OPEN SESSIONS OF THE STANDING TECHNICAL AND RESEARCH COMMITTEES OF THE EuFMD COMMISSION

APPLIANCE OF SCIENCE IN THE PROGRESSIVE CONTROL OF FMD

29-31 October 2012
Jerez de la Frontera, Spain
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The 2012 Sessions will bring together people and organizations involved in the areas of FMD control, laboratory services, research and development, predominantly from Europe and its neighbourhood regions, but with important participation from free and endemic countries from across the world. In addition to the two Open Sessions – of the EuFMD Standing Technical and Research Committees - the Annual Network Meeting of the OIE/FAO FMD reference Laboratory Network, and the Meeting of the Global Foot-and-Mouth Disease Research Alliance (GFRA), will use this opportunity to meet.

The Open Sessions, as the name suggests, are Open to all persons having FMD control as a part of their job description. The Standing Technical Committee has responsibility to provide strategic guidance and advice to the EuFMD on technical issues and bring attention to new developments which are important for policy making. In 2011, the Commission established a Special Committee on Research (SCR), with 12 elected members, replacing the former “Research group of the Standing Technical Committee”. The SCR has a role to review the technical developments reported at the Open Session which are important to FMD surveillance and control in the member states and may be commissioned, or propose, studies to the STC. In 2009-12, studies were commissioned on full genome sequencing, epidemiology in wild boar, telemetry and non-invasive sampling of wild boar, NSP and LPBE diagnostics for SAT viruses, serotype specific PCR assays, software for surveillance design, vaccine effectiveness in the field, and a global survey on FMD Research (through GFRA). It is a requirement of these supported studies to report to the Open Sessions.

More epidemiology, more control: since 2002, attendance at Open Session has grown from around 100 to about 250, with a shift from predominantly lab based studies to field based - with over half the papers in 2012 coming from epidemiology and risk management studies. What is behind this? More capacity to partner between north and south institutions? The success of projects linking the field and advanced labs? Better communications and networking? The ideas coming out of Open Sessions and other events that bring people together? The new confidence in endemic regions that comes from applying ideas locally? Or because FMD is becoming recognized in endemic countries as important and preventable?

The Open Sessions have had a history of developing new ideas that are taken up by the international organizations – for example the Erice Session (2008) provided the concept of virus pools needing specific regional programmes, and the “Progressive Control Pathway (PCP-FMD)” developed by EuFMD with FAO as a framework for developing sustainable national programmes. The PCP-FMD has, since 2011, been a joint tool with the OIE, and provides a framework for the Global Strategy for FMD Control launched by FAO and OIE in Bangkok in June 2012. The Strategy has the aim of all endemic countries advancing two PCP Stages in the next 15 years; in other words, at the end of this period all countries will have at the least a control programme protecting their vulnerable sectors. So in the 4 years since Erice, a lot has happened that has lead to a revised system for promoting progressive control. An increased amount of field work is needed as part of the PCP application at national level, and in parallel a lot more basic and applied research is needed in almost all disciplines.

The 2012 Open Session recognizes networking is essential, to develop and spread ideas. It recognizes that science will help us progress FMD control in every part of the affected world, and that what you publish and report at this Conference is part of the process of progressive control – your work is transforming the possibilities.

So bring on the Open Session at Jerez de la Frontera – at the frontiers of FMD science, our ideas should not observe boundaries!

Keith Sumption
Secretary EuFMD
Acknowledgements

The EuFMD Commission gratefully acknowledges the support of the European Commission and the EuFMD Member States, for funding the Committee meetings, Working Groups, and Research Studies. Professor David Paton and Dr Aldo Dekker are thanked for their work as Chairpersons of the Standing Technical and Research Committees, for their ideas and enthusiasm for the Open Sessions and behind the scenes in the work of the Committees.

The Open Session 2012 is made possible through the support of Dr Ulrich Herzog, President of the EuFMD Commission, Dr Alf Füssel (DG SANCO), and our host, Dr Lucio Carbajo Goñi, Marta Cainzos and their team. We would like to acknowledge on your behalf the EuFMD team, and in particular Enrique Anton, who managed and undertook most of the many, major tasks involved with the Jerez Session. The city of Jerez is thanked for its hospitality.

Organization of the 2012 Open Session
Chairman of the Standing Technical Committee:
Professor David Paton

Members of the STC:
Dr Christianne Bruschke
Dr Matthias Kramer
Prof David Paton
Dr Preben Willeberg

Chairman of the Special Committee on Research:
Dr Aldo Dekker

Members of the SCR:
Dr Bernd Haas (Germany)
Dr Emiliana Brocchi (Italy)
Dr Naci Bulut (Turkey)
Dr Stefan Zientara (France)
Dr Labib Bakkali (France)
Dr Jeff Hammond (WRL, Pirbright, UK)
Dr Georgi Georgiev (Bulgaria)
Dr Marisa Arias (Spain)
Dr Eoin Ryan (Ireland)
Dr Graham Belsham (Denmark)
Dr Kris de Clercq (Belgium)
Dr Michel Bellaiche (Israel)

The EuFMD Team in Rome:
Dr Keith Sumption (Secretary)
Dr Eoin Ryan (Animal health officer)
Dr Vesna Milicevic (Animal health officer, STP program)
Dr Dimitrios Dilaveris (Animal health officer, STP program)
Ms Nadia Rumich (Communications officer)
Ms Rossana Cecchi (Operations officer)
Ms Manuela Zingales (Clerk)
Mr Leonardo Leon Perez (Clerk)
Mr Enrique Anton (Manager of the Jerez meeting)
Ms Claudia Ciarlantini and her team (graphic designer)
The generous support of our sponsors has greatly assisted to reduce the costs of the event, enabling us to widen participation, and is greatly appreciated.

And special thanks to our Hosts and Local Organiser:

![Sponsors logos]
Day 1 :: Monday 29 October

**PLENARY ROOM**

**Open Session of the Standing Technical Committee (STC): Focus on issues affecting FMD control in FMD free regions**

**SESSION I. OPENING**

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<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>08:15 – 08:45</td>
<td>Welcoming/Opening remarks. Government of Spain (Marm), FAO, EuFMD</td>
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<tr>
<td>08:45 – 09:15</td>
<td>Frenkel lecture: Frenkel’s legacy: What is keeping us? (P.W. de Leeuw)</td>
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<tr>
<td>09:15 – 09:45</td>
<td>Global surveillance – European neighbourhood and global FMD situation (J. Hammond)</td>
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<tr>
<td>09:45 – 10:00</td>
<td>Minister of Agricultura, Alimentación y Medio Ambiente (M. Arias Cañete)</td>
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<tr>
<td>10:00 – 10:30</td>
<td>Coffee/Tea break</td>
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**PLENARY SESSION**

**SESSION II. FOCUS ON ISSUES AFFECTING FMD CONTROL IN FMD FREE REGIONS**

What should the waiting periods be for reinstatement of FMD-free status after vaccination-to-live?

