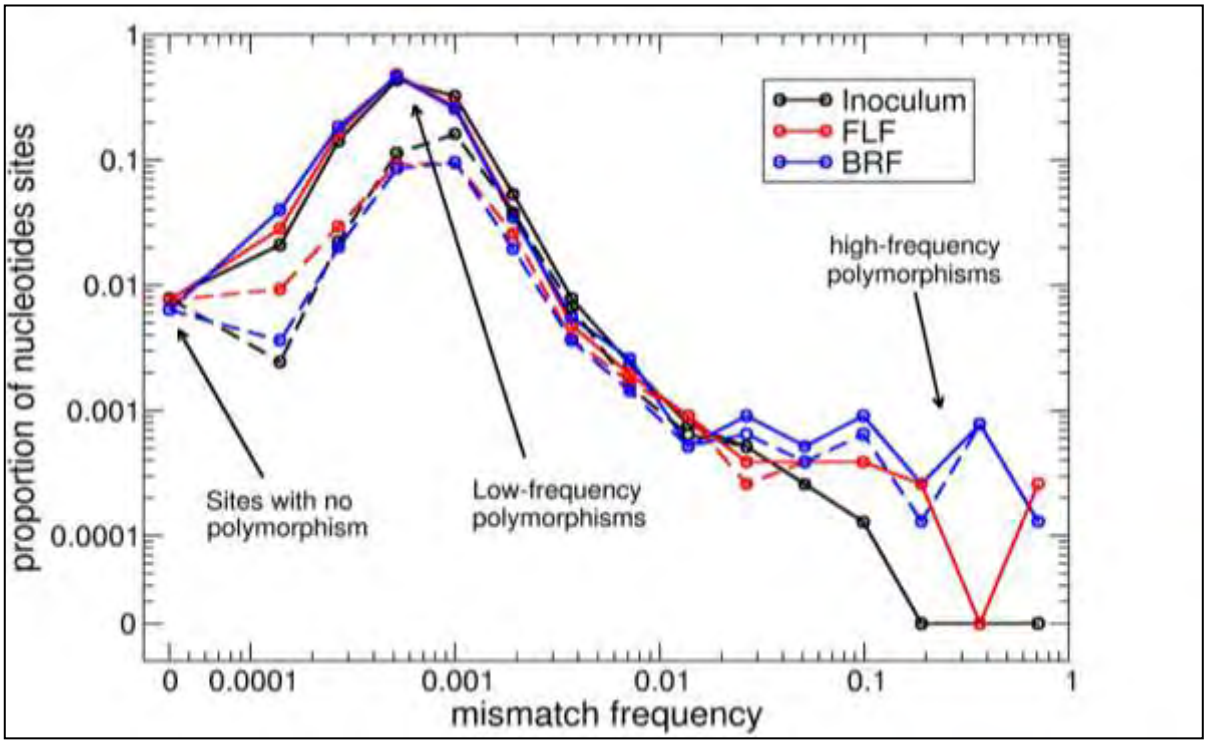


Figure 5: Frequency distribution of the weighted averaged mismatch frequencies between the two runs, for the three samples (the ordinate represents the frequencies of sites showing that fraction of mismatches). Solid lines: all sites receiving minimum coverage of 100 in both runs (7,755 sites for Inoculum, 7,730 for FLF and 7,710 sites for BRF). Dashed lines: sites receiving coverage of 100 or more in both runs, and classified as validated site specific polymorphisms (SSPs) (2,622 sites for Inoculum, 1,434 for FLF and 1,703 for BRF). All lines show a similar trend: a small fraction of the sites (<1%) display no variability in both runs, most of the sites show a very mild amount polymorphism in the viral population (between 0.01% and 1%), while a very small fraction of the sites (0.14% for Inoculum, 0.22% for FLF and 0.39% for BRF) present variation at a level above 1

Statistics of polymorphic sites

The presence of validated polymorphisms generating STOP codons within the ORF provides evidence of progeny from intra-cellular replication prior to the onset of selective pressures (such mutations are lethal for the virus and would therefore be removed from the population during infection of another cell). STOP codons were found at 24 sites in the Inoculum, 9 sites in FLF and 21 sites in BRF, mostly at frequencies around 0.1% (with a single exception in BRF where a mutation generating a STOP codon is present in 0.7% of the reads).

The presence of STOP codons can be used to obtain an upper limit on the mutation rate (per nucleotide per transcription event) of this virus. Using these data and the results linking mutation frequency to mutation rate obtained by Thebaud et al. [28], we obtained an upper bound for the mutation rate (μ) of 7.8×10^{-4} per nucleotide per transcription event (95% CI: 7.4×10^{-4} to 8.3×10^{-4}), in line with previous estimates (e.g. [29-31]).

Finally, we can ask whether these results are broadly consistent with those acquired from cloning studies. In ref. [4], Cottam et al. generated 26 viral capsid clones from an FMDV sample taken from a single lesion of a bovine host. We simulated 10,000 sets of 26 viral capsid 'clones', essentially bootstrapping from the nucleotide frequencies revealed by the NGS alignments to be present at each site within the capsid genes. Of these 26 clones, the median number of sequences in each of the 10,000 simulated data sets that were identical to the consensus was 12 (95% CI: 5-17), compared to 15 observed in ref. [4]. The median number of simulated clones containing 1, 2, 3, and 4 differences compared to the consensus were 9 (95% CI: 4-14), 3 (95% CI 1-7), 1, (95% CI: 0-3), and 0 (95% CI: 0-1) respectively. These numbers correspond well with those obtained by Cottam et al., [4] which were 6, 3, 2, and 0 respectively.

Full results and Supplementary material can be found within Wright et al, 2011 (online access - doi: 10.1128/JVI.01396-10).

DISCUSSION

This study describes a novel use of Illumina NGS to investigate the population genetic structure of FMDV. These experiments generated an unprecedented amount of sequence data and required a new systematic approach to confidently distinguish between sequences that were actually present in the samples from artifacts introduced during amplification and sequencing. Results obtained here were consistent with the findings of previous investigations, providing validation of the use of NGS in the study of FMDV evolution within a host. Carrillo et al. [32] reported an average of 1 to 5 substuitutions per animal passage during an infection experiment in pigs, in line with the 2 substitutions we found in the FLF. However, the case of the BRF sample points out a more complex scenario that could not have been observed with consensus sequences only: the drift of mutations above and below the threshold needed to appear in the consensus. Apparent loss and subsequent regain of mutations during the transmission of infection across hosts [32]. Our results are also comparable to those reported for Human Rhinovirus infection of HeLa cells by Cordey et al. [20], in terms of mutations fixed at the consensus level. Additionally, randomizations of the diversity measured in the capsid region allowed us to obtain simulated clones whose characteristics in terms of mutation were analogous to those found in [4], validating the use of NGS in the study of FMDV microevolution. Confidently observing the mutant spectrum of the viral population at such fine resolution will provide a more sophisticated understanding of evolutionary processes shaping its variability within-host.

Comparisons between the sequences recovered from the Inoculum and clinical lesions provide new insights into the impact of early replication events on viral evolution within-host. This study reveals that only a few sites displayed mutations present in a large fraction of the population, i.e. high frequency polymorphisms (>1%), while the vast majority of the polymorphisms were present at lower frequencies. We hypothesize the high frequency polymorphisms have been selected over multiple rounds of replication within cells, and that the lower frequency polymorphisms most likely directly reflect the high rate of mutation experienced by these viruses, as our estimate of an upper limit for the genome-wide mutation rate suggests. In this study we used a cell-culture adapted virus as the reference giving us the opportunity to monitor changes at specific loci associated with the HS binding that were under selective pressure during initial replication in a mammalian host. Examination of these sites (collated in the Supporting Table S1 within Wright et al, 2011) reveals for the first time the presence of intermediate stages in the evolution of the viral population between a tissue culture adapted genome and a host-adapted genome.

Figure 5 reveals that the viral population sequences are highly heterogeneous supporting the findings of previous cloning studies [33, 34]. However, the massively increased coverage achieved by NGS enables the nature of this heterogeneity to be established at much greater resolution. This is important for understanding viral evolutionary dynamics such as those described by the quasi-species theory [see [1, 33] and references therein], where heterogeneity is a necessary but not sufficient condition [35, 36]. Taking FMDV as an example, given that there are ~25,000 one-step mutant variants to any one sequence (3 alternative nts at each position of the ~8,300 nucleotide genome), NGS approaches are clearly a powerful tool for examining directly whether viral populations are structured in a way that is consistent with a quasi-species dynamic.

A viral population within a host undergoes complex processes, including the onset of infection, cellular replication, selection, and migration to different tissues. In particular, it is not clear how the diversity generated within a cell propagates through a host to give rise to the amount of diversity we observe. The data collected in studies like this can be used for building models aimed at understanding the link between the micro-evolution of FMDV at the cellular scale with the population heterogeneity at the host scale. We anticipate that a model of viral replication across several cell generations within a host will produce a more stringent upper bound to the genome-wide mutation rate.

Although further work is required, these findings strongly suggest that data generated through the use of this methodology can provide novel insights into viral evolutionary dynamics at a greater resolution than previously achieved for a positive-stranded virus such as FMDV. In particular, the genome wide assessment of polymorphic frequencies is likely to be an important asset in the parameterization of models that can evaluate the role of quasi-species dynamics in RNA virus evolution. A full discussion of the study results can be found within Wright et al, 2011 (online access - doi: 10.1128/JVI.01396-10).

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DEVELOPMENT OF A FOOT-AND-MOUTH DISEASE DIAGNOSTIC MULTIPLEX IMMUNOASSAY

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INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed livestock (including cattle, sheep, goats and pigs) which can also affect a large number of wildlife species. The causal agent of the disease is a small non-enveloped RNA virus classified within *Aphtovirus* genus, as a member of the *Picornaviridae*. FMD virus shows a high genetic and antigenic variability. Seven serotypes (A, O, C, Asia 1, and SAT 1, 2, and 3) have been identified serologically, and multiple subtypes occur within each serotype. FMD is widely distributed throughout the world and has a great potential for causing severe economic losses and trade disruptions in animals and animal products (Grubman and Baxt, 2004). Thus, FMD is an old disease but still an actual threat as exemplified still recently by outbreaks in Japan in 2010. Therefore, rapid and accurate detection of all FMDV serotypes is essential for immediate implementation of outbreak control measures and development of an effective vaccination strategy. This is of particular importance for FMD since other vesicular diseases, including swine vesicular disease, vesicular stomatitis and vesicular exanthema cause vesicular lesions in swine and cattle that cannot be distinguished from those caused by FMD. Moreover FMD vaccines may stimulate production of antibodies indistinguishable from those produced by infected animals in response to live virus (Rodriguez and Grubman, 2009). Because vaccinated animals can be infected and become carriers of FMDV, it is important to develop diagnostic test allowing differentiation between vaccinated and infected animals.

The objective of this project is the development of a microsphere-based multiplex immunoassay for FMD diagnosis by using the Luminex liquid array technology. Such an assay was previously designed by Clavijo and colleagues (Clavijo et al; 2006) but only to test immune response to FMDV non structural antigens.The luminex technology utilizes polystyrene microbeads embedded with unique ratios of two fluorophores (red and infrared). Each unique dye ratio results in a distinct emission profile. This system will allow in the same analysis the identification of different proteins by the separation of the beads according to their endogenous fluorescence and the detection of fluorescence signal corresponding to the antibodies reacted with the proteins fixed on the beads. FMDV VP1 proteins of all serotypes, FMDV non structural proteins (3A, 3B, 3C, 3D and 3ABC) and antigenic proteins of other viruses inducing vesicular diseases will be coupled to microspheres labeled with different proportions of fluorescent dye. Proteins not available will be produced in *E.Coli* and/or in insect cells as his-tagged recombinant proteins. This assay will allow, in a single reaction and from a single sample, simultaneous detection of antibodies against vesicular disease viruses and FMDV structural and non structural proteins, coupled to microspheres labeled with different proportions of fluorescent dye. Such a test will thus allow differential diagnosis of FMD but also differentiation between infected and vaccinated animals (Figure1).

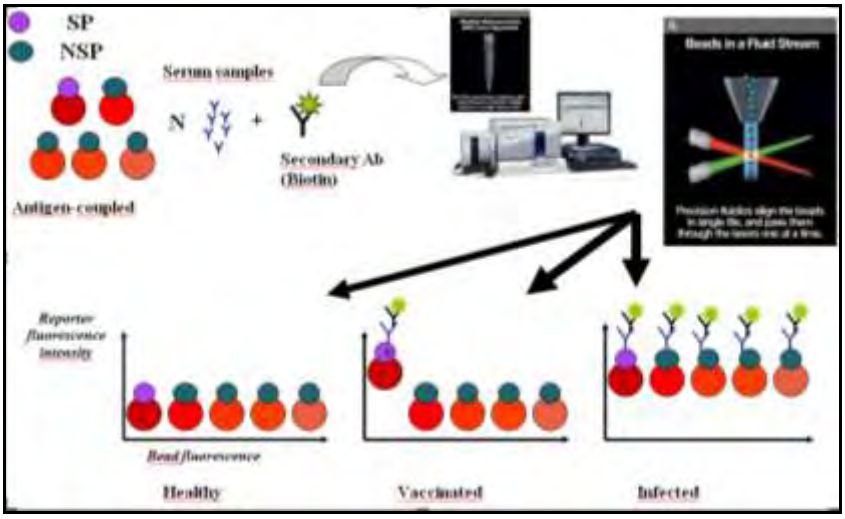


Figure 1: Multiplex immunoassay principle

MATERIALS AND METHODS

Preliminary experiments have been performed, using EMCV VP1 structural protein as model, in order to optimize both antigen purification and antigen-coupling conditions.

Antigen: expression and purification

EMCV VP1 gene was cloned into pET28b vector (Novagen). Recombinant EMCV VP1 protein was produced in BL21 (DE3) *E.coli* cells and its expression was induced by IPTG.The EMCV VP1 protein is double his tagged (NH2 and COOH extremities) and accumulated in bacteria as inclusion bodies. Purification of VP1 protein was down following two different protocols for the solubilisation step: urea 8M or guanidine hydrochloride 6M (Hijnen et al., 2005), as summarized in figure 2. Both protein extraction methods were adapted in order to avoid the presence of components disturbing antigen/beads coupling, in the final protein suspension in PBS pH 7.4. Indeed, Imidazole interferes with Ni-NTA/6His-tag interaction and sodium azide, BSA, glycine, tris or amine containing additives disturb carbodiimide reaction with bioplex beads
The soluble recombinant protein was then stored in aliquots at -80°C.

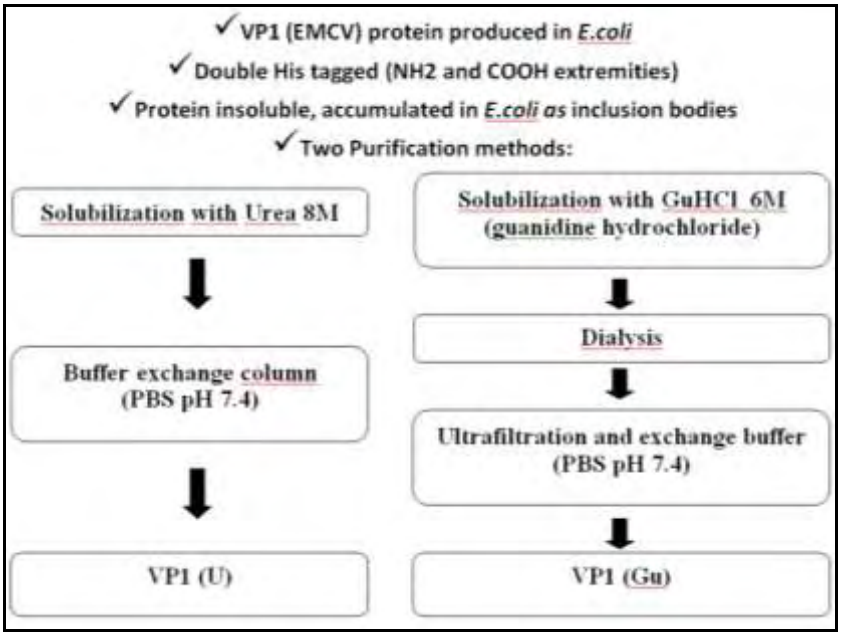


Figure 2: Optimization of recombinant antigen purification

Antigen-bead coupling and luminex immunoassay

EMCV VP1 was coupled to two different bead sets (figure 3): carboxyl beads from Biorad (covalent binding) or Ni-NTA beads from Qiagen (Ni-NTA-6xHis-tag interaction). Antigen-carboxyl beads coupling was performed using the Bio-PlexAmine coupling kit from Biorad. For each bead set, both protein purification methods were assayed and two distinct amounts of protein (12 or 24µg). The different resulting VP1-bead complexes (2500 beads per assay) were incubated with anti-EMCV-VP1 monoclonal antibody (mAb), anti-HisTag mAb (invitrogen) or immune and non-immune Pig serum. The VP1 coupled microspheres reacting against serum samples or monoclonal antibody were then incubated with biotinylated anti-species antibodies (Abcam) and then stained with streptavidin-phycoerythrin (Qiagen). Reactions were then analyzed using the Bioplex Luminex 200 system (Biorad).

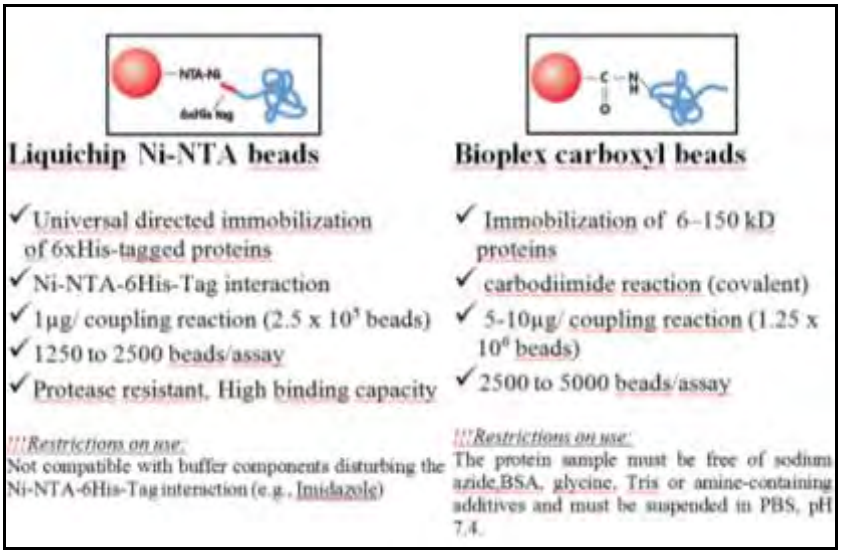


Figure 3: Optimization of bead/antigen coupling

RESULTS

EMCV VP1-bead coupling efficiency:

EMCV VP1 was coupled to two different bead sets: carboxyl beads (covalent binding) or Ni-NTA beads (Ni-NTA-6xHis-tag interaction). For each beads set (Biorad carboxyl beads or Qiagen Ni-NTA beads) and purification method (Urea or guanidine hydrochloride), protein coupling efficiency was assayed using different protein quantity, and anti-VP1 monoclonal antibody (mAb). As schown in figure 4, comparable results were obtained except for Ni-NTA-VP1 (Urea) coupled beads. For bioplex beads, 12µg of protein per coupling reaction are sufficient, whatever the solubilisation method employed.

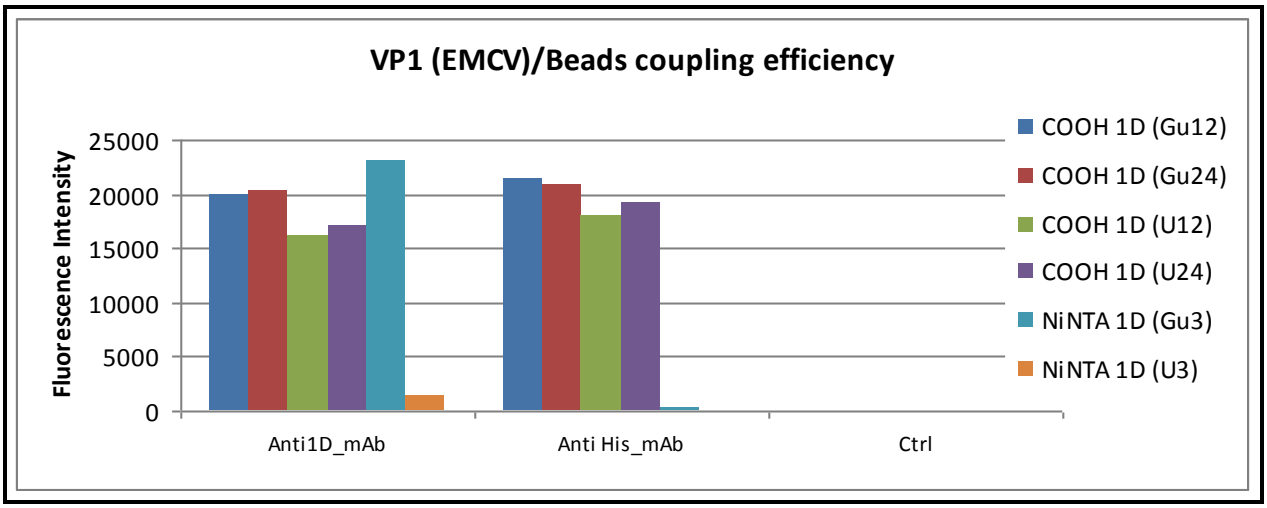


Figure 4: VP1 (EMCV)/Beads coupling efficiency

Luminex serological assay:

Serological assays were then performed using VP1-coupled beads and immune or non-immune pig serum as analytes. As shown in figure 5, the best results were obtained for the serological test using carboxyl beads coupled to VP1 solubilized in GuHCl but the assay needs to be improved to reduce background. According to these preliminary results, optimal antigen coupling conditions are Bioplex-VP1 (GuHCl) or NiNTA-VP1 (GuHCl).

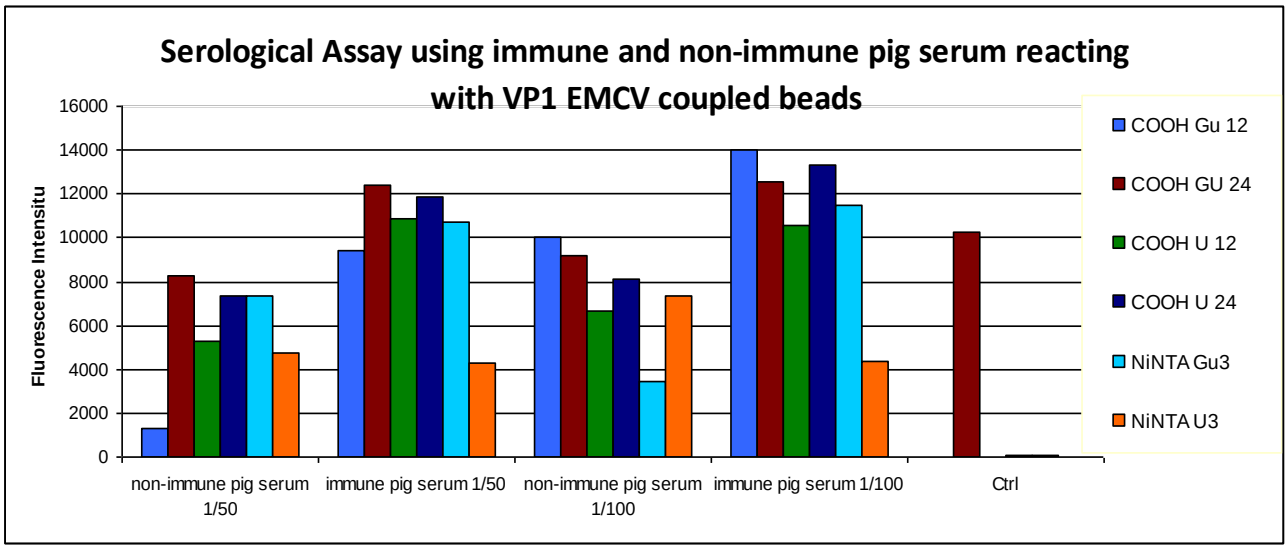


Figure 5: Serological Assay using immune and non-immune pig serum reacting with VP1 EMCV coupled beads

CONCLUSIONS AND PERSPECTIVES

The results obtained with EMCV VP1 recombinant protein as model for FMDV structural protein allowed us to define the optimal coupling conditions. Because very little distinct colored Ni-NTA bead sets are available from the Qiagen company, and in order to optimize antigen purification by an additional step using his-trap column and imidazole elution, we decided to follow the development of our test using carboxyl beads. These data were obtained using polystyrene carboxyl beads but we will switch to magnetic bio-plex carboxyl beads, allowing automated and more accurate washing steps, improving precision and thus performance of the test. Results obtained using pig sera are encouraging even if additional work needs to be done in order to (i) decrease background and thus increase the specificity of this immunoassay (ii) Improve and standardize GuHCl solubilization protocol to increase yields and purity of recombinant proteins (currently in progress for the structural VP1 type O and VP1 type Asia1 of FMDV) (ii) Produce and extract proteins of interest (FMDV VP1 for the other serotypes, FMDV NSP 3ABC, 3A, 3B, 3D and peptide 2B, VSV and SVDV SP). For this purpose, gateway cloning strategy and codon-optimization for expression in bacteria are currently in progress for several FMD antigens.

Analog serological experiments will be then performed using FMDV VP1 (serotype O) and sera from naïve, infected or vaccinated animals. Simplex immunoassays will be firstly optimized and then the multiplex immunoassay allowing differential FMD diagnosis will be developed. Simplex luminex based immunoassays will be compared with ELISA.

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DEVELOPMENT OF A SIMPLE ELISA KIT FOR FMDV ANTIGEN DETECTION AND SEROTYPING

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ABSTRACT

A simple ELISA kit has been developed and evaluated for the diagnosis of FMDV types O, A, C and Asia 1. The stabilized kit was designed using selected MAbs coated onto ELISA plates as catching antibodies and a unique pan-FMDV MAb (1F10) as a detector conjugate. The kit also includes an additional pan-FMDV test (using the 1F10 MAb both for coating and conjugation), to complement the specific typing and to detect virus isolates which might escape binding to the selected type-specific MAb. The stabilised kit was kept as simple as possible incorporating antigen controls already trapped onto the plate. Type-specific catching MAbs with the broadest intra-typic reactivity for each of the four FMDV serotypes were selected after analyses of available panels of MAbs with a wide spectrum of FMDV isolates, namely 130 MAbs for type O, 108 for type A, 53 for Asia 1 and 33 for type C. Only a minority of strains, in general old historical viruses no longer circulating, were missed by the selected MAbs.

Diagnostic performances of the prototype kit were analysed using 298 epithelial suspensions representative of the antigenic and molecular variation within each of the FMDV serotypes. Overall, the results proved evidence that the diagnostic performances of the new ready-to-use kit are similar or better (for type A) than those of the more complex polyclonal ELISA. The kit is simple, rapid and stable; maximum simplification was achieved with the use of a unique pan-FMD detector MAb combined with type-specific catching MAbs. FMDV antigenic diversity is sufficiently covered by the selected MAbs.

INTRODUCTION

Sensitive, rapid and accurate diagnosis is an important element of the measures adopted to control foot-and-mouth disease (FMD). Despite advance in molecular tests characterised by high sensitivity, the antigen detection ELISA still affords an effective and rapid diagnosis on clinical samples and remains the test of choice for the identification (serotyping) of field virus isolates after amplification in cell cultures. The worldwide used method consists of an indirect sandwich ELISA employing polyclonal immune sera as both capture (rabbit) and detecting (guinea pig) antibodies (Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988). The test recognizes FMDVs of wide antigenic and molecular diversity but suffers from poor specificity, which often leads to difficulties in test interpretation for defining the causative FMDV serotype. Moreover, the reproducibility of batches of antisera, that is essential for quality assured diagnostics, cannot be guaranteed.

Appropriate combinations of capture and detecting monoclonal antibodies (MAbs) may be used instead of polyclonal antisera, providing benefits in assay specificity and standardization; a simple sandwich ELISA based on these tools has been extensively used in Italy for the diagnosis of FMD outbreaks of type O, A and C which occurred during the 1980's (Brocchi et al., 1986) and in 1993.

A further need for improvement regards assay simplification and accessibility for FMD laboratories in developing countries. Kits for antigen detection based on polyclonal antisera and the in-house assay have for long been made available by the IAH, Pirbright. Furthermore, rapid tests based on lateral flow (LF) chromatography have been validated (Ferris et al., 2009, Ferris et al., 2010) and have also recently become commercially available; they prove useful either when used as a field test or as a rapid laboratory tool to complement the panel of in-house FMD diagnostics (Ryan et al., 2008). However, the currently available LF devices do not permit FMDV serotyping except for FMDVs of type SAT 2.

In order to overcome shortcomings of assay complexity associated with the former kit and deficiency in serotype coverage, this work describes the development of a simple, ready-to-use ELISA kit using selected MAbs for the diagnosis and serotyping of FMDV serotypes O, A, Asia 1 and C. Common properties of such kits are their robustness, with limited assay steps and user-friendly; these properties allow FMD diagnosis to be performed in laboratories with simple equipment and are also strategic for endemic countries, particularly for implementation of the Progressive Control Pathway (http://www.fao.org/ag/againfo/commissions/en/eufmd/pcp.html# and FAO 2009). In addition, these kits may also represent a valuable resource for emergencies, for example they could be part of a reagents bank.