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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>10:30 – 10:55</td>
<td>Aligning waiting periods for vaccinate to-Live &amp; vaccinate-to-die (D. Geale)</td>
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<tr>
<td>10:55 – 11:20</td>
<td>A quantitative approach to determining waiting periods for fmd freedom. (A. Cameron)</td>
</tr>
<tr>
<td>11:20 – 11:30</td>
<td>Evaluation of the benefits and feasibility of a vaccination-to-live strategy in fmd free countries (D. Hadorn)</td>
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European Wild boar and their role in disease transmission: Lessons learnt and policy implications

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>11:30 – 12:00</td>
<td>FMD in wild boar and policy implications (K. Depner)</td>
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<tr>
<td>12:00 – 12:30</td>
<td>FMD in wild boar: can the virus be maintained in wildlife? Experiences and consequences from Thrace (S. Khomenko)</td>
</tr>
<tr>
<td>12:30 – 12:40</td>
<td>Discussion</td>
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<td>12:40 – 13:40</td>
<td>Lunch break</td>
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</table>

**SESSION III. FOCUS ON ISSUES AFFECTING FMD CONTROL IN FMD FREE REGIONS**

<table>
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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>13:40 – 14:10</td>
<td>Fmd lab bio-risk management: what have we learnt from application of the 2009 minimumum standards? (B. Haas)</td>
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</table>

**Approaches to the evaluation of FMD emergency management options and control measures in Europe**

<table>
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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>14:10 – 14:40</td>
<td>Economic evaluation of fmd management options: Implications for science and policy (R. Bergevoet)</td>
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<tr>
<td>14:40 – 15:10</td>
<td>Simple decision tools informed by model predictions when considering fmd emergency vaccination strategies (P. Willeberg)</td>
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<tr>
<td>15:10 – 15:20</td>
<td>Panel discussion: With speakers and other invited panelists, to discuss ideas of where the work should go</td>
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<tr>
<td>15:20 – 15:50</td>
<td>Coffee/Tea break</td>
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(cont. on page viii)
PARALLEL SESSION
Research Group (RG): Immunology and Vaccine Research

SESSION P1. IMMUNOLOGY AND VACCINE RESEARCH 1

11:15 – 11:30  **Keynote:** The cellular innate immune response during acute infection of cattle and swine with FMDV (W. Golde)
11:30 – 11:45  Adaptive immune responses in the respiratory tract of fmd-vaccinated cattle after oronasal infection (M. Pérez Filgueira)
11:45 – 12:00  Characterization of opsonizing antibodies against fmd Virus (A. Summerfield)
12:00 – 12:15  Whole 140s FMDV particles are needed to elicit specific cellular immunity in vivo and to stimulate recall responses *in vitro* (A.V. Capozzo)
12:15 – 12:30  Intraserotype Chimeric Foot-and-Mouth Disease Vaccine Antigen elicit protection in cattle (F. Maree)
12:30 – 12:40  **Discussion**
12:40 – 13:40  Lunch break

SESSION P2: IMMUNOLOGY AND VACCINE RESEARCH 2

13:40 – 13:55  Rational development of FMD Virus Vaccines (B. Charleston)
14:10 – 14:25  Development and evaluation of an adenovirus vector based intranasal FMDV capsid vaccine in mice for increasing immune responses (A. Babu)
14:25 – 14:40  Alternative FMD vaccine potency tests based on serology and payload (T. Willems)
14:40 – 14:55  Improving challenge-free FMD vaccine batch acceptance (R. Reeve)
14:55 – 15:10  Relation between antibody response and protection in FMD vaccine depends on vaccine quality (A. Dekker)
15:10 – 15:20  **Discussion**
15:20 – 15:50  **Coffee/Tea break**
15:50 – 16:05  Cross protection against current Asia 1 field isolates is provided by a high potency Asia 1 Shamir Vaccine (Y. Li)
16:05 – 16:20  Testing the efficacy of 01 Manisa high potency vaccine against challenge with 0/Vietnam/2010 (0 MYA98 topotype) in pigs (W. Vosloo)
16:20 – 16:35  Antibody titres in fmd type a strains: comparison of methodologies to predict cross-protection (T. Tesfaalem)
16:35 – 17:00  **Discussion**
17:20 – 18:20  Poster session
Day 1 :: Monday 29 October (cont.)

Open Session of the EuFMD Research Group (RG): Focus on science contributing to the roll-out of progressive control of FMD

SESSION IV. PROGRESSIVE CONTROL PATHWAY FOR FMD (PCP): RESULTS, TECHNICAL DEVELOPMENTS AND ISSUES

15:50 – 16:20 PCP Stage 1–3: Results, technical developments and issues. (C. Bartels, N. de Haan, M. McLaws)
16:20 – 16:50 Socioeconomics: Enhanced fmd control through the integration of socio-economic approaches (C. Bartels, N. de Haan, M. McLaws)
16:50 – 17:20 The role of the OIE in fmd prevention and control. How to translate science in standards and guidelines, how to develop tools and ensure their convergence (J. Domenech)
17:20 – 18:20 Poster session
18:30** Dinner offered by the Spanish MARM (buses from the venue to the ‘Yeguada’ la Cartuja)
Open Session of the EuFMD Research Group (RG): Focus on science contributing to the roll-out of progressive control of FMD

SESSION V. FMD EPIDEMIOLOGY: TRANSMISSION, VIRUS CIRCULATION, RISK FACTOR

08:30 – 09:00 Keynote: Local differences in circulation, what have we learnt from patterns of fmd persistence and spread? (N. Knowles)

09:00 – 09:15 Limited transmission of foot-and-mouth disease virus from infected sheep to naïve calves (C. Bravo de Rueda)

09:15 – 09:30 FMDV infection in vaccinated and non-vaccinated sheep: transmission to contact animals and diagnostic aspects (P. Eblé)

09:30 – 09:45 Transmission of fmdv from infected buffalo (Bubalus bubalis) to vaccinated and naïve buffalo and cattle (M. Madhanmohan)

09:45 – 10:00 Within herd transmission and evaluation of the performance of clinical and serological diagnosis of foot and mouth disease in vaccinated cattle (J.L. Gonzales)

10:00 – 10:15 Discussion

10:15 – 10:45 Coffee/Tea break

SESSION VI. EPIDEMIOLOGY 2

10:45 – 11:00 Foot and Mouth Disease Virus (FMDV) in the african buffalo (Syncerus caffer) in Kenya (S. Wekesa)

11:00 – 11:15 Seroprevalence profile of foot-and-mouth disease in wildlife populations of West and Central African Regions with special reference to syncerus caffer subspecies (A. di Nardo)