MATERIALS AND METHODS

Viruses/Samples

A selection of 324 FMDV cell culture isolates of type O, A, C and Asia 1 that originated from cases of FMD which occurred around the world from the 1940s to 2010 were used for the preliminary selection of type-specific MAbs with the best intra-type reactivity (Table 1). A further 18 isolates of each of the three FMDV SAT serotypes were also examined to exclude heterologous reactivity.

Epithelial suspensions from 322 FMD suspect cases submitted to the FAO World Reference Laboratory for FMD and confirmed positive by VI and/or PCR for one of the different FMDV serotypes or swine vesicular disease virus (SVDV), as detailed in Table 2, were evaluated by the ready-to-use kit developed for FMDV antigen and serotype detection. The samples originated from different animal species and were representative of different regions, time periods and of the variety of genotypes/topotypes and antigenic diversity within each serotype. In addition, 14 negative samples and 119 FMDV isolates grown in cell cultures were included in the evaluation.

Virus detection tests

The majority of tests for virus detection were undertaken at the time of sample receipt and encompassed the following procedures. Virus isolation was performed using primary calf thyroid cells and a permanent cell line of IB-RS-2 cells; indirect sandwich ELISAs for FMDV, SVDV and VSV were used to characterize the specificity of the virus serotype in original material and cell culture antigens derived from them (Roeder and Le Blanc Smith, 1987; Ferris and Dawson, 1988; Ferris and Donaldson, 1988); in addition, real-time RT-PCR procedures (King et al., 2006; Shaw et al., 2007) were undertaken on samples received post-August 2001 (such assays had yet to be implemented and used routinely for diagnosis prior to this date).

Monoclonal Antibodies (MAbs)

A large number (several hundred) of type-specific MAbs to FMDV strains of serotypes O, A, C and Asia 1, produced by similar methods to those described by Brocchi et al., 1986, were initially screened by ELISA for their reactivity against strains of homologous and heterologous serotypes of FMDV. Consequently, the following MAbs were chosen for inclusion in the ELISA kit: 3B11 for type O (raised against O_1 Manisa), 4D12 (A Iran 96) and 5F6 (A_{24} Cruzeiro) for type A, 3E9 (C Brescia 64) for type C and 3D8 (Asia 1 NEP 29/97) for type Asia 1, in addition to a previously described pan-FMDV MAb 1F10 (virus strain?; Nordengrahn et al., 2008, Ferris et al., 2009).

MAb profiling ELISAs

Two trapping ELISAs were used for the evaluation of the MAb intra- and inter-type reactivity, and which differed between the capture ligand used to bind the FMDV isolates (supernatant of infected cells) onto the solid-phase: one used type-specific rabbit antiserum (Samuel et al., 1991), while the other one used a recombinant αvβ6 integrin, pan-reactive to FMDVs of all seven serotypes (Ferris et al., 2005). Essentially, each MAb was reacted with pre-titrated concentrations of viruses which had been trapped by the capture ligand; the reactivity of field isolates with each MAb was then measured in relation to the reaction of the parental strain.

ELISA kit for FMDV detection and serotyping

Microplates were prepared pre-coated with the selected catching MAbs. On two of the rows of each plate, one positive inactivated control antigen for each of the FMDV types O, A, C and Asia 1 and a negative control were additionally pre-coated (trapped by the respective catching MAb or by the pan-FMDV MAb) while the remainder of the plate allowed for 6 test samples to be examined.

Test samples (epithelium suspensions or vesicle fluids, pre-diluted 1/2 in diluent buffer) were incubated in duplicate or triplicate with each coated MAb. After washing, a unique pan-FMDV MAb (1F10), peroxidase-conjugated (detector), was added. After incubation and washing the reaction was developed by distribution of the substrate/chromogen solution (TMB) and stopped after 20 min at room temperature by adding the stop solution (sulphuric acid 0.6 M).

The positive controls were calibrated to yield optical density (OD) values of 1.5 units or higher in the type-specific reactions and in the pan-FMDV reaction, while the negative control usually developed OD values lower than 0.1 in all wells. OD readings ≥0.1 were regarded as positive.

Fifty µl volumes were used throughout the procedure, samples and conjugate incubations were carried out at room temperature for 1 h and plate washings were performed with three cycles of PBS-Tween 0.05%, pH 7.4.

RESULTS AND DISCUSSION

Test design and kit stabilization

The principle of the test developed for FMDV antigen detection and serotyping is that of a simple sandwich ELISA based on two MAbs, one coated onto the solid-phase as virus catching antibody, the second conjugated with peroxidase as detector antibody. To keep the execution as simple as possible, the kit was designed with type-specific catching antibody for each of the four serotypes, O, A, Asia 1 and C, combined with a unique conjugate, corresponding to the pan-FMDV MAb 1F10. These combinations enable detection and specific typing of the four FMDV serotypes. In addition, the kit also includes a pan-FMDV test, obtained using the MAb 1F10 both for coating and detection, to complement the specific typing and to detect virus isolates which might escape binding to the selected type-specific MAbs.

Since ready-to-use kits normally provide pre-coated solid-phases, ELISA microplates sensitized with the different catching MAbs were submitted to a stabilisation process: this consisted of treatment with stabilising buffers based on sugars and proteins, vacuum drying and thermo-sealing in alufoil envelops. The process prevents deterioration by long storage, humidity, oxidation and light.

The peroxidase-conjugated MAb was prepared at a 10-fold concentration by dilution in a stabilizing fluid; other chemical solutions, i.e. diluents, TMB chromogen and blocking solution were prepared and included ready-to-use.

In addition, for maximum simplicity, FMDV antigen controls were already incorporated and trapped onto plates by the relevant MAb and minimizes the likelihood that the test operator may make mistakes with test operation. The kit remained stable at 5°C for over one year.

Selection of appropriate monoclonal antibodies

An accurate selection of catching MAbs is crucial for the diagnostic performance of the kit; a fundamental criterion for selection is the capability to cover the antigenic diversity within each serotype, achievable with catching MAbs displaying broad intra-typic reactivity.

The selection of appropriate catching MAbs was achieved as a consequence of the following two steps. Firstly, data available from previous studies conducted to characterize the available panels of type-specific MAbs, including the analysis of a restricted spectrum of FMDV isolates, were used to select a subset of MAbs , for each of the four serotype considered, with broad intra-typic reactivity. For example, for type A approximately 150

MAbs raised against six reference strains were analyzed with a spectrum of about 20 FMDV different isolates of type A (Brocchi et al., 2008). Secondly, these pre-selected MAbs were next analyzed against a wider range of isolates, representative of the existent antigenic and molecular diversity within each of the serotypes evaluated. The specificity of MAbs was also cross-checked by analyzing each MAb against heterologous viruses, including several isolates of the SAT serotypes.

The results of these studies are summarized in Table 1 and led to the final selection of one or two MAbs for each serotype which were subsequently used for the production of prototype kits for antigen detection ELISA. For FMDV type A a spectrum of 130 isolates from the 1940s to 2010 was analyzed. Of three MAbs showing the broadest reactivity, 4D12 detected the greatest number of isolates, i.e. 122/130. The eight missed isolates were very old and mainly from South America, with the exception of one recent isolate originating from Kenya in 2008. This MAb was then selected for inclusion in the kit. A second candidate MAb, 5F6, detected less viruses in total, i.e. 113/130; however, since the two MAbs did not miss the same but different isolates, with the exception of three very old strains, the two MAbs were used in parallel for the initial validation. Studies are in progress to check the synergy of pooling the two MAbs together.

For type O, three MAbs recognized 105/108 isolates collected during last 60 years (from 1950 to the present) and among them MAb 3B11 was preferred because of a better antigen capture capacity.
For the serotype Asia 1, a unique MAb was found that identified all the 53 isolates (from 1954 to 2009) tested.
For type C, more MAbs showed a similar profile, detecting all but one isolate of the 33 examined and one of them, 3E9, was selected for incorporation into the kit.

Table 1. Studies of intra-typic reactivity for the selection of best appropriate catching MAbs

FMDV Serotype	N. isolates tested	Period covered		Final selection of catching Monoclonal Antibodies						
Type A	**130**	**1943 2010**	**MAbs**	**4D12**	5F6	1C11				
			N. isolates reactive	**122**	113	112				
Type O	**108**	**1950 2010**	**MAbs**	**3B11**	A8	7E1	3C8			
			N. isolates reactive	**105**	105	105	81			
Type Asia 1	**53**	**1954 2009**	**MAbs**	**3D8**	1F10	4G6	3C6	5E10		
			N. isolates reactive	**53**	51	48	43	35		
Type C	**33**	**'60s 2005**	**MAbs**	**3E9**	2B1	5C4	4D7	2E5	3E5	4C4
			N. isolates reactive	**32**	32	32	31	30	27	27

Evaluation of diagnostic performance of the prototype ELISA kits

The diagnostic performance of the new ELISA kit for antigen detection was compared with those of the classic polyclonal ELISA for the analysis of FMDV positive epithelial suspensions from 318 FMD and four SVD cases (Table 2), as well as 14 negative samples and 119 FMDV isolates grown in cell cultures; however, the two tests were not carried out simultaneously: the original value obtained by the polyclonal ELISA usually performed at time of sample receipt was used for comparison.

Table 2. Samples (epithelial suspensions) positive by VI/PCR used for evaluation of the ELISA kit

FMDV type	O	A	C	Asia 1	SAT 1	SAT 3	SAT 3	SVDV
No. tested	136	103	29	30	9	8	3	4

Diagnostic sensitivity

Figure 1 summarizes the results observed with 136 epithelial suspensions positive for FMDV type O. The scatter plots show the relationship between OD values recorded with the polyclonal ELISA and the prototype ELISA kit; for each sample analyzed the kit provides two values, one observed with the type O specific MAb 3B11 and another one recorded with the pan-FMDV MAb 1F10.

A direct correlation between the scores from the two MAb-based ELISAs (Figure 1, scatter plot below) is evident, reflecting the amount of antigen present in the sample at the time of testing. A general agreement in terms of positive/negative results was obtained also between the polyclonal test and the type O MAb-based kit, but the correlation between OD scores was not always good (scatter plot above). As a result, 115 samples were concordantly positive and six concordantly negative in the two assays, giving an 89% concordance. Fourteen samples were missed by the type O MAb-based ELISA, however 12 of them were also negative with the pan-FMDV test, suggesting that detectable antigen was no longer available in these samples at the time of testing using the kit. Therefore, any failure of reaction may be attributed to deterioration of antigen rather than to an incapability of the type O MAb to recognize these isolates; supported by the finding that these same isolates were reactive after amplification in cell cultures (results not shown). Only two very recent samples, both originating in Ecuador in June 2010, were indeed not recognized by the type O MAb, while being positive with the pan-FMDV test. Interestingly, these strains belonging to the EURO-SA topotype were barely detected even by the polyclonal ELISA but were recognized by another type O MAb (3C8, McCullough et al., 1987) raised against an old European type O vaccine strain. The diagnostic sensitivity of the MAb-based kit for FMDV type O was 85%, but with evidence that it was underestimated compared to 95% of the polyclonal test, for the reasons discussed above.

Figure 2 shows the results achieved from testing 103 epithelial suspensions positive for FMDV type A. As observed for the serotype O, the correlation between the two MAb-based ELISAs of the new kit, performed with the type specific and the pan-FMDV MAb respectively, was almost perfect, suggesting again a direct relationship between OD scores and the quantity of antigen present in the samples. In contrast, the correlation between the polyclonal and the type A MAb-based ELISA was poor providing evidence of a significantly better sensitivity of the MAb-based ELISA, both in regard to the intensity of the OD values and for the overall number of samples detected. In fact, 29 samples were scored positive by the MAb-ELISA and negative in the polyclonal test, with a resulting sensitivity of 40% for the polyclonal-ELISA as opposed to 65% for the MAb-based assay, when compared to a combination of VI and PCR. It would thus appear that the type A MAb (4D12) used in the kit, being directed against a very conserved epitope, covers the great antigenic diversity within isolates of serotype A better than the polyclonal immune sera. In spite of this, three samples were missed by the type A specific MAb-based ELISA: two of them, just borderline in the polyclonal ELISA, were undetectable also by the pan-FMDV test but did react positively after amplification in cell cultures. However a third sample from Kenya 2008, detected by the pan-FMDV MAb-ELISA as well as by the polyclonal-ELISA, did not react in the type A MAb-ELISA, even after amplification in cell cultures. Interestingly this isolate was clearly recognized by the second candidate catching MAb for type A (5F6), and for this reason studies to evaluate the synergy of the two MAbs used together or in tandem have been undertaken.

Figure 3 shows the results observed with 30 epithelial suspensions positive for FMDV serotype Asia 1. Again, a direct correlation between the results of the two MAb-based ELISAs (Asia 1-specific and pan-FMDV) provided in the kit was observed, as well as a general agreement, with 86% concordance, between the Asia 1 monoclonal and polyclonal tests, although the OD values were not always directly correlated. The 2x2 table gives evidence of only four discordant samples that were not detected by the MAb-based kit; however, these samples were borderline in both tests, with OD values just below or above the threshold respectively. Inclusion of these borderline samples, in which FMDV antigen might have been deteriorated when tested by the new kit, adversely affected the estimate of sensitivity for the MAbs-based ELISA 73% compared to 86% for the polyclonal test.

Finally, the results observed with 29 epithelial suspensions positive for type C (Figure 4) showed a similar correlation both between the polyclonal and monoclonal ELISAs and between the two MAbs-based ELISAs performed with the pan-FMDV and the type C-specific MAb respectively. Only one sample behaved discordantly, not being detected by the type specific MAb-based test. This sample did react with the pan-FMDV ELISA, so that it was recognized as FMDV even if not typed.

By collating the results from tests on all the 298 samples, each positive for one of the four serotypes O, A, C and Asia1, the overall sensitivity was in favour of the new test kit, with sensitivity of 75% and 76% for the type-specific and the pan-FMDV test respectively, compared to 73% of the polyclonal ELISA.

Assay specificity

In regard to analytical and diagnostic specificity, estimates were derived from cross-testing of all the above samples by each serotype-specific test. It was found that the samples were all negative in heterologous tests except that there was a tendency of some FMDV isolates of type O, mainly as cell cultures grown matrixes, to give cross-reactions, although low and inconsistent, with the type A MAb-ELISA performed with the MAb 4D12 as catcher. Nevertheless, the homologous reaction was clearly higher and cross-binding to the second candidate type A MAb 5F6 did not occur, allowing a correct interpretation of the virus serotype to be made.,.

In addition, the specificity was further evaluated by testing 24 epithelium suspensions, each one positive for SAT 1 or 2 or 3 or SVDV as well as 14 negative samples. All of them were negative.

CONCLUSSION

The results illustrate that the Mab-based ELISA kit is suitable for the antigen detection of FMDV serotypes O, A, C and Asia 1, having a similar or better sensitivity to a conventional polyclonal antigen ELISA. The assay effectively has two test operator stages (prior to assay completion with substrate/chromogen solution and acid stopper) and all assay components are included with the kit making it rapid and simple to use. The kit is also robust. The additional inclusion of a FMDV pan-reactive test provides further insurance that FMDV will be detected if present in samples in sufficient concentration.

ACKNOWLEDGEMENTS

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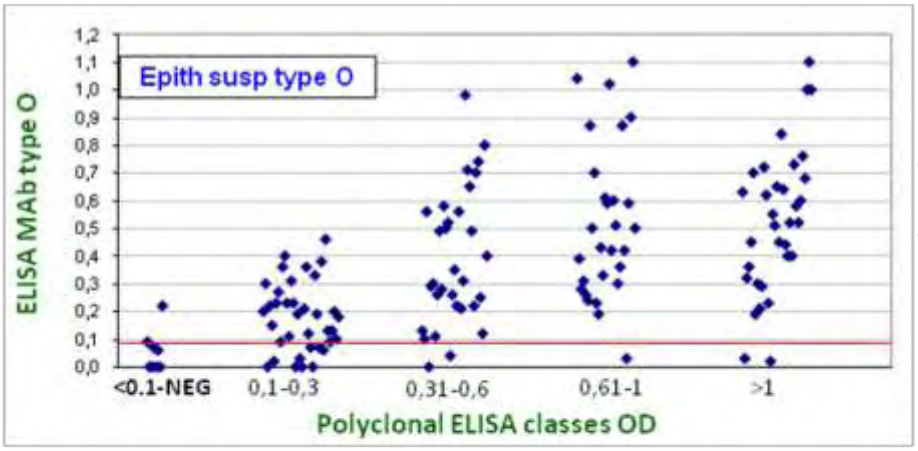
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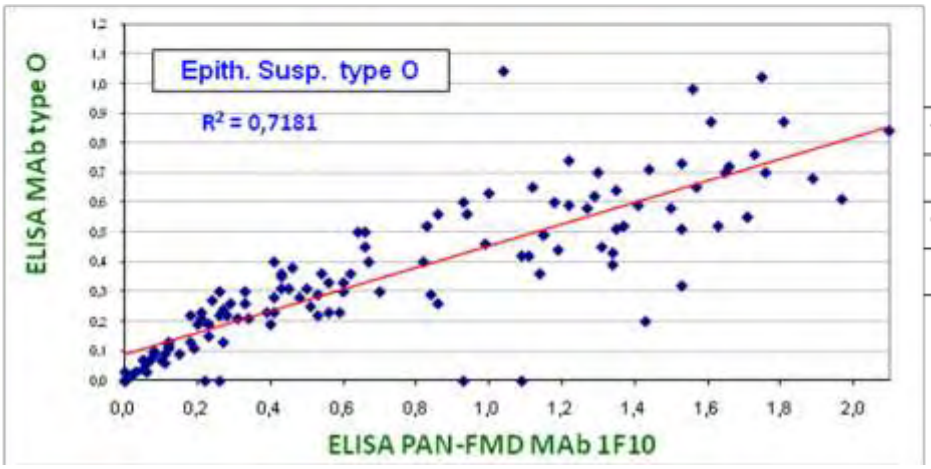
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Figure 1. Results of FMDV Ag detection ELISA - N. 136 epithelial suspensions positive for FMDV type O (y and x axes : OD values)



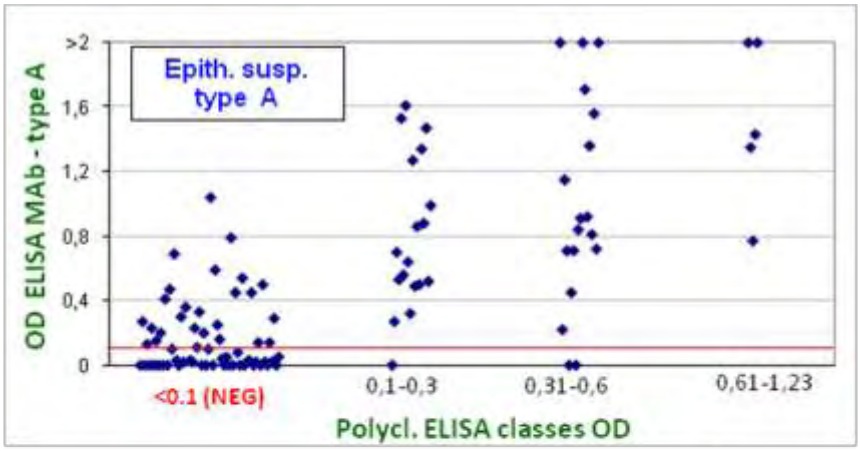
Type O Epith. Susp.		Type O MAb ELISA +	Type O MAb ELISA -	Total
ELISA polyclonal	+	115	14	129
	-	1	6	7
Total		116	20	136



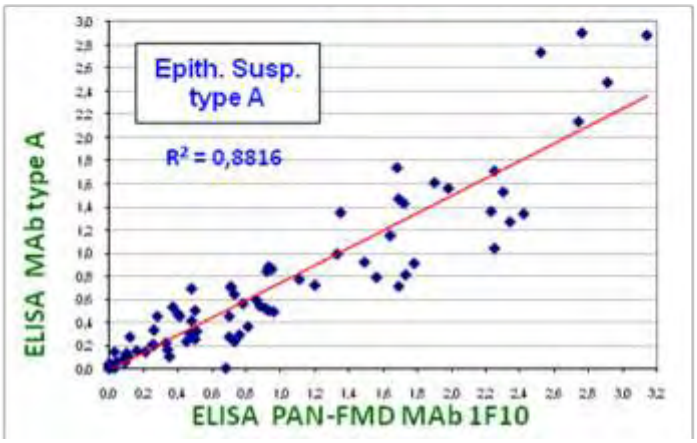
TEST	N.pos/N.tested	Se*
Polyclonal ELISA	129/136	95%
Type O Mab ELISA	116/136	85%
PAN-FMD ELISA	118/136	87%

Se* re. to VI/PCR

Figure 2. Results of FMDV Ag detection ELISA - N. 103 epithelial suspensions positive for FMDV type A (y and x axes : OD values)



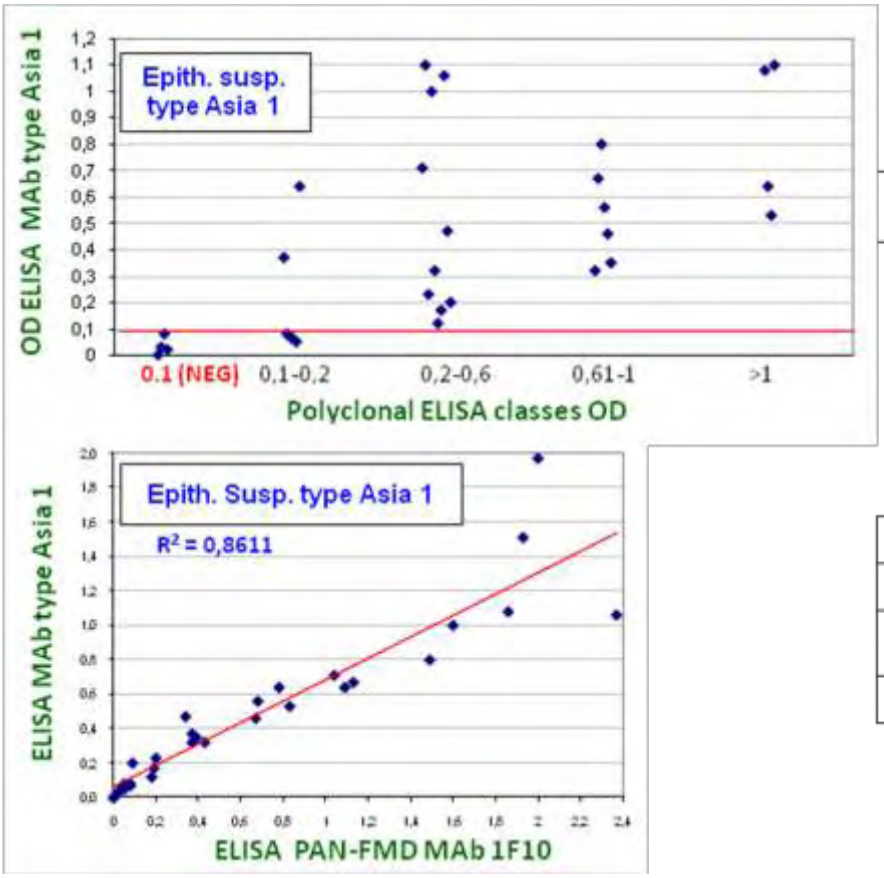
Type A Epith. Susp.		Type A Mab ELISA +	Type A Mab ELISA -	Total
ELISA polyclonal	+	38	3	41
	-	29	33	62
Total		67	36	103



TEST	N.pos/N.tested	Se*
Polyclonal ELISA	41/103	40%
Type A Mab ELISA	67/103	65%
PAN-FMD ELISA	67/103	65%

Se* re. to VI/PCR

Figure 3. Results of FMDV Ag detection ELISA kit - N. 30 epithelial suspensions positive for FMDV type Asia 1 (y and x axes : OD values)

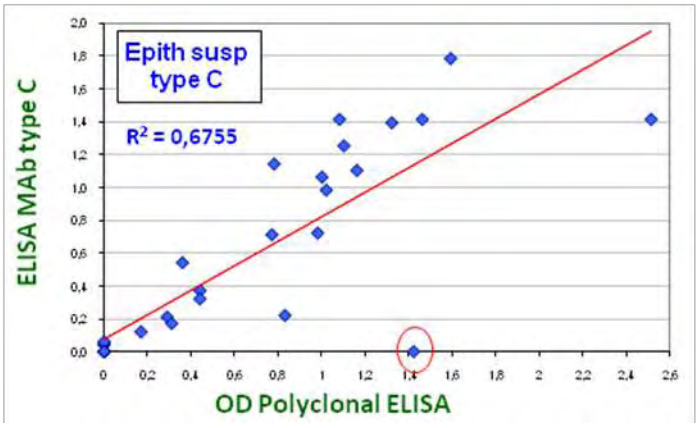


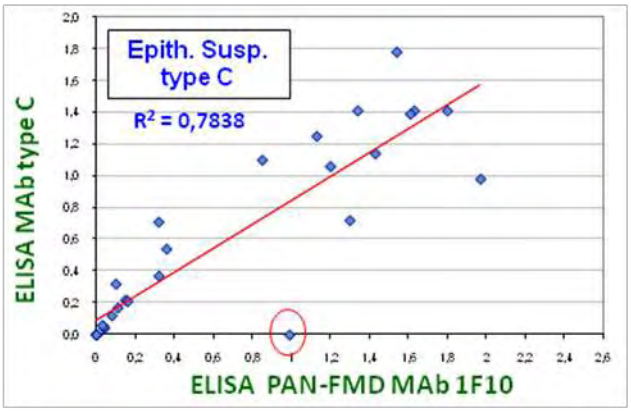
Type Asia 1 Epith. Susp.		Type Asia 1 MAb ELISA +	Type Asia 1 MAb ELISA -	Total
ELISA polyclonal	+	22	4	26
	-	0	4	4
Total		22	8	30

TEST	N.pos/N.tested	Se*
Polyclonal ELISA	26/30	86%
Type Asia 1 MAb ELISA	22/30	73%
PAN-FMD ELISA	21/30	70%

Se* re. to VI/PCR

Figure 4. Results of FMDV Ag detection ELISA kit - N. 29 epithelial suspensions positive for FMDV type C (y and x axes : OD values)





Type C Epith. Susp.		Type C MAb ELISA +	Type C MAb ELISA -	Total
ELISA polyclonal	+	19	1	20
	-	0	9	9
Total		19	10	29

TEST	N.pos/N.tested	Se*
Polyclonal ELISA	20/29	69%
Type C MAb ELISA	19/29	66%
PAN-FMD ELISA	20/29	69%

OVERVIEW OF THE FMD AND SVD COMBINED PROFICIENCY SCHEME STUDIES 2009 (FAO PHASE XXII)

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ABSTRACT

The aim of this exercise was to complete a proficiency testing study (PTS) for virological and serological diagnosis for FMD and SVD in 2009 as one of the annual PTS organised jointly by WRL FMD and European reference laboratories for FMD and SVD to assist National FMD Laboratories in developing and maintaining accurate and reproducible FMD diagnostic tests. This presentation is focused on the overview of the exercise. For FMD the priority serotypes were type O. This study was focused on the detection of and the differentiation between FMD and SVD.

Four panels including infectious materials, BEI inactivated materials, FMD serology and SVD serology with different hypothetical cases were made available to participants. Participating labs were invited to select tests and interpret results as if these samples were from suspected vesicular disease infection cases. Panels were dispatched to participants on request along with a template for results and a set of instructions. The participants were requested to interpret the results for each sample and an overall interpretation for each case. They were also asked to provide information on their tests to evaluate the homogeneity of methods and to enable some analysis of possible causes for discrepant results.

Test results have been submitted from participating laboratories and they are being decoded and collated. The laboratory performance for each panel was analysed. Most or all of laboratories (81%, 80% and 100% for panel 1, panel 3 and panel 4, respectively) have met the criteria preset for all tests from a panel except for panel 2, only 46% of laboratories has fully met the preset criteria.

INTRODUCTION

This study was to complete a combined proficiency testing study (PTS) for virological and serological diagnosis for FMD and SVD in 2009 as one of the annual PTS organised jointly by the FAO FMD World Reference Laboratory (WRL) for FMD and the European Reference Laboratories (EURL) for FMD and SVD in the Pirbright laboratory of the Institute for Animal Health (IAH-P). The aim of the exercise was to continue the assistance to National Reference Laboratories (NRL) of Member States of the European Commission for the Control of FMD (EUFMD) and elsewhere in developing and maintenance accurate and reproducible FMD diagnostic tests, also in harmonisation testing between different laboratories to enable the comparison of the results generated from different reference laboratories. The WRL has conducted a series of proficient testing studies for FMD diagnosis known as the "Phase" studies (Paton *et al*., 2002, 2003; Li *et al.* 2006; Li et al. 2008). The current study focused on the detection of FMD and SVD by both virological and serological diagnosis.

This study consisted with the hypothetical cases for infectious materials and serology panels to incorporate the situation of a suspected vesicular disease. The priority for FMD serotypes were O and SAT 2. Particular tests were not specified, but labs were invited to select tests and interpret

results as if the samples were from suspected vesicular disease cases (according to specification given with the panel).

MATERIALS AND METHODS

Four panels including infectious materials and non-infectious materials for virological and serological testing for FMD and SVD were prepared. All samples from each panel have been tested 10 times independently by tests to be evaluated to gain the confidence on sample status and to establish the criteria for assessment of the laboratory performance. Each sample is uniquely coded and labelled before dispatching.