11:15 – 11:30 Epidemiological patterns and risk factors for Foot-and-Mouth Disease exposure in traditional livestock-keeping systems of Northern Tanzania (T. Lembo)

11:30 – 11:45 Retrospective serosurvey of Foot and Mouth Disease (FMD) in free ranging domestic pigs and wild suids in Sub-Saharan African Countries (M. Arias)

11:45 – 12:00 Risks associated with unofficial livestock movements in the greater Mekong region (A. Cameron)

12:00 – 12:15 Risk factors for foot and mouth disease in beef cattle herds in Israel (E. Klement)


12:30 – 12:45 Discussion

12:45 – 13:45 Lunch break

SESSION VII. EPIDEMIOLOGY 3

13:45 – 14:00 Prevalence and risk factors for FMD-NSP-antibodies in cow and buffalo calves, and small ruminants in Egypt (V. Maanen)

14:00 – 14:15 Determining the level of vaccine-induced versus field-virus induced antibodies in livestock in West-Azarbaijan, Iran (C. Bartels)

14:15 – 14:30 Spatio-temporal origin and transmission of the Foot-and-Mouth Disease Virus outbreaks in Burgas Region (Bulgaria) in 2011 (B. Valdazo)

14:30 – 14:45 Molecular epidemiology of Foot-and-Mouth Disease Virus in the african buffaloes in Southern Africa (C. Kasanga)

14:45 – 15:00 Discussion

(cont. on page xii)
SESSION P3. IMMUNOLOGY AND VACCINE RESEARCH 3

08:45 – 09:00 Antigenic cartography for analysis of antigenic variations in FMD Virus (B. Pattnaik)
09:00 – 09:15 Evidence of further neutralisation after removal of five neutralising antigenic sites in serotype O FMDV (A. Asfor)
09:15 – 09:30 Discussion
09:30 – 09:45 Determining the Epitope dominance on the capsid of a SAT2 Foot-and-Mouth Disease Virus by mutational analysis (P.A. Opperman)
09:45 – 10:00 Study of antigenic site variation in fmd virus serotype 0 grown under vaccinal serum antibodies in vitro (B. Pattnaik)
10:00 – 10:15 Discussion
10:15 – 10:45 Coffee/tea break

SESSION P4. FMD MANAGEMENT 1

10:45 – 11:00 Modelling into policy: How can an ‘Intelligent Customer’ ensure appropriate use of evidence? (F. Gauntlett)
11:00 – 11:15 Scaling up from 1-to-1 animal transmission experiments to epidemiological models of national outbreaks (D. Schley)
11:15 – 11:30 Epidemiological models of FMD in two different austrian regions (J. Hiesel)
11:30 – 11:45 Multi-criteria decision analysis for evaluating control options during FMD outbreaks (K. Mintiens)
11:45 – 12:00 Meta-Analysis on the efficacy of Foot-and-Mouth Disease Emergency Vaccination (T. Halasa)
12:00 – 12:15 Evaluating vaccination for Foot-and-Mouth Disease Control – an international study (M.G. Garner)
12:15 – 12:45 Discussion
12:45 – 13:45 Lunch break

SESSION P5. COMPLEMENTARY RESEARCH 1

13:45 – 14:00 A new approach to the oldest disease developing an antiviral drug strategy for the containment of Foot-and-Mouth Disease outbreaks (N. Goris)
14:00 – 14:15 The Pyrazinecarboxamide Derivative T-1105 offers protection against O1 Manisa Virus infection in Guinea pigs (De Vleeschauwer)
14:15 – 14:30 Discussion

SESSION P6. FMD MANAGEMENT 2

15:30 – 16:00 Coffee/Tea break
16:00 – 16:15 Simulated effects of introducing emergency vaccination or depopulation during fmd outbreaks in Denmark (A. Boklund)
16:15 – 16:30 Modelling the spread of fmd in endemic regions (M. Tildesley)

(cont. on page xiii)
Day 2 :: Tuesday 30 October  (cont.)

SESSION VIII. VACCINATION PROGRAMMES

15:00 – 15:30  **Keynote**: Effectiveness of vaccination programmes (*P. Fine*)
15:30 – 16:00  **Coffee/tea break**
16:00 – 16:15  FMD Asia-1 vaccine effectiveness in Turkey (*T. Knight-Jones*)
16:15 – 16:30  An investigation of vaccination effectiveness in two Cambodian villages facing an outbreak of Foot-and-Mouth Disease (*A. Cameron*)
16:30 – 16:45  The field effectiveness of inactivated vaccine for prevention of foot and mouth disease (*E. Klement*)
16:45 – 17:00  Foot and Mouth Disease: Vaccine impact and progressive control in India (*S.N. Singh*)
17:00 – 17:15  A high throughput liquid phase blocking elisa for quantitative estimation of antibody titers against structural proteins of Foot-and-Mouth Disease Virus (*G.K. Sharma*)
17:15 – 17:30  **Discussion**
19:30**  Meeting at the Alcazar for the “Afta” session

**AFTA – Thoughts Night session** (also known as the *Fred Brown* event)
16:30 – 16:45 Assessing and comparing control strategies for FMD in endemic countries: adaptation of the North American animal disease spread models (NAADSM) (M.D. Salman)
16:45-17:00 Geographically-grounded, cost-benefit based control policies: Built as equal circles or considering local connecting networks? (A.L. Rivas)
17:00-17-15 Costs and benefits of FMDS practises in commercial dairy farms in central Ethiopia (A.F. Beyi)
17:15-17:30 Discussion
Day 3 :: Wednesday 31 October  PLENARY ROOM

Open Session of the EuFMD Research Group (RG): Focus on science contributing to the roll-out of progressive control of FMD

**SESSION IX. DIAGNOSTIC DEVELOPMENTS AND LABORATORY NETWORKS**

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<th>Session Topic</th>
<th>Speaker(s)</th>
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<tbody>
<tr>
<td>08:30 – 09:00</td>
<td>Keynote: New Elisa’s for FMD (E. Brocchi)</td>
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<tr>
<td>09:00 – 09:15</td>
<td>FMD and SVD combined proficiency test studies 2011 (B. Armson)</td>
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<td>09:15 – 09:30</td>
<td>New roles for “Auxiliary labs” in the diagnosis of FMD? (B.Haas)</td>
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<tr>
<td>09:30 – 09:45</td>
<td>Laboratory capacity for diagnosis of Foot-and-Mouth Disease in Eastern Africa: Implication on progressive control pathway (A. Namatovu)</td>
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<tr>
<td>09:45 – 10:00</td>
<td>Evaluation of FTA® cards as a laboratory and field sampling device for the detection and serotyping of Foot-and-Mouth Disease Virus (M. Madhanmohan)</td>
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<td>10:00 – 10:15</td>
<td>Development of RNA transfection method for rescue of FMD virus in susceptible cell (B. Pattnaik)</td>
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<td>10:15 – 10:45</td>
<td>Coffee/Tea break</td>
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<tr>
<td>10:45 – 11:00</td>
<td>Open fmd a resource for automatic and curated nomenclatures and tools for the FMD (epiphylogeography?) community (P. Claes)</td>
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<tr>
<td>11:00 – 11:15</td>
<td>Development and evaluation of a real-time reverse Transcription-Loop-Mediated isothermal amplification assay for rapid serotyping of Foot-and-Mouth Disease Virus (M. Madhanmohan)</td>
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**SESSION X. DIAGNOSTIC DEVELOPMENTS AND APPLICATIONS**