The samples in each panel were detailed in Table 2. The description for panels was as the following:

Panel 1: infectious material from 2 cases of suspected vesicular disease for virus detection
Case 1a: 4 epithelial suspension samples from cattle in a herd affected with a vesicular condition.
Case 1b: 2 epithelial and 2 faecal suspension samples from pigs in a herd affected with a vesicular condition.

Panel 2: 10 double BEI treated non-infectious samples from cattle or pigs, with each originating from a different case of a herd with a vesicular condition.

Panel 3: BEI treated non-infectious material for FMD serology
Case 3a: 4 bovine sera from a suspected FMDV infection case in UK. The cattle showed no vaccination history.
Case 3b: 4 bovine sera from a suspected FMDV infection case in Africa. The cattle were vaccinated against FMDV O1 Manisa and SAT 2 Eritrea.

Panel 4: 8 BEI treated sera from pigs from a suspected SVDV historical infection case.

An invitation letter for Phase XXII was sent to the potential participants in early 2009. Panels were dispatched to laboratories on request along with a template for result reporting and a set of instructions. The participants were requested to interpret the results for each sample and an overall interpretation for each case. They were also asked to provide information on their tests to evaluate the homogeneity of methods and to enable some analysis of possible causes for discrepant results. On receipt, the results were sent back to the participant for their confirmation and then decoded. The results from all participants for each assay from each panel have been collated and analysed.

RESULTS

A total of 71 laboratories from all EU member state countries, EUFMD but not EU countries, and countries in other regions were invited to take part in Phase XXI studies. Out of them, 45 agreed to participate in this study with 57.8% from EU and 42.2% from Non-EU countries. Up to the completion of the study, 1, 4, 3 and 1 lab haven't sent their results back to WRL/EURL for panel 1, 2, 3 and 4, respectively (Table 1).

Participants tested the panel 1 and 2 by virus isolation, Antigen (serotyping ELISA) and/or RT-PCR; Antigen ELISA and/or RT-PCR, respectively. Tests used for testing panel 3 included FMD NSP ELISA with either commercial available kit or in house ELISA, PrioCHECK type O ELISA (Prionics

Lelystad B.V.), LPBE, SPCE and VNT. SVD serology panel 4 were tested by ELISA including 5B7 MAC ELISA, PrioCHECK SVD ELISA (Prionics Lelystad B.V.), Isotype ELISA and in-house ELISA and VNT.

The analysis of laboratory performance for each panel was categorised into four groups: meet criteria; miss sample; false positive and mistyping. Cross serotype reactivity was also analysed for panel 2 as correct serotyping was the essential criteria for this panel. As shown in Figure 1-4, 81% (17 out of 21 labs), 46% (17 out of 37 labs), 80% (32 out of 40 labs) and 100% (34 out of 34 labs) of participating laboratories have met the criteria preset for all tests from panel 1, panel 2, panel 3 and panel 4, respectively.

For FMD serology panel 3, 38 out of 40 labs tested the panel by NSP ELISA. VNT appeared to be the most serotype specific, followed by SPCE. LPBE has given the most cross-serotype activities (Figure 5).

DISCUSSION AND CONCLUSIONS

The PTS 2009 (Phase XXII) for FMD and SVD was focused on the detection of and differentiation between FMD and SVD. An advisory board was constituted with four members: Dr Kris De Clercq (Belgium), Dr Aldo Dekker (Netherland), Dr Emiliana Brocchi (Italy) and Dr Bernd Hass (Germany). Participants have reached the maximum number since 1977 (Phase I) until 2009 (Phase XXII) (Figure 6). As shown in Figure 1-4, most of the laboratories have met the pre-set criteria for all panels. However, some labs still needed to improve the performance of their tests. Laboratories testing panel 4 were all provided correct results and have met the pre-set criteria.

For panel 1, 4 and 3 labs needed to improve the sensitivity of cell cultures and the sensitivity of FMD PCR, respectively. 1 lab needed to improve the specificity of SVD PCR.

The cross-serotype reactivity appeared to be the main fault for panel 2. 11 out of 37 labs have mis-serotyped one or more samples. 6 labs needed to improve the sensitivity or specificity of FMD PCR. 1 lab hasn't tested the panel for FMDV. 5 labs haven't tested the panel for the presence of SVDV.

For panel 3, 7out of 40 labs were not able to pick SAT 2 samples possibly due to the lack of SAT tests in the lab. 2 laboratories have only tested the antibodies against non-structural proteins. 6 labs haven't tested the panel for non-type O antibodies. 5 laboratories needed to improve the sensitivity and/or the specificity for VNT and/or LPBE or NSP ELISA, respectively.

In conclusion, 45 labs participated in PTS 2009. In general, most labs showed a good level of consistency for all tests; 81%, 46%, 80% and 100% of labs meet the criteria preset for all tests from panel 1, panel 2, panel 3 and panel 4, respectively. 4 labs have received the panels they requested but have not reported their results. 5 labs have not tested all samples in a panel.

RECOMMENDATIONS

- Improvement the specificity of the serotyping ELISA
- Establishment of the tests to detect the antibodies against all serotypes or non-type O FMDV structural proteins for laboratories which haven't got these tests currently available.
- Establishment of the tests to type or to detect antibodies against FMDV SAT serotypes for laboratories which haven't got these tests currently available.

ACKNOWLEDGEMENTS

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Table 1. Participants and results receiving from panels for PTS 2009 (Phase XXII)

Phase XXII (country/lab)	**EU**	**Non-EU**
Country invited	26/27	42/44
No. of lab participated	25/26	18/19
No. of labs	**Received**	**Reported results**
Panel 1	22	21
Panel 2	41	37
Panel 3	43	40
Panel 4	35	34

Table 2. PTS 2009 (Phase XXII) samples from four panels

Panel 1	**Case**	**P1a-1**	**P1a-2**	**P1a-3**	**P1a-4**		**P1b-1**	**P1b-1**	**P1b-1**	**P1b-1**	
	Sample	**1**	**2**	**3**	**4**		**5**	**6**	**7**	**8**	
		O UKG 685/2007	O UKG 685/2007	Neg epi	Neg		SVDV faeces	Neg faeces	SVDV epi	Neg epi	
Panel 2	**Case**	**P2a-1**	**P2a-2**	**P2a-3**	**P2a-4**	**P2a-5**	**P2a-6**	**P2b-1**	**P2b-2**	**P2b-3**	**P2b-4**
	Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
		O IRN 34/2007	A IRN 37/2007	Asia 1 HKN 3/2005	SAT 2 BOT 2/2007	Neg	Neg	SVDV ITL 9/2008	SVDV ITL 9/2008	Neg	Neg
Panel 3	**Case**	**P3a-1**	**P3a-2**	**P3a-3**	**P3a-4**		**P3b-1**	**P3b-2**	**P3b-3**	**P3b-4**	
	Sample	**1**	**2**	**3**	**4**		**5**	**6**	**7**	**8**	
		NBS	NBS	NBS	NBS		O1 Manisa Vaccinated and challenged	O1 Manisa Vaccinated only	SAT 2 Eritrea Vaccinated and challenged	SAT 2 Eritrea Vaccinated only	
Panel 4	**Sample**	**1**	**2**	**3**	**4**		**5**	**6**	**7**	**8**	
		Invitrogen NPS	SO11 7dpi	Sigma NPS	PAA NPS		VO52 22dpi	SL98 6dpi	Local Abbatoir NPS	SL82 9dpi	

epi: epithelium
dpi; days post infection.
NBS: Normal bovine serum.
NPS: Normal pig serum.
Neg: Negative.

Figure 1. Analysis of laboratory performance from PTS 2009 (Phase XXII) panel 1

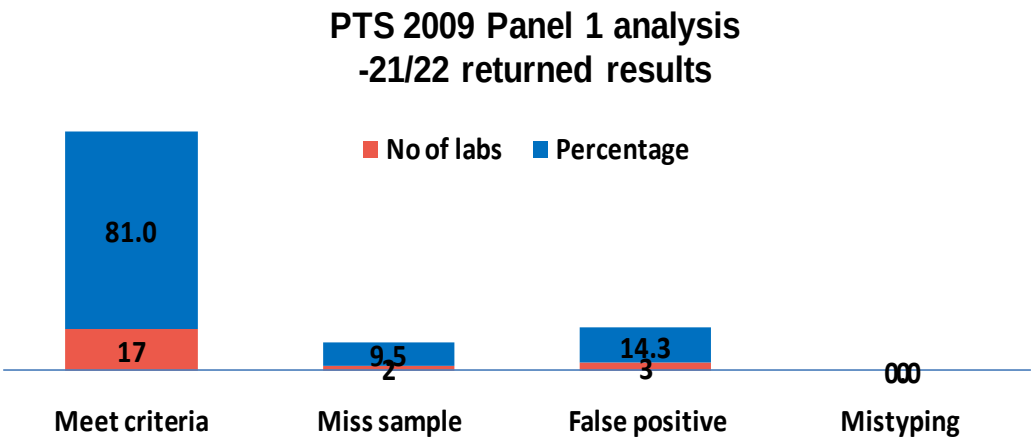


Figure 2. Analysis of laboratory performance from PTS 2009 (Phase XXII) panel 2

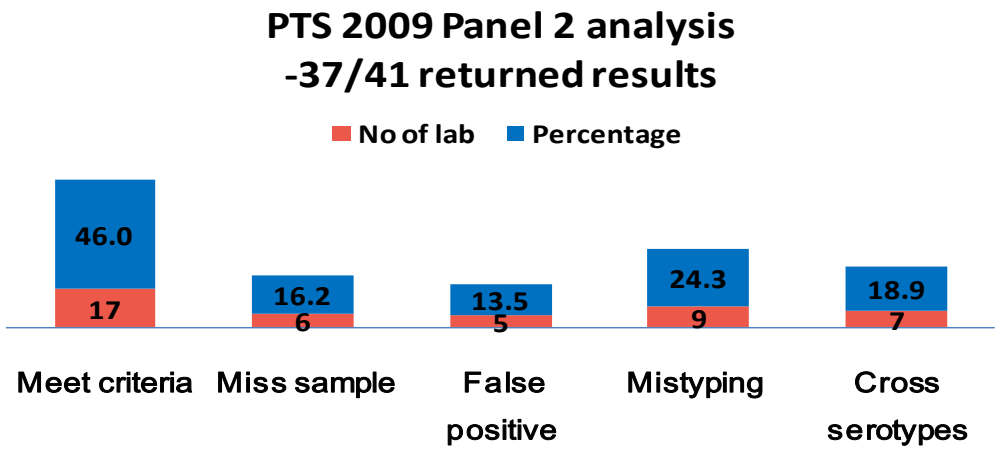


Figure 3. Analysis of laboratory performance from PTS 2009 (Phase XXII) panel 3

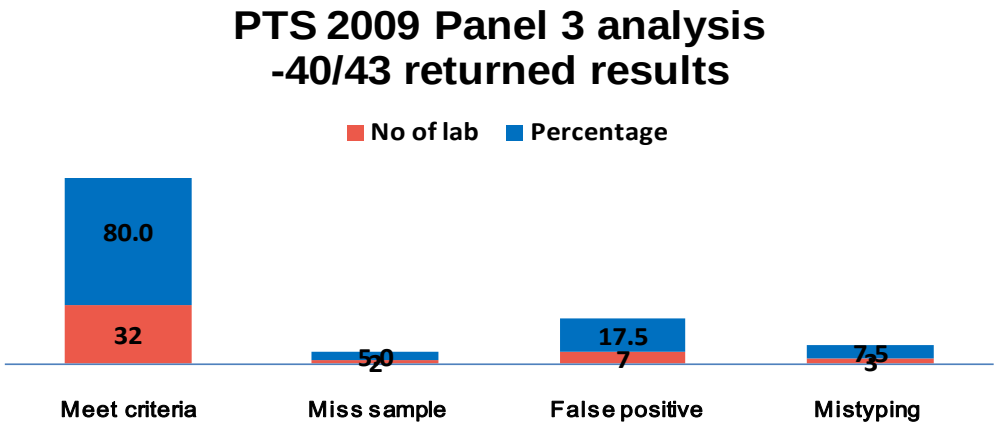


Figure 4. Analysis of laboratory performance from PTS 2009 (Phase XXII) panel 4

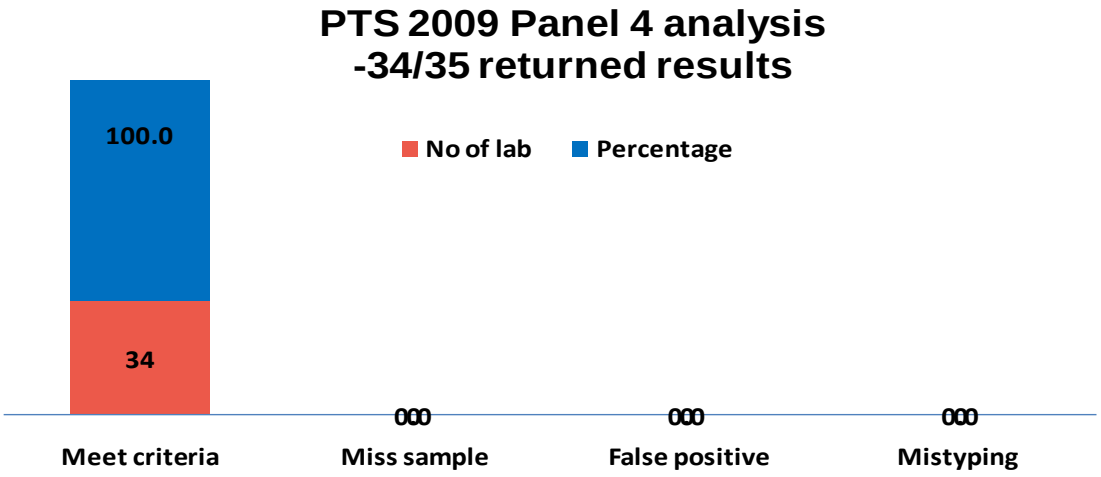


Figure 5. Analysis of cross serotype reactivity from VNT, SPCE and LPBE for PTS 2009 (Phase XXII) FMD serology panel 3