<table>
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<tr>
<td>11:15 – 11:30</td>
<td>Diagnostic performance of an immunochromatographic lateral-flow strip test using generic rapid assay device for detection and serotyping of Foot-and-Mouth Disease Virus serotypes 0 or Asia 1 in clinical samples (Z. Zhan)</td>
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<tr>
<td>11:30 – 11:45</td>
<td>Development and evaluation of a one-step duplex real time RT-PCR for diagnosis of Foot-and-Mouth Disease (K. Gorna)</td>
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<tr>
<td>11:45 – 12:00</td>
<td>The development and evaluation of a SAT-adapted 3ABC Diva Test for Foot-and-Mouth Disease Virus in the Southern Africa context (M. Chitray)</td>
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<tr>
<td>12:00-12:15</td>
<td>Detection, isolation, and typing of Foot-and-Mouth Disease Virus from oral swab samples collected from balochistan province of Pakistan. (M. Assad Ullah)</td>
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<td>12:15-12:45</td>
<td>Discussion</td>
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<td>12:45-13:45</td>
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**SESSION XI. WRAP UP AND CLOSURE**

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<tr>
<td>13:45</td>
<td>FINAL Session of papers – or The way ahead. Conclusions given by leaders (keynote speakers) Recommendations Appreciations and prizes</td>
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<tr>
<td>15:30</td>
<td>Closure</td>
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GFRA MEETING: An African Perspective

SESSION P7. GFRA MEETING

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<th>Time</th>
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<tr>
<td>08:30</td>
<td>GFRA: State of the alliance overview (F. Marée)</td>
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<tr>
<td>09:00</td>
<td>Role of buffalo in the maintenance of FMDV (B. Charleston)</td>
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<td>09:15</td>
<td>FMD virus ecology: Collaborative studies (L. Rodriguez)</td>
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<tr>
<td>09:30</td>
<td>Development of a safe antigenic marker FMD vaccine platform (E. Rieder)</td>
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<tr>
<td>09:45</td>
<td>Top priorities for research (D. Paton)</td>
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<tr>
<td>10:00</td>
<td>GFRA workshop in 2013 and concluding remarks (F. Marée)</td>
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<td>10:15</td>
<td>Coffee/tea break</td>
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Open Session of the EuFMD Research Group (RG): Focus on science contributing to the roll-out of progressive control of FMD

SESSION P8. FMD MONITORING AND SURVEILLANCE: EXPERIENCE, METHODS AND APPROACHES

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<td>Estimating the incidence of foot and mouth disease (M. McLaws)</td>
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<tr>
<td>11:45</td>
<td>The use of oral fluids from pig herds for pre-clinical diagnosis and monitoring in a FMD emergency: Current research and future directions (Z. Zhang)</td>
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<tr>
<td>12:00</td>
<td>Foot-and-Mouth Disease Virus transboundary movements between Subsaharan Africa, North Africa and the Middle East (N. Knowles)</td>
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<tr>
<td>12:15</td>
<td>Maximising efficiency with a surveillance strategy for Foot-and-Mouth Disease during an outbreak in a previously fmd-Free country (K. Walker)</td>
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<tr>
<td>12:30</td>
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FRENKEL’S LEGACY: WHAT IS KEEPING US?

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In 1947, Dr. H. S. Frenkel reported growth of Foot and Mouth Disease (FMD) virus in cultured explants of cattle tongue epithelium. This method was subsequently developed for large-scale production of the virus, thus creating possibilities for FMD vaccine production on a much wider scale than hitherto. Frenkel-type vaccines were first used for large scale cattle vaccinations in the Netherlands in 1953. The number of FMD cases dropped dramatically, but rose again in the early 60-ties, especially in pigs. It was not until the late 60-ties, when the neighboring countries started to vaccinate routinely, that FMD disease was gradually brought under control. The lessons from this early experience are twofold: first FMD can be controlled, even with the classical vaccines in countries with a dense population of susceptible animals, and second such efforts are best undertaken as part of a regional approach. In other regions the lessons learned were similar, for instance in South America where remarkable progress with FMD control has been achieved.

Despite the existing possibilities to control FMD or at least reduce its impact, the disease is still endemic in large parts of the world. FMD not only hampers regional and global trade and constitutes a risk for FMD-free countries; it also negatively affects the productivity of food producing animals, draught animals and sector development, in particular in developing countries - resulting in less food security and negative effects on family’s livelihoods.

With the above in mind, FAO and OIE developed the Global FMD Control Strategy launched in Bangkok in June 2012. The Global Strategy builds on experience and current scientific insights. The basic notion is that better FMD control is possible with the present means and methods, provided there is sufficient political will and financial support. The support should come from FMD-affected countries, but also from the world community in the true spirit of “fighting the disease at source”. The Global Strategy also emphasizes that improved FMD control will result in and has to go hand in hand with expertise and system improvements, i.e. Veterinary Services, resulting in wider positive effects.

The Global FMD Control Strategy combines the instruments of FAO and OIE, with emphasis on the FMD Progressive Control Pathway (PCP) for FMD and the Performance of Veterinary Services (PVS) pathway. It strives to strengthen the laboratory and epidemiology support structures at national, regional and global level and emphasizes the need to improve or maintain independent vaccine quality control. The Strategy has a clear long term vision and 5-year goals.

Control strategies of animal diseases cannot be implemented without adequate scientific support, for instance to signal and interpret unexpected findings. Furthermore it is anticipated that the Global FMD Control Strategy will be accelerated by new scientific achievements such as improved, safe and cheap vaccines in the not too distant future. Frenkel brought innovations to application in only 6 years; we need to assist translation of future breakthroughs in similar periods of time.