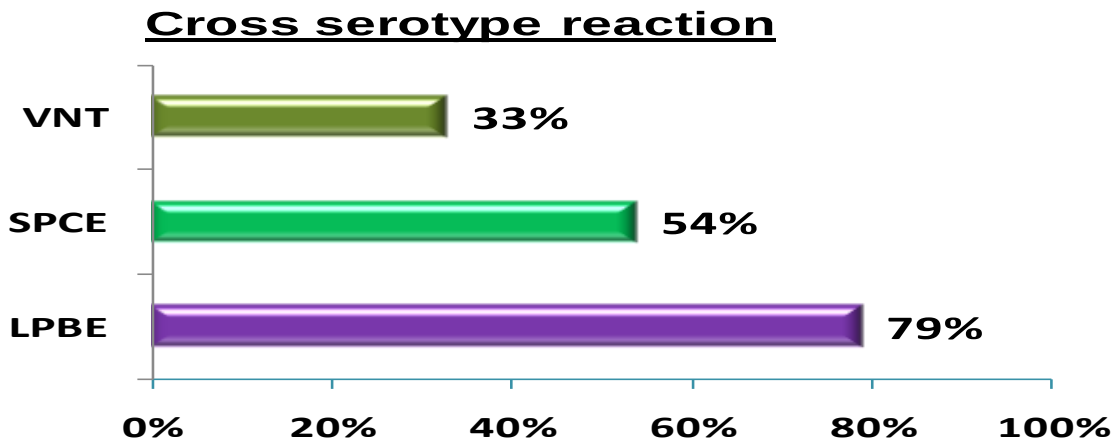
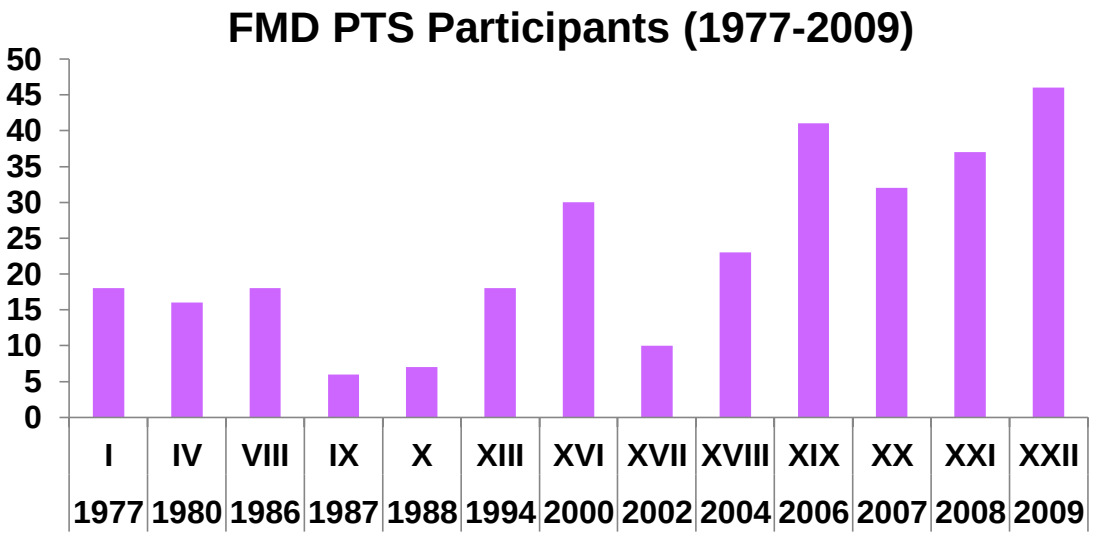


Figure 6. Participants for WRL/EURL FMD PTS FMD from Phase I to Phase XXII



A MOLECULAR BIOLOGICAL STUDY ON RECENT SUDANESE FOOT AND MOUTH DISEASE VIRUS ISOLATES

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SUMMARY

The aim of this study was to characterise foot-and-mouth disease (FMD) viruses collected between 2004 and 2008 from Sudan. Using virus isolation and antigen ELISA, three FMD virus serotypes (O, A and SAT 2) were detected in 24 samples that were submitted to the FAO World Reference Laboratory for FMD. Pan-serotypic real-time RT-PCR assays targeting the 5' untranslated region (5'UTR) and 3D genes of FMD virus were also used to contribute to the laboratory diagnosis of these cases. Phylogenetic analyses of VP1 sequences from these viruses were used to determine the relationships with 23 older viruses from Sudan and other viruses from West and East Africa. For serotype O, there was close genetic relation between concurrent and historical Sudanese isolates. A similar pattern was also evident for serotype A and SAT2 viruses. These findings provide the first insight into FMD viruses that are circulating in Sudan.

INTRODUCTION

Foot-and-mouth disease virus (FMDV; family *Picornaviridae,* genus: *Aphthovirus*) exists as seven serotypes and causes a highly contagious disease of ruminants and swine. FMD is endemic in most of the African continent where serotypes O, A, SAT1 and SAT2 predominate (Kitching, 1998; Rweyemamu et al., 2001). FMD is endemic in Sudan (Eisa and Rweyemamu, 1977, Abu Elzein, 1983). Recently, three serotypes (O, A and SAT 2) of FMD virus (FMDV) have been threatening the livestock in the country (Habiela et al 2008). Phylogenetic analysis is widely used to characterize FMDV strains and nucleotide sequencing data is an important tool in tracing the source of outbreaks. This study was carried out to define the genetic relationships between FMD viruses isolated recently from outbreaks in the Sudan and to compare these viruses to others collected from sub-Saharan Africa.

MATERIALS AND METHODS

Clinical samples

This study made use of (47) FMDV samples collected from Sudan over the period 1974-2008 which had been sent to the WRLFMD Pirbright for virological investigation. Twenty four of these samples were collected from recent FMD outbreaks in Sudan (2004-2008). (Table 1). Additional FMDV-positive samples (*n* = 23) that had been tested and stored from earlier Sudanese outbreaks (1974-1999) were also included for comparative purposes in this study (Table 2).

FMD virus detection assays

Samples were tested using VI (primary bovine thyroid cultures: BTY and a renal swine cell line: IB-RS-2: De Castro et al., 1964) and antigen-detection ELISA (Ferris and Dawson, 1988). Recent samples (>2004) were also tested at submission by one-step real-time RT-PCRs targeting the 5' UTR and 3D regions of the FMDV genome (Shaw et al., 2007; Callahan et al., 2002) using an automated robot (MagNA Pure LC, Roche) to extract total nucleic acids. Where available, older archived material was retrospectively tested using these real-time RT-PCR assays.

Sequencing of 5' untranslated region (5' UTR) targeted by real-time RT-PCR

Sequence analysis of the 5' UTR target for O/SUD/2008 isolates (n=3) was undertaken using a previously described method (King et al., 2006). Briefly, for each sample, a 542 bp fragment (spanning nucleotides 581-1123 of AY593823) encompassing the diagnostic real-time RT-PCR target (corresponding to nucleotides 898-994 of AY593823) was amplified by RT-PCR with primers FOR [5'-CGT CHG CGC ACG AAA CGC-3'] and REV [5'-RCG ATR AAR CAG TCR GTY R-3']. After post-PCR removal of unincorporated nucleotides and primers (GFX PCR purification Kit, GE Healthcare) these RT-PCR products were sequenced (ABI 3730) on both strands using the primers used for PCR amplification (see above).

Table 1: FMDV samples included in this study (2004-2008)

Sample ID (WRL code)		Location, State	Species	Sample type	Collection Date	Virus isolation		Serotype (Ag-ELISA)	Real-time RT-PCR (C_T)		VP1 sequence accession number
						BTY	RS		5'UTR	3D	
1	SUD/1/2004	Warab	Cattle	EPI	5/9/04	POS	POS	O	21.39	12.39	GU566045
	SUD/3/2004	Warab	Cattle	EPI	5/9/04	POS	POS	O	24.67	12.73	GU566046
	SUD/4/2004	Warab	Cattle	Serum	5/9/04	POS	-	O	-	-	GU566047
	SUD/9/2004	Warab	Cattle	EPI	5/9/04	POS	NVD[a]	O	23.25	14.65	GU566048
	SUD/12/2004	Warab	Cattle	EPI	5/9/04	POS	NVD	O	27.72	12.17	GU566049
	SUD/14/2004	Warab	Cattle	EPI	5/9/04	POS	POS	O	25.85	12.37	GU566050
	SUD/15/2004	Warab	Cattle	Serum	5/9/04	POS	-	O	-	-	GU566051
	SUD/16/2004	Warab	Cattle	EPI	5/9/04	POS	POS	O	24.55	8.21	GU566052
2	SUD/25/2004	Warab	Cattle	EPI	7/9/04	POS	NVD	O	23.49	10.18	GU566053
	SUD/26/2004	Warab	Cattle	Serum	7/9/04	POS	-	O	-	-	GU566054
	SUD/30/2004	Warab	Cattle	Serum	7/9/04	POS	-	O	-	-	GU566055
3	SUD/1/2005	South Darfur	Cattle	EPI	3/1/05	POS	POS	O	24.29	12.30	GU566056
	SUD/2/2005	South Darfur	Cattle	Probang	3/1/05	POS	POS	O	No Ct	13.21	GU566057
4	SUD/3/2005	North Darfur	Cattle	EPI	9/1/05	POS	POS	O	No Ct	16.43	GU566058
5	SUD/1/2006	Kasala State	Cattle	EPI	9/4/06	POS	POS	A	22.99	22.27	GU566069
6	SUD/3/2006	Gezira state	Cattle	EPI	26/4/06	POS	POS	A	26.11	20.11	GU566070
7	SUD/1/2007	Khartoum	Cattle	EPI	21/1/07	POS	NVD	SAT 2	33.05	17.84	GU566071
8	SUD/1/2008	Gezira state	Cattle	EPI	4/2/08	POS	POS	SAT 2	16.48	14.01	GU566072
	SUD/2/2008	Gezira state	Cattle	EPI	4/2/08	POS	POS	SAT 2	14.37	12.05	GU566073
9	SUD/3/2008	White Nile State	Cattle	EPI	11/2/08	POS	NVD	O	27.19	14.96	GU566059
	SUD/4/2008	White Nile State	Cattle	EPI	11/2/08	POS	POS	O	25.97	12.71	GU566060
10	SUD/5/2008	White Nile State	-	EPI	11/2/08	POS	POS	O	25.40	16.42	GU566061
	SUD/6/2008	White Nile State	Cattle	EPI	11/2/08	POS	NVD	O	No Ct	20.35	GU566062
11	SUD/8/2008	Gezira state	Cattle	EPI	9/5/08	POS	NVD	O	22.13	11.58	GU566063

- Data not collected or diagnostic test not undertaken

[a] NVD – no virus detected

VP1 RT-PCR and sequencing

Samples were submitted for VP1 sequence analysis using a one-step RT-PCR method as previously described (Knowles et al., 2009). Oligonucleotide primers used for PCR amplification: For serotype O, forward primers were either O-1C244F [5'-GCA GCA AAA CAC ATG TCA AAC ACC TT-3'] or O-1C272F [5'-TBG CRG GNC TYG CCC AGT ACT AC-3'] and reverse primer EUR-2B52R [5'-GAC ATG TCC TCC TGC ATC TGG TTG AT-3']. Primers used for serotype A were either A-1C562F [5'-TAC CAA ATT ACA CAC GGG AA-3'] or A-1C612F [5'-TAG CGC CGG CAA AGA CTT TGA-3' as a forward primer, with EUR-2B52R as a reverse primer. Primers used for serotype SAT 2 RT-PCR used either SAT2-1C445F [5'-TGG GAC ACM GGI YTG AAC TC-3'] or SAT2-P1-1223F [5'-TGA ACT ACC ACT TCA TGT ACA CAG-3'] as a forward primer and SAT-2B208R [5-ACA GCG GCC ATG CAC GAC AG-3'] as a reverse primer. For serotype A, amplification conditions were as previous described (Knowles et al., 2009) while the annealing temperatures of 50°C and 60°C were used for SAT 2 and O viruses respectively. RT-PCR products were sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using the following oligonucleotides: NK72 [5'-GAA GGG CCC AGG GTT GGA CTC-3'] for all serotypes; O-1D296F [5'-ACA ACA CCA CCA ACC CAA C-3'] and O-1D628R [5'-GTT GGG TTG GTG GTG TTG T-3'] for serotype O; A-1C612F [5'-TAG CGC CGG CAA AGA CTT TGA-3'] for serotype A, and SAT2-D [5'-GGT GCG CCG TTG GGT TGC CA-3'] and SAT2-1C513aF [5'-CAC ACA CAC AGA CAC ACC CGC GAT GGC-3'] for SAT 2.

Table 2: Additional archived FMDV samples from Sudan (1974-1999) included in the study

Sample ID (WRL code)	Location, State	Species	Sample type	Collection Date	Virus isolation		Serotype (Ag-ELISA)	Real-time RT-PCR (C_T)		VP1 sequence accession number
					BTY	RS		5'UTR	3D	
SUD/5/74	Upper Nile	-	CC	1974	POS	-	O	-	-	AY344602[a]
SUD/3/77	Kadaro, Khartoum	-	-	1977	-	-	A	-	-	GU566064
SUD/6/77	-	-	-	1977	-	-	SAT 2	-	-	AY343939
SUD/9/77	-	-	-	1977	-	-	SAT 2	-	-	AY442014
SUD/1/76	Khartoum	-	EPI	2/1976	POS	NVD[b]	O	15.18	14.59	AY344599[a]
SUD/2/80	-	-	-	1980	POS	-	O	-	-	AY344608[a]
SUD/1/81	Blue Nile	Cattle	EPI	1981	POS	-	A	-	-	GU566065
SUD/1/82	-	Cattle	EPI	30/8/82	POS	-	A	25.26	24.45	GU566066
SUD/2/83	-	-	-	1983	POS	-	O	-	-	AY344607[a]
SUD/3/83	-	-	-	1983	POS	-	O	-	-	GU566036
SUD/2/84	Khartoum	Cattle	EPI	4/11/84	POS	-	A	28.38	16.86	GU566067
SUD/1/85	Soba, Al Jazirah	Cattle	EPI	6/12/84	POS	-	A	20.21	15.90	GU566068
SUD/2/86	Khartoum	Cattle	EPI	7/11/86	POS	POS	O	13.51	10.09	DQ165075
SUD/2/89	Soba, Al Jazirah	-	-	1989	POS	NVD	O	-	-	GU566037
SUD/3/89	Khartoum	-	EPI	20/11/87	POS	NVD	O	16.53	16.40	GU566038
SUD/4/89	Soba, Al Jazirah	-	-	5/12/87	POS	NVD	O	-	-	GU566039
SUD/5/89	Khartoum	-	EPI	13/12/87	POS	POS	O	14.95	13.85	GU566040
SUD/6/89	Khartoum	-	EPI	26/1/88	POS	NVD	O	28.01	24.29	GU566041
SUD/7/89	Gerawid, Blue Nile	-	EPI	13/3/89	POS	POS	O	17.22	15.19	GU566042
SUD/1/99	-	-	EPI	1999	POS	NVD	O	30.98	21.55	DQ165076
SUD/3/99	-	-	-	1999	POS	NVD	O	-	-	GU566043
SUD/4/99	-	-	-	1999	POS	NVD	O	-	-	GU566044
SUD/5/99	-	-	-	1999	POS	NVD	O	-	-	AY344609[a]

- Data not collected or diagnostic test not undertaken

[a] Partial VP1 sequence available

[b] NVD – no virus detected

Phylogenetic analysis

Sequences (generated using ABI 3730) were assembled using SeqMan (DNAStar Lasergene 8.0) and complete VP1 nucleotide sequences were aligned using BioEdit 7.0.5.3 (Hall, 1999) and Clustal W 1.83 (Thompson et al., 1994). These alignments were used to construct distance matrices using the Kimura 2-parameter nucleotide substitution model (Kimura, 1980) as implemented in the programme MEGA 4.0 (Tamura et al., 2007). Midpoint-rooted Neighbor-joining trees were then constructed using MEGA 4.0. The robustness of the tree topology was assessed with 1000 bootstrap replicates as implemented in the program.

Individual FMD viruses were classified into geographically restricted clusters, also known as topotypes, as previously described (Knowles and Samuel, 2003; Vosloo et al., 2004)

RESULTS

Nineteen out of twenty four of samples were typed using Ag-ELISA as serotype O (Table 1), while the remainder of the viruses comprised either serotype SAT2 (*n* = 3) or serotype A (*n* = 2). Using a positive cut-off C_T value of 32.0 for real-time RT-PCR(Shaw et al., 2007), all of the samples were positive using the 3D assay, while 5' UTR Assay generated poorer signal in 3 serotype O samples, and a further serotype SAT 2 sample gave a signal below the designated assay C_T cut-off used at the WRLFMD. Subsequent sequence analysis of the 5' UTR target for the serotype O viruses revealed 4 nucleotide mismatches: three with the forward primer (at positions 12, 20 and 22) and one with the probe (at position 15). Further serotype O viruses (from 2008, 2005, 2004 as well as 1999) sharing close VP1 genetic relationships (see below in Figure 1), showed similar discrepancy in the C_T values between the 5' UTR and 3D real-time RT-PCR assays.

Phylogenetic analysis of serotype O viruses

The 19 recent serotype O isolates from Sudan were all classified as belonging to the EAST AFRICA-3 (EA-3) topotype (Figure 1). Within this topotype, the Sudanese FMD viruses form a lineage that is distinct from recently characterised viruses from Ethiopia (Ayelet et al., 2009). With the exception of a single isolate (O/SUD/3/83) that was related to a virus from Nigeria (WEST AFRICA [WA] topotype), all of the archived FMD isolates from Sudan were also characterised within this genetic sub-lineage of the EA-3 topotype.

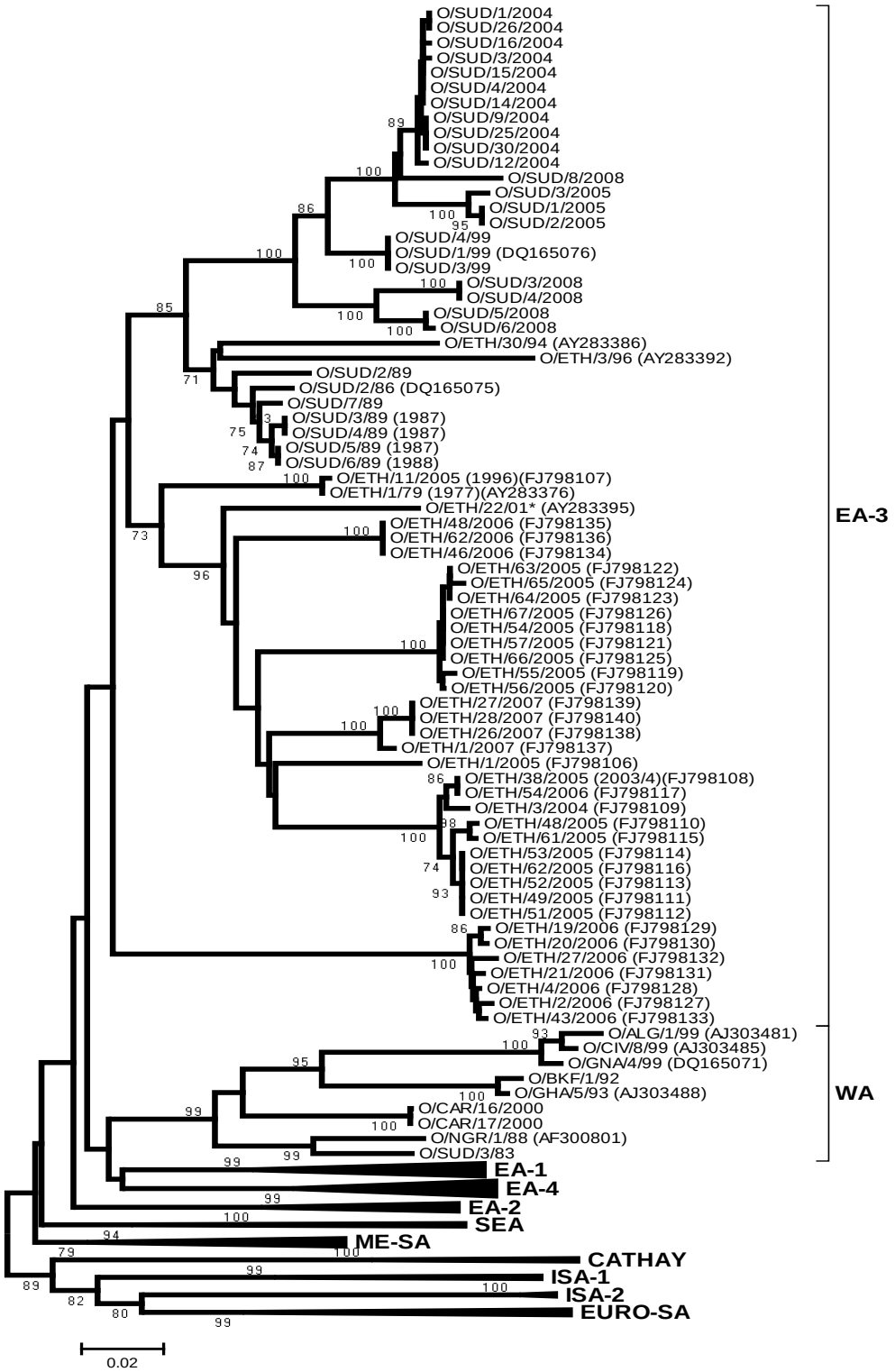


Fig. 1. Midpoint-rooted neighbour-joining tree showing the relationships between the serotype O viruses collected from Sudan. Extent of serotype O topotypes (EA-3 and WA) is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. *Reference number not assigned by the World Reference Laboratory for Foot-and-Mouth disease.

Phylogenetic analysis of serotype A viruses

The two serotype A samples (A/SUD/1/2006 and A/SUD3/2006) fell within the G-IV genotype of the AFRICA topotype (figure 2). This group also contained most of the older FMDV isolates from the country and an isolate (A/CAR/15/2000) that was sampled from Cameroon. A single isolate collected in 1982 (A/SUD/1/82) did not cluster with these viruses and was characterised as a member of the G-VII genotype of the AFRICA topotype.

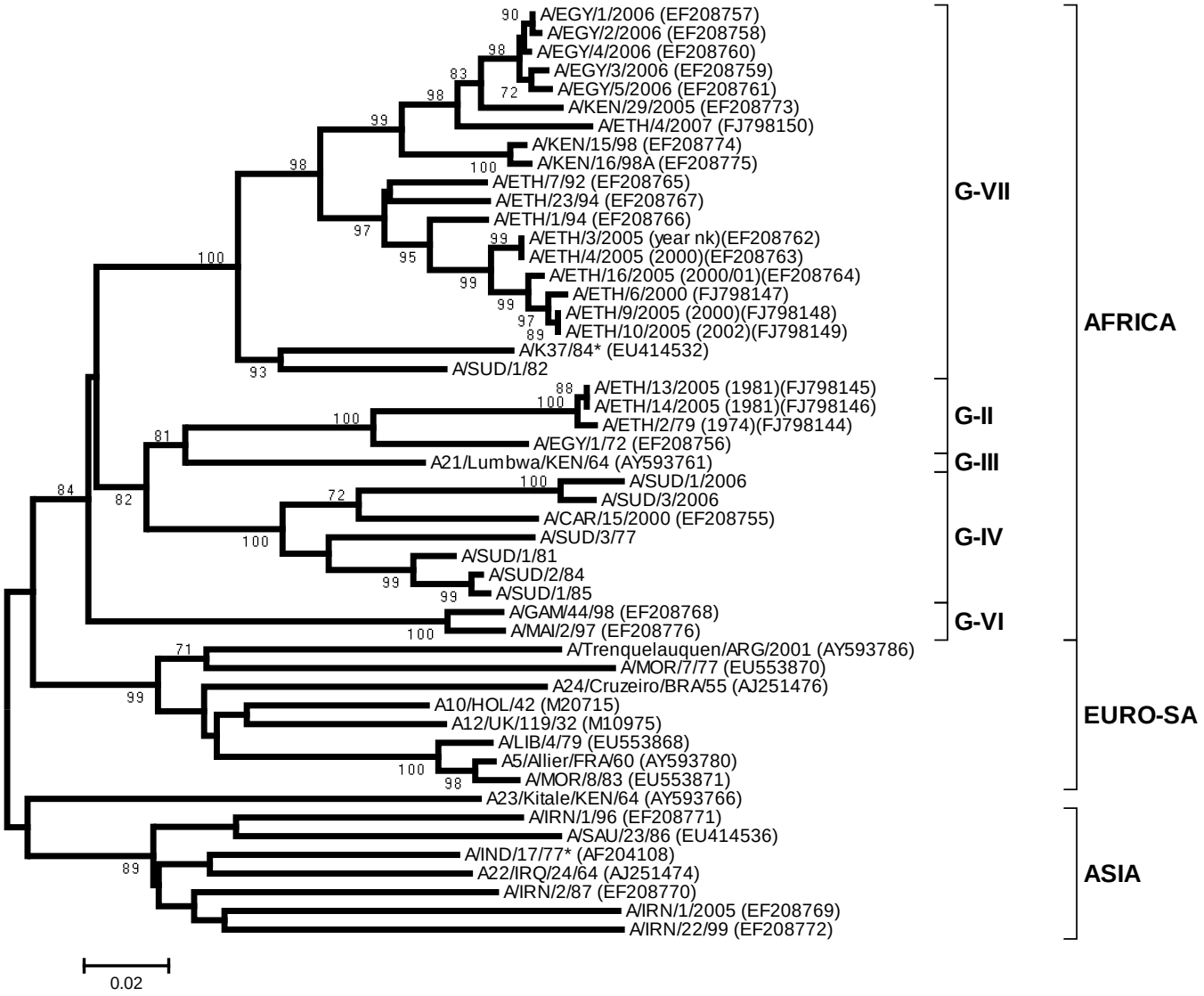


Fig. 2. Midpoint-rooted neighbour-joining tree showing the relationships between the serotype A viruses collected from Sudan. Extent of serotype A topotypes (AFRICA, EURO-SA and ASIA) and proposed African genotypes are shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. *Reference number not assigned by the World Reference Laboratory for Foot-and-Mouth disease.

Phylogenetic analysis of serotype SAT 2 viruses

FMD SAT 2 viruses have been previously divided into 14 topotypes (Bastos et al., 2003; Sangare et al., 2004; Vosloo et al., 2004). The phylogenetic analysis for Sudanese SAT 2 viruses is shown in Figure 3. The two isolates collected during 2008 (SAT2/SUD/1/2008 and SAT2/SUD/2/2008) were closely related to each other and to a SAT 2 virus (SAT2/ETH/2/2007) recovered from an FMD outbreak in Ethiopia in 2007 (respective nucleotide identities of 98.3% and 98.5%). These viruses were grouped together with older viruses collected during 1977 from Sudan in topotype XIII of SAT 2. A further recent SAT 2 virus from Sudan (SAT2/SUD/1/2007) was grouped in SAT 2 topotype VII. Viruses from this

lineage have also been identified from several other countries in sub-Saharan Africa (Cameroon, Eritrea [Bastos et al., 2003], Nigeria, Niger and Libya) and beyond (Saudi Arabia).

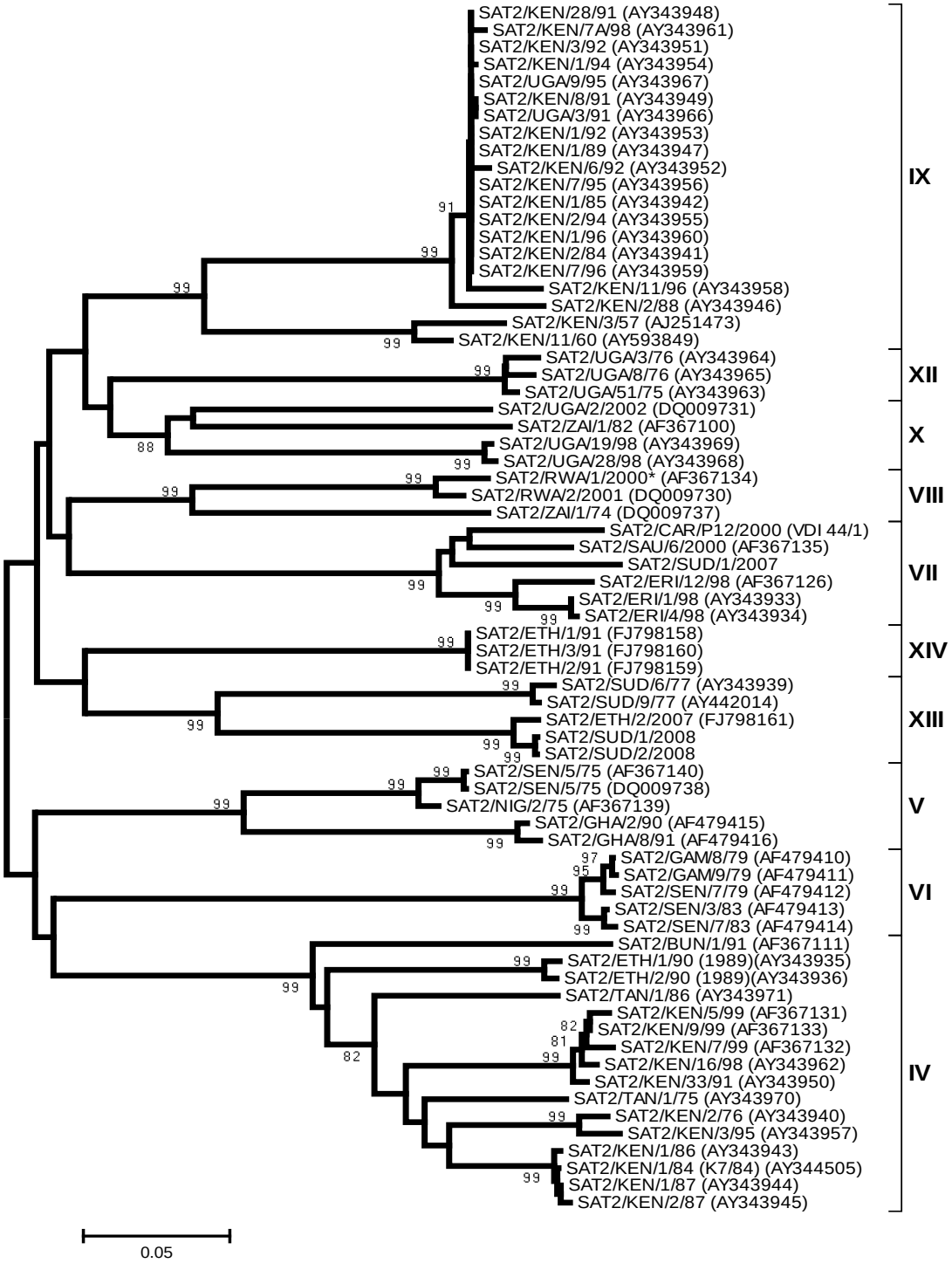


Fig. 3. Midpoint-rooted neighbour-joining tree showing the relationships between the serotype SAT2 viruses collected from Sudan. Extent of proposed SAT2 topotypes are shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. *Reference number not assigned by the World Reference Laboratory for Foot-and-Mouth disease.