Appliance of science in the progressive control of FMD
Open session of the EuFMD, Jerez de la Frontera, Spain. 29-31 October 2012
GLOBAL SURVEILLANCE: EUROPEAN NEIGHBOURHOOD AND GLOBAL FMD SITUATION

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Foot-and-mouth disease (FMD) is highly contagious, infects a wide variety of domestic and wildlife hosts and occurs as seven virus serotypes with multiple subtypes known as topotypes. Its presence reduces production and restricts trade opportunities for endemic countries and poses a constant threat to those countries free of the disease. FMD viruses are not randomly dispersed throughout the world but are associated with particular ecological niches. The distribution is affected by recurring upsurges in the prevalence of particular strains that may be associated with viral evolution, waning population immunity and/or opportunities presented by the increasing and more frequent movements of animals and their products. This can give rise to pandemic spread affecting new regions. Current global surveillance for FMD aims to identify the current hazards and to predict heightened risk so that appropriate diagnostics and vaccines can be made available for their detection and control.

The World Reference Laboratory for FMD (WRLFMD®) at The Pirbright Institute, UK, is the centre of an OIE/FAO FMD Reference Laboratory Network that regularly receives samples for FMD diagnosis from many parts of the world. FMD virus isolates are identified by serotyping, vaccine matching with a range of current FMD vaccine strains and by nucleotide sequencing to provide precise characterisation of new isolates and tracing of their origin by comparison with viruses held in the extensive WRLFMD® and other collections. This analysis assists the monitoring of the ‘real time’ emergence and spread of FMD virus globally.

Studies on FMD virus occurrence over many years have provided the information to suggest the clustering or grouping of FMD viruses into 7 virus pools, with 3 pools covering Europe, the Middle-East and Asia, 3 pools covering Africa and 1 pool covering the Americas. This concept has provided the platform to enable a targeted approach to progressive FMD control at the national, regional and global level.

This presentation will focus on the global FMD surveillance provided by the OIE/FAO network of FMD reference laboratories and highlight the regional differences in virus populations and current needs for diagnosis and control.
ALIGNING WAITING PERIODS FOR VACCINATE-TO-LIVE & VACCINATE-TO-DIE

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Introduction:

The CVOs of Australia, Canada, New Zealand and the USA initiated a scientific review to evaluate if waiting periods to regain OIE status of FMD free not practising vaccination could be 3 months irrespective of whether vaccinate-to-live or vaccinate-to-die policies were applied.

Materials and Methods:

The authors reviewed the following designated areas reflecting their expertise [historical review of waiting periods; Carriers; Vaccinology; DIVA technology; Post Outbreak Surveillance and Animal Products].

Results:

Current science supports eligibility to return to OIE status of FMD free country where vaccination is not practised in 3 months following an outbreak where stamping-out and emergency vaccination using higher potency vaccines are applied irrespective of whether vaccinate-to-live or vaccinate-to-die policies. This assumes aspects of vaccination affecting population immunity such as insufficient match, inadequate coverage, incorrect storage, application, maternal antibody etc are addressed.

The alignment of the 3 month waiting period applies only to animal products as in 2006, the Code restricted export of live vaccinated animals from a FMD free country not practising vaccination. However, countries with OIE status, FMD free country where vaccination is practised may accept vaccinated animals and those with no OIE FMD status should not refuse them as per the OIE Code User Guide Part C a). Bilaterally negotiated additional risk mitigation measures may be needed to meet individual importing countries’ Appropriate Level of Protection (ALOP) as in any application of the Code.

Discussion:

It is surveillance intensity rather than time that establishes the risk of the presence of residual FMDV. Thus, in addition to the conclusion, the scientific review recommends that rather than stipulating a 3, 6, 12 or 18 month waiting periods in Article 8.5.9, the OIE set an acceptable level of statistical certainty for surveillance to (i) substantiate the absence of FMDV infection for a FMD free country where vaccination is not practised OR (ii) substantiate the absence of FMDV circulation for FMD free country where vaccination is practised.

Support for the concept of threshold of surveillance by Europe is evidenced by the 2007 Tervuren workshops’ conclusions and from a recent expert task force (SANCO/7070/2010).
A QUANTITATIVE APPROACH TO DETERMINING WAITING PERIODS FOR FMD FREEDOM

A. Cameron

Global and regional standards for the demonstration of freedom from FMD often include mandatory waiting periods. For example, the OIE Code for initial declaration of free status requires a waiting period of 12 months since the last outbreak for a country or zone without vaccination, and 2 years for a country with vaccination. Waiting periods for regaining free status after an outbreak vary between 3, 6 and 18 months, depending on the eradication policy. The purpose of these waiting periods is not explicitly explained in the Code, nor is the way in which they were determined. They appear to be arbitrary figures based on an intuitive understanding of factors influencing confidence in freedom.

These periods contribute to confidence in free status in two ways. Firstly, if undetected disease is present at very low levels, it provides time for the disease to spread until it reaches a detectable prevalence (exceeds the specified design prevalence). In the case of FMD, the highly contagious nature of the disease means that in most cases this spread would occur rapidly (except in vaccinated populations). Secondly, it provides time for the accumulation of surveillance evidence. There is a general requirement for the presence of a passive clinical reporting system (amongst other forms of surveillance). The waiting period provides time for evidence from this surveillance to accumulate to a level providing acceptable probability of freedom.

Quantitative standards for surveillance are usually expressed in terms of ‘confidence’ which may be more accurately described as the surveillance sensitivity, or the probability that a surveillance activity would detect at least one case of disease, if it were present at a specified prevalence (the design prevalence). Estimation of surveillance sensitivity may take multiple factors into account including sample size, the sensitivity of screening and confirmatory tests, and the effect of risk-based sampling.

More recently, the probability of freedom has been used as a quantitative standard for surveillance. This is the population-level analogy of the negative predictive value of a test on an individual animal, and can be calculated from the surveillance sensitivity using a Bayesian approach. In addition to the above factors, the probability of freedom takes further factors into account: the combined sensitivity of multiple surveillance activities (e.g. sero-surveillance and clinical surveillance), the probability of re-introduction of disease per unit time (i.e. biosecurity measures in place), and the accumulation of surveillance evidence over time. The ability to quantitatively capture time-related aspects of the probability of freedom provides a mechanism to evaluate appropriate waiting periods for FMD freedom. For example, lower sensitivity of clinical surveillance, the absence of concurrent surveillance activities and the use of a low design prevalence (due to the presence of vaccination) will all result in a longer time period until a specified target probability of freedom is achieved. In contrast, the use of multiple surveillance activities, passive clinical surveillance with high sensitivity and a high design prevalence in an unvaccinated population will result in a very short time period. This paper illustrates the use of this approach, providing examples of how the appropriate waiting periods may be calculated, based on different approaches to eradication and surveillance.
EVALUATION OF THE BENEFIT AND FEASIBILITY OF A VACCINATION-TO-LIVE STRATEGY IN FMD FREE COUNTRIES

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Introduction: Within the frame of the Swiss Animal Health Strategy 2010+ (www.bvet.admin.ch), the Swiss Federal Veterinary Office initiated a project in order to evaluate a vaccination-to-live strategy against Foot and Mouth Disease (FMD). Within the scope of this project, the benefit of emergency vaccination within 3 km (V3) and 10 km (V10) around an infected premises (IP) was evaluated and the technical feasibility of such an emergency vaccination was analyzed regarding the corresponding EU FMD directive (COUNCIL DIRECTIVE 2003/85/EC).