DISCUSSION

This report describes the first detailed laboratory analysis of FMD virus isolates collected from Sudan. Since 2004, three FMDV serotypes (O, A and SAT 2) were detected; although the majority of the samples were characterised as serotype O (79 % of these recent isolates).

FMDV was also detected in the samples using real-time RT-PCR assays targeting the 5'UTR and 3D regions of the FMD virus genome. Although the diagnostic sensitivity of these tests is high, this study found examples of FMD viruses that were poorly detected by the 5'UTR assays. This data shows the importance of the continuous review of real-time RT-PCR targets to ensure that the assays used are able to detect circulating FMDV field strains.

From the phylogenetic trees constructed it is possible to infer how FMD viruses might be dispersed between countries in the region. Figure 1 shows that the phylogenetic group containing most of the recent Sudanese viruses also contained two sequences reported to be from older viruses from Ethiopia (O/ETH/30/94 and O/ETH/3/96), suggesting that the ancestral history of this lineage is shared between Ethiopia and Sudan. Furthermore, a single isolate from Nigeria (O/NIG/1/2007) was also a member of this group (data not shown).

The phylogenetic trees for serotype A and SAT 2 isolates highlight close relationships between Sudanese viruses and FMD viruses collected elsewhere in the region suggesting that long-distance animal movement can also contribute to FMD dispersal across sub-Saharan Africa. Previously, close epidemiological links have been reported between SAT 2 viruses in Sudan and Cameroon (Bronsvoort et al., 2004).

However, it is important to recognise that the sampling density of FMD outbreaks in domesticated livestock is generally low across sub-Saharan Africa and there are clear gaps in some countries that neighbour Sudan (Chad, Central African Republic) where FMD outbreaks are rarely reported, beside transhumance and lack of data on FMD in wild animals (mainly buffaloes: *Syncerus caffer aequinoctialis*), therefore, the relationships outlined in the phylogenetic trees should be interpreted with caution since it is likely that there is considerable un-sampled FMDV transmission occurring within both domesticated livestock and wildlife. Further collection and analysis of samples with improved local epidemiological investigation of FMD outbreaks countries in sub-Saharan Africa is required to improve our understanding of the complex epidemiology of FMD in the region.

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DEVELOPMENT OF A FOOT-AND-MOUTH DISEASE INFECTION MODEL IN SEVERE COMBINED IMMUNODEFICIENT MICE FOR THE PRELIMINARY EVALUATION OF ANTIVIRAL DRUGS

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ABSTRACT

Recent European guidelines facilitate the use of emergency vaccines during outbreaks of foot-and-mouth disease (FMD). Antiviral drugs could be used as a complementary measure. The present study aimed at developing a small animal model to assess the *in vivo* activity of early antiviral lead molecules with anti-FMDV activity *in vitro*. In a first attempt, several FMDV strains were titrated in Balb/c mice. Inoculations with O_1 Manisa or C_1 Noville did not induce clinical disease, whereas Asia1 Shamir induced death too rapidly (i.e. within 4 days post inoculation (dpi)). Therefore, we switched to severe combined immunodeficient (SCID) mice which are frequently used as a model for viral infections and experimental therapeutics. Strain O_1 Manisa did not induce clinical disease but titrations with A_{22} Iraq, C_1 Noville or Asia1 Shamir resulted in virus-induced morbidity (including respiratory problems and weight loss) with subsequent mortality. Inoculations with strain A_{22} Iraq resulted in a reproducible mean time of death of 6 dpi (this was shorter for the other strains). In this newly developed rodent model, strain A_{22} Iraq seems the most suited to assess the *in vivo* anti-FMDV activity of selective inhibitors of FMDV.

INTRODUCTION

Foot-and-mouth disease virus (FMDV) is an antigenically diverse virus that exists in 7 serotypes, i.e. O, A, C, Asia1 and the South African Territories (SAT) types 1, 2 and 3 and multiple subtypes (Grubman and Baxt, 2004). FMDV infection of cloven-hoofed animals causes major economic losses worldwide. After the epizootic FMD outbreaks in the UK, Ireland, France and the Netherlands in 2001, in which nearly 4.5 million animals were destroyed (OIE, 2010), European guidelines were amended to facilitate the use of emergency vaccination in case of outbreaks of FMD (Council Directive 2003/85/EC). However, emergency vaccines only confer complete clinical protection 7 days post vaccination (Golde et al., 2005). Moreover, FMD vaccines are serotype and to a lesser extent subtype specific, and thus have to be carefully selected prior to use (Paton et al., 2005). Antiviral drugs could decrease this one week post vaccination immunity gap in a serotype non-specific manner and could be used as an adjunct to emergency FMD vaccination (Goris et al., 2007; Goris et al., 2008). Currently, we are optimizing the in vitro anti-FMDV activity of a promising class of non-nucleoside analogues that were originally identified in a screen against human enteroviruses (Neyts J., unpublished data). Before assessing the efficacy of such viral inhibitors in a target species (e.g. pigs) it would be of great value to be able to evaluate the potential antiviral activity in a small animal model for FMDV infection. Therefore, we aimed at developing an FMD infection model in mice for the Eurasian serotypes O, A, C and Asia1.

MATERIALS AND METHODS

The animal experiments in this study were authorized and supervised by the Ethical Committee of the VAR under reference number 091110-01, according to the Council Directive 86/609/EEC and APPENDIX A to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS NO. 123). In a first series of experiments, 7- to 9-week-old Balb/c mice were intraperitoneally titrated with 10-fold dilutions of FMDV. For strain Asia1 Shamir, mortality was uniformly observed between 3 to 4 days post inoculation (dpi). This is considered to be too fast to assess the antiviral activity of early antiviral leads.

On the other hand, we were not able to induce clinical disease with FMDV strains O_1 Manisa or C_1 Noville in Balb/c mice. Therefore, we aimed at developing an FMDV infection model in severe combined immunodeficient (SCID) mice [3- to 4-week-old; male and female (50/50); mean body weight (B.W.) 13.0 $\pm$ 2.8g]. SCID mice have impaired B- and T-cell responses and are frequently used as a model for viral infections for antiviral drug testing (NIAID, 2010). Due to their crippled adaptive immune response, the % of protected SCID mice can directly be correlated with the activity of the antiviral agent, since survival cannot result from a protective adaptive immunity. Briefly, 10-fold serial dilutions [ranging from $10^{5.0}$ to $10^{0.0}$ cell culture infectious $dose_{50}$ ($CCID_{50}$)] of O_1 Manisa, A_{22} Iraq, C_1 Noville or Asia1 Shamir were inoculated intraperitoneally in 3 to 4 mice per inoculation dose and promising inoculation doses were independently repeated 3 or 4 times. The inoculation doses that did not immediately result in a high proportion of diseased mice were not repeated due to ethical reasons.

RESULTS

All 14 PBS-inoculated and all 18 O_1 Manisa-inoculated mice remained healthy until the end of the trial at 14 dpi and were negative for viral RNA in serum at 2 dpi, as determined with two semi-quantitative real time RT-PCRs (Goris et al., 2009). Infections with A_{22} Iraq, C_1 Noville or Asia1 Shamir evolved quickly into clinical disease and mice were humanly euthanized because of ethical reasons. The results of these experiments are summarized in Table 1. Forty-tree mice were inoculated with A_{22} Iraq, 41 with C_1 Noville and 43 with Asia1 Shamir. The lowest inoculation dose that resulted in 100% mortality for all 3 strains was $10^{5.0}$ $CCID_{50}$ per mouse. Mean times of death or euthanasia were approx. 3 dpi for Asia1 Shamir, approx. 4 dpi for C_1 Noville and approx. 6 dpi for A_{22} Iraq. A graphical representation of the clinical symptoms is given in Figure 1. Hyperacute death at 1 or 2 dpi was observed in 2/41 mice (5%) inoculated with C_1 Noville and in 4/43 mice (9%) inoculated with Asia1 Shamir. These 6 mice were inoculated with $10^{3.0}$ $CCID_{50}$ or higher. Between 2 to 4 dpi, 25 out of 43 A_{22} Iraq-inoculated mice (58%), 25 out of 41 C_1 Noville-inoculated mice (61%) and 29 out of 43 Asia1 Shamir-inoculated mice (67%) were less active or apathic. At that time point, 20 out of 43 A_{22} Iraq-inoculated mice (47%), 15 out of 41 C_1 Noville-inoculated mice (37%) and 25 out of 43 Asia1 Shamir-inoculated mice (58%) had moderate to severe respiratory problems. In these mice, moderate to severe oedema of the lungs was consistently observed. Respiratory problems were observed with every inoculation dose, except $10^{0.0}$ $CCID_{50}$. Twenty-eight mice survived until 5 to 10 dpi: 22/43 A_{22} Iraq-inoculated mice (51%), 4/41 C_1 Noville-inoculated mice (10%) and 2/43 Asia1 Shamir-inoculated mice (5%). In the 28 mice surviving until 5 to 10 dpi, progressive weight loss was the most evident clinical sign. These mice were humanly euthanized once they lost 20% or more of their B.W. None of the 127 FMDV-inoculated mice became ill after 10 dpi and 13 FMDV-inoculated mice survived until the end of the trial at 14 dpi: 7 A_{22} Iraq-inoculated mice and 6 Asia1 Shamir-inoculated mice. Ten of those 13 mice were negative for viral RNA in serum at 2 dpi, but unexpectedly, 3 A_{22} Iraq-inoculated mice had Ct values between 25 and 30. Two of them were inoculated with a dose of $10^{1.0}$ $CCID_{50}$, one with $10^{4.0}$ $CCID_{50}$. Viral RNA was not detected in the organs of the mice that survived. In randomly selected mice that died or were euthanized, viral RNA was systematically detected in the lungs, heart, spleen, liver and kidneys, with Ct values ranging from 20 (very high) to 38 (cut-off).

DISCUSSION

In general, these results confirm that FMDV can quickly induce a severe, generalized disease in mice and that the clinical outcome depends on several factors including age, mouse strain, FMDV serotype and strain (Salguero et al., 2005; Kamstrup et al., 2006). A high inoculation dose seems to be required to ensure that all animals are infected (Salguero et al., 2005; Kamstrup et al., 2006), although the results from the present study suggest that differences may exist between FMDV serotypes and strains. In particular, the present study shows that 3- to 4-week-old SCID mice are much more sensitive to clinical infection with strain A_{22} Iraq than 7- to 10-week-old Balb/c mice (Kamstrup et al., 2006) or C57BL/6 mice (Salguero et al., 2005). Under the conditions of the present study, neither SCID mice nor Balb/c mice were susceptible to clinical infection with O_1 Manisa, the latter confirming previous results from Kamstrup et al. (2006). Further, these experiments suggest that infections with Asia1 Shamir or C_1 Noville in 3- to 4-week-old SCID mice evolve too quickly to allow the evaluation of antiviral drugs against FMDV in the lead optimization phase, even when low inoculation doses are used. Infection of SCID mice with A_{22} Iraq seems to allow adequate time to document an antiviral response against FMDV. The A_{22} Iraq SCID mice model may thus represent a valuable system for the preliminary evaluation of antiviral drugs.

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Table 1
Mortality and viral RNA load in FMDV-inoculated SCID mice.

Virus strain	Inoculum (log_{10} $CCID_{50}$)	Mortality				Cumulative mortality		Mean no. of days until death ± SD[a]	Mean viral RNA load ± SD[a] in serum at 2 dpi	
		Exp. 1	Exp. 2	Exp. 3	Exp. 4	Ratio	%		Ct (5'-UTR)	Ct (3D)
A_{22} Iraq	0	ND [b]								
	1	0 / 3				0 / 3	0	-	> 45	> 45
	2	2 / 3				2 / 3	67	7.0 ± 0.0	27.9 ± 1.2	26.0 ± 2.4
	3	3 / 3	4 / 4	1 / 3	3 / 3	11 / 13	85	5.9 ± 1.5	23.1 ± 3.8	23.5 ± 5.2
	4	2 / 2	3 / 3	3 / 4	3 / 3	11 / 12	92	5.7 ± 1.8	24.9 ± 3.3	23.2 ± 3.2
	5	2 / 2	4 / 4	3 / 3	3 / 3	12 / 12	100	5.6 ± 1.9	22.2 ± 3.4	22.3 ± 3.9
C_1 Noville	0	3 / 3				3 / 3	100	6.0 ± 3.5	40.0[c]	35.9[c]
	1	3 / 3				3 / 3	100	4.7 ± 2.1	39.0[c]	31.8[c]
	2	3 / 3	3 / 3			6 / 6	100	3.8 ± 0.4	36.0 ± 3.9	29.3 ± 4.5
	3	3 / 3	4 / 4	3 / 3		10 / 10	100	3.9 ± 1.3	31.8 ± 3.4	25.5 ± 3.0
	4	3 / 3	4 / 4	3 / 3		10 / 10	100	3.6 ± 0.5	32.2 ± 4.2	25.5 ± 3.9
	5	3 / 3	3 / 3	3 / 3		9 / 9	100	3.4 ± 1.5	30.2 ± 4.6	26.9 ± 6.7
Asia1	0	0 / 3				0 / 3	0	-	> 45	> 45
Shamir	1	3 / 3				3 / 3	100	5.3 ± 4.0	21.7[c]	28.9[c]
	2	1 / 3	2 / 3			3 / 6	50	4.3 ± 2.3	25.6 ± 1.5	30.7 ± 1.9
	3	3 / 3	3 / 3	3 / 3		9 / 9	100	2.1 ± 0.6	18.3 ± 4.7	25.6 ± 2.3
	4	3 / 3	4 / 4	3 / 3		10 / 10	100	2.5 ± 0.7	24.9 ± 1.6	28.9 ± 1.6
	5	3 / 3	3 / 3	3 / 3		9 / 9	100	2.7 ± 0.5	22.0 ± 2.6	28.4 ± 0.6
	6	3 / 3				3 /3	100	3.0 ± 0.0	ND [b]	ND [b]

[a] Results from surviving mice were not taken into account.
[b] ND = not done.
[c] Two of the 3 mice were too small to take blood.

Figure 1
Percentage of animals with clinical symptoms in function of the inoculation dose.