Materials and Methods: We used the Davis Animal Disease Simulation (DADS) model (Carpenter et al., 2011; Durr et al., 2012) in order to compare the conventional disease control strategy with and without an additional emergency vaccination strategy (V3 and V10, respectively). In a second step, we analyzed the implementation of a vaccination-to-live strategy with regard to its feasibility and economical consequences.

Results: It was shown that emergency vaccination in a low-livestock density country like Switzerland would be only beneficial in a situation where the epidemic is already widely distributed (V10 strategy). On the other hand, our feasibility study with respect to the vaccination-to-live strategy revealed that the animal movement restrictions within the vaccination zone would lead to a significant increase in welfare culling especially in the pig production sector due to the long duration of the restrictions.

Discussion: The expected increase in welfare culling due to the long duration of animal movement restrictions within the vaccination zone actually impedes the implementation of a vaccination-to-live strategy for ethical and economic reasons. Therefore, the implementation of animal movement restrictions during the different phases of a vaccination-to-live process has to be re-examined and adjusted accordingly.
FMD IN WILD BOAR: CAN THE VIRUS BE MAINTAINED IN WILDLIFE? EXPERIENCES AND CONSEQUENCES FROM THRACE

Klaus Depner, Sofie Dhollander, Anette Bøtner and Angele Breithaupt

Following an index case of FMD in wild boar in Bulgarian Thrace in January 2011, the clinical course of FMD in wild boar was studied by Breithaupt et al. (2012)\(^1\). After an incubation period of 2 to 4 days, the first clinical signs were noticed. The severity of the clinical course was rather mild compared with disease in domestic pigs. Although severe foot lesions were seen, the animals’ mobility was not impaired. Viraemia started two days after exposure and lasted until 6 days post exposure (DPE). Virus shedding started also during the incubation period and lasted up to 9 DPE. Viral RNA was constantly detected in tissue samples and oropharyngeal fluids for up to 27 DPE. Antibodies were detected after the first week of infection. Besides the mild clinical signs, the early onset of viral excretion may indicate that the wild boar could play a potential role to spread FMD virus, particularly in areas with a high wild boar density.

The results of the wildlife surveillance activities in Thrace carried out by EU-FMD, detected seropositive wild boar only within 50 km zone around FMD outbreaks. and the seroprevalence declined during the surveillance period that lasted one year after the outbreaks. This in combination with lack of further outbreaks in domestic animals indicated that the wildlife population was most likely not able to sustain the virus circulation.

Besides the experimental studies and the serosurveillance results, an epidemiological model developed by Lange (2012)\(^2\) studied the potential maintenance of FMD virus in Thrace. The conclusions were that FMD will not be sustainable within a wild boar and deer host system alone, but limited spread of FMD virus in time and space may occur.

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Introduction: Extensive serological and virological FMD surveys in wild boar were implemented to prove freedom from the disease in Bulgaria/Turkish Thrace (2011) and better understand its role the FMD enzootic areas in Anatolian Turkey (2011-2012, commissioned by the EuFMD Standing Technical Committee and funded by EC through EuFMD).

Materials and methods: Samples were collected from shot wild boar: in Thrace mostly on the border between Bulgaria and Turkey (n=1004), and in 4 provinces with different disease situation in Anatolian Turkey (n=252). Information on sex, age, group size, GPS coordinates and other details was recorded. Results of laboratory test were compiled into georeferenced database and explored together with information on spatio-temporal occurrence of FMD in livestock.

Results: Only one virus (serotype Asia-1/ lineage Sind08) was detected in Gümüşhane Province in Turkey. It was most closely related to the recent livestock isolates. Average seroprevalence (SP) in all animals sampled in 2011 in Thrace was 7.8 %. It was higher closer to outbreak locations in livestock (17.9 % (12.6 - 24.3)) and declined further away, reaching zero beyond 50 km radius. Juveniles had significantly lower SP of 5.6% (3.4 – 8.5) as compared to adults (9.1 % (6.9 – 11.6)). No difference in SP was found between sexes. In Anatolia FMD positive animals were found in all 4 provinces. Average SP was 13.1 % (9.2-17.9) with large regional variation (Rize 4.8 % (0.1-24) and Erzrum 41 % (18-67)).

Discussion: Wild boar get involved into FMD transmission of multiple serotypes (O, Asia-1, SAT-2) and exchange viruses with livestock. Disease prevention, control and eradication strategies should account for this complication where wild boar density is high. Timely detection of FMD incursions to wildlife requires non-invasive sampling methods.
FMD LAB BIO-RISK MANAGEMENT: WHAT HAVE WE LEARNT FROM APPLICATION OF THE 2009 MINIMUM STANDARDS?

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According to currently EU legislation, the laboratory diagnosis of suspect cases of FMD involving handling of materials from infected holdings requires a laboratory listed in Annex XII of Council Directive 2003/85/EC which has to meet the FAO EuFMD "Minimum Standards". The same applies to any in-vitro research with infective FMDV. It has turned out that in most countries not all the diagnostic tasks in the framework of FMD control can be carried out in such listed FMD laboratories, because there are too few of them available and these labs are usually research facilities with a limited sample throughput. Therefore regional or “auxiliary laboratories” have become part of many contingency plans. They are dealt with in No. 13 of Annex XV of Council Directive 2003/85/EC and in Annex II of the “Minimum Standards”. In the later regulation, it is stated that such “auxiliary laboratories” only have to meet very limited containment requirements, i.e. quarantine for staff in respect to susceptible animals and autoclaving of waste, but no negative pressure is required and makeshift shower facilities are acceptable. According to Annex II of the “Minimum Standards”, in an outbreak situation, such auxiliary labs can process samples from holdings without clinical signs (serological samples in the framework of disease surveillance) as well as samples from holdings with clinical signs (PCR tests to detect virus), the later kind after inactivation on the premise. In contrast to the expectation when the “Minimum Standards” were drafted, currently still no validated protocol exists for the inactivation of FMD samples on the premise and there are doubts whether this really is the best approach to the problem. While it is possible to inactivate virus in swabs by putting them into a buffer containing guanidinium-isothiocyanate, this procedure may not always completely inactivate the virus in larger solid pieces of tissue and it is not feasible for serum samples one would like to test also for antibodies. Therefore, during the 2010 FMD epidemic in Bulgaria, the EU has tolerated that also non-inactivated samples from holdings with clinical signs were examined in a Bulgarian laboratory not meeting the requirements of the “Minimum Standards”. It can now be concluded that this approach worked very well. The Bulgarian lab quickly produced valuable results on the FMD situation in the country which were crucial for the control of the disease and there is no reason to assume that the activities of the lab posed any inappropriate risk to the environment. The alternative to tolerating the examination of Bulgarian field samples in this laboratory would have been to send all suspect samples to a foreign laboratory, which in particular in times of crisis, is a logistical and communication nightmare and would have substantially increased the turn-over time while reducing the throughput. While originally, “auxiliary laboratories” were mainly considered as a supplement to a listed FMD lab within a country, it now appears that we have to reconsider their role. In accordance with Article 66 of Council Directive 2003/85/EC, the EU FVO carried out a series of audits on the bio-risk management systems applied at laboratories authorized to handle live FMD virus between 2009 and 2012. One of the findings was that some laboratories need considerable investment, mainly into their effluent treatment and air handling plants. Unfortunately, not all member states are able to afford such an investment. Furthermore, it appears that for economic reasons not even all the European countries with a high livestock density will maintain a FMD laboratory meeting the “Minimum Standards”. However, for effective and swift disease control, it is crucial that official vets as well as the national crisis centres can contact a diagnostic laboratory with staff that is familiar with national legislation and conditions without a language barrier at any time. An absolute requirement to send any samples for FMD testing to a foreign laboratory would considerably raise the psychological threshold for sending any samples at all. Furthermore, in case of an FMD outbreak in a country with a high livestock density and export volume, any major European FMD laboratory which theoretically may be able to help will soon be stressed to the limit by the examination of suspect samples from its own country, even if the country is not (yet) affected. Therefore suggestions are made for modifications of No. 13 of Annex XV of Council Directive 2003/85/EC and Annex II of the “Minimum Standards” for “auxiliary laboratories”. While we have to insist that “auxiliary labs” not meeting the “Minimum Standards” must not handle live FMDV in “peace times” and only examine suspect samples send in by the national veterinary service by methods that don’t require live FMDV as reagents, small risk-based adoptions of the legal documents would put something that is already done on a sound regulatory basis and actually help to decrease and control the risk posed by FMD.
ECONOMIC EVALUATION OF FMD MANAGEMENT OPTIONS: IMPLICATIONS FOR SCIENCE AND POLICY

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Outbreaks of FMD in the EU can have devastating effects on the livestock sector and serious economic as well as social consequences in affected countries. Therefore strict international and EU regulation exists to prevent spread between member states (MS). This regulations can be considered as the minimum measures to be applied. Given the differences in structure of the livestock sector within the EU and even within MS’s, on top of these measures several MS’s apply specific measures tailored to their local circumstances.

Recent development with respect to vaccine development (DIVA), alternative ideas for Cost and Responsibility Sharing Schemes (CRSS) amongst stakeholders and experiences with recent outbreaks might trigger Competent Authorities to evaluated alternative policy options.

In this paper aspects that have an impact on the economic evaluation of FMD management options will be discussed. Also attention will be given to implications for science and policy.

The economic implications of FMD for MS’s and the EU are determined by:
1. the probability of occurrence of an outbreak in one or more MS’s,
2. the size and duration of the outbreak and the economic effects of the outbreak and
3. the control measures taken by Competent Authorities and well as the reaction of stakeholders and trade partners.

Outbreaks result in costs for government (EU and national) as well as for the livestock sector and society in general. Distribution of cost and benefits amongst the might be different in different FMD management options.

In the presentation the economic implications of alternative strategies on these 3 aspects will be illustrated and discussed. The role of existing and potential schemes for co-financing of emergency measures as well as the economic impact of an alternative CRSS is illustrated.

For an evaluation of different policy options epidemiological and economic simulation models are increasingly used. These models can assist the policy maker in making a well-balanced decision by answering questions like:

- What would be the implications and risks of applying differentiated strategies for sparse and densely populated areas across the EU?
- How much difference does the waiting period after vaccination versus non-vaccination make to the cost of vaccination to live option?
- What are the implications for FMD research?

In conclusion, economic evaluation of different FMD management options:
- should to be based on universal principles,
- need to be tailored to local circumstances in discussion with stakeholders,
- is likely to result in different solutions for different countries e.g. due to difference in livestock population density, trade patterns or acceptance of product originating from vaccinated animals, and
- should be supported by epidemiological and economic models.
SIMPLE DECISION TOOLS INFORMED BY MODEL PREDICTIONS WHEN CONSIDERING FMD EMERGENCY VACCINATION STRATEGIES

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Introduction: Through a series of proposed workshops, EuFMD will assist Veterinary Services in Europe to adopt model-based decision tools for their contingency planning efforts. This activity was supported by the meeting of EuFMD CVOs in June 2012, and it was argued that Veterinary Services should at least be able to function as “intelligent customers” in dealing with models, i.e., they should acquire a basic experience with animal disease models and how they can be applied in their work.

An example is a simple quantitative decision tool using the first 14-days incidence (FFI) of FMD outbreaks to predict the duration and the cumulative number of outbreaks at the end of the epidemic.

We evaluated the applicability of this simple method to improve contingency planning and emergency preparedness of veterinary services, with a view to substantiate important and difficult management decisions, such as modifying the control strategy to include emergency vaccination.

Materials and methods: We used modified FFI procedures to analyze output from 5,000 simulations using a series of FMD models with current Danish population data in a modified DADS model (DTU-DADS). In addition we analyzed the FMD outbreaks in Argentina in 2001 with the 17 affected provinces as the units of observation.

The primary independent variable was the number of outbreaks detected during the first 14 days of the epidemic. The primary dependent variable was the number of outbreaks occurring after day 14, and various simple tools were used to show the relationship between the two: correlation, regression, 2x2-tables and selected cases comparing model output for the basic control strategy to that of a suppressive vaccination strategy.

Results: Statistically significant positive associations and useful predictive values were found with both data sets.

Discussion: Emergency vaccination might be considered if an alarming cumulative size of the epidemic is predicted by the model under the basic control scenario. It is imperative, however, that the model uses appropriate national information, since the outcome is highly dependent on input parameters and national priorities.
PCP STAGE 1 - 3: RESULTS, TECHNICAL DEVELOPMENTS AND ISSUES

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Introduction:
Since the Progressive Control Pathway for Foot and Mouth Disease (PCP-FMD) was first elaborated in 2008, countries in West Eurasia have been actively implementing this approach. This presentation describes some of the lessons learned to-date from working in this region, and emphasizes that, throughout the Stages, the PCP-FMD requires a dual focus on risk assessment and risk management activities.

Discussion:
At each Stage of the PCP-FMD, risk assessment activities are critical in order to target the limited resources available for surveillance and control most effectively. These activities involve both qualitative (e.g. workshops on value chain analysis, description of identified risk hot-spots, outbreak investigation case studies) and quantitative (e.g. surveys and risk factor analysis) methods.

However, risk assessment alone will not control FMD. Therefore, the information gained through risk assessment activities must be translated into an FMD control strategy – this is risk management. Effective risk management requires a strong organizational infrastructure based on a multi-disciplinary approach, and the active participation of stakeholders. Progress should be regularly assessed by monitoring indicators, defined explicitly in the control strategy.

As countries progress through the PCP-FMD, they must build capacity in the laboratory sciences, epidemiology and socio-economics in order to continue to enhance the knowledge base about the nature of FMDV circulation in that specific environment. Concurrently, the organizational capacity also requires progressive strengthening to ensure that the activities that make up newly-developed/revised control strategies are implemented as intended.

In this presentation, these concepts will be described with examples from the field relating to PCP-FMD Stages 1-3.
ENHANCED FMD CONTROL THROUGH THE INTEGRATION OF SOCIO-ECONOMIC APPROACHES

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Introduction:
For any animal disease control strategy to be effective and efficient, participation of the people affected by the disease and its control is a fundamental requirement. Recent experiences from rinderpest and highly pathogenic avian influenza (HPAI) control have shown that participation can be improved through the integration of socio-economic analysis into an animal disease control strategy at an early stage. Social sciences and economics offer a variety of tools to understand and enhance the role and engagement of livestock owners and other people involved in animal disease control. However, the process of integrating these tools effectively within disease control strategies is still in its infancy and remains a challenge.

The paper will therefore discuss recent experiences of interdisciplinary work with socio-economics on FMD control in order to develop systems of best practice.

Discussion:
Social and economic approaches, such as impact assessments, value chain studies and cost-benefit analyses of interventions, can contribute to disease control in several ways including: as advocacy tools at national-level and production-level; as identification tools of those impacted and the degree to which they will be impacted; and as tools to ensure the engagement of pertinent stakeholders and champions. The value of other tools and approaches such risk analysis and linkages with epidemiology, and initial stakeholder analysis will also be discussed.

The paper provides several examples of experiences in integrating these methodologies within FMD and other animal disease control strategies. It also takes a critical look at challenges being faced with implementation of these approaches within the progressive control pathway for FMD control (PCP-FMD), and provides solutions and recommendations for the way forward.
THE ROLE OF THE OIE IN FMD PREVENTION AND CONTROL. HOW TO TRANSLATE SCIENCE IN STANDARDS AND GUIDELINES, HOW TO DEVELOP TOOLS AND ENSURE THEIR CONVERGENCE

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The OIE standards and recommendations are based on scientific evidences and they provide to Member Countries very clear guidance to prepare policies and strategies for disease control programmes. They are published in the “Terrestrial Code” and in the “Terrestrial Manual”. The OIE standard setting is based on responsive, transparent and rapid procedures. The process starts with the involvement of the best recognized and independent experts who are invited to participate to groups which report to the Specialist Commissions. The major source of OIE experts is the OIE- Reference Centres. All draft texts are sent to member countries for comments prior to their presentation to the World Assembly for adoption.

OIE disseminates scientific information through publishing scientific and technical journals or organizing international conferences. The OIE has also the mandate to disseminate animal health information through the WAHIS and WAHID systems.

The development of international and regional collaborations with relevant organisations is an integral key component of the strategic plan and many tools, methods and strategies have been prepared jointly with partners among them are FAO (the preparation of the Global FMD Control Strategy being one of the best recent example of this collaboration) and WHO.

Besides of the adoption of constantly updated and new standards and guidelines, the OIE contributes to the emergence or strengthening of new tools such as Reference Centers and regional or global networks (e.g. the OIE/FAO FMD Reference Laboratory Network ), the FAO/OIE/WHO Global Early Warning System (GLEWS), the FAO/OIE Crisis Management Center for Animal Health (CMC-AH) or the identification of a minimum curriculum in veterinary education (minimum competencies required by veterinarians for countries to meet the OIE quality standards for Veterinary Services).

OIE has strongly promoted the principles that good governance of animal health systems based on a close public/private partnership is crucial and that effective official Veterinary Services (VS) have to be considered as public goods. The quality and compliance with OIE standards can be evaluated and the progress made over time be assessed using the OIE Tool for the evaluation of Performance of Veterinary Services (OIE PVS Tool).

Ensuring convergence between the tools developed by the OIE and partner organisations is an objective which has been considered during the design of the Global Strategy. Linking the OIE PVS levels of Critical Competencies (CCs) to the PCP-FMD stages is one the examples of why and how to improve this tool convergence. The basic principle is that a country embarking on the PCP-FMD should acquire the appropriate capacity and capability of the VS to conduct activities aimed at the control or elimination of FMD (and other TADs). This is referred to as the ‘enabling environment’ in the PCP. Bridging the PCP stages with the CCs of the OIE PVS tool is an important element in the successful implementation of the Global Strategy. It requires the reinforcement of the VS to be tailored to the needs and timeframe of the PCP stages. Overall, it is very important to note that the ‘relation’ PCP-OIE PVS works both ways: a country will be granted with a PCP stage only if the requirements in terms of enabling environment will be met as well (level 3 achieved for all the FMD related CCs for that particular PCP Stage); reversely, the national PCP ‘history/continuum’ (pace of progress; possible regression etc.) will be key if the country wants to have its national FMD control programme endorsed by the OIE at the end of Stage 3 or further embark for PCP Stages 4 and above.
LOCAL DIFFERENCES IN CIRCULATION: WHAT HAVE WE LEARNT FROM PATTERNS OF FMD PERSISTENCE AND SPREAD?

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Geographically, seven foot-and-mouth disease (FMD) virus pools have been defined with different ranges of serotypes and topotype occurring in each. In the last few decades, the study of virus movements, principally using phylogenetic analyses of VP1 nucleotide sequence data, has provided new insights into FMD virus evolution and epidemiology. For example, in Pool 1 (East and Southeast Asia), Southeast Asian countries have been shown to be the source of outbreaks of both FMD types O and A in countries in the north of the region. In Pool 3 (Western and Central Asia), new variants first appear in Afghanistan-Iran-Pakistan and spread westwards eventually reaching Turkey and sometimes even North Africa. In Pool 4 (East Africa), FMD type O viruses regularly appear in the Yemen Arab Republic. In Pool 6 (Southern Africa), FMD is generally well controlled; however, a few hotspots are present where it is suspected that FMD outbreaks in cattle originate from buffalo.

However, many important questions remain to be answered, e.g.:

1) What are the precise mechanisms which result in the generation and expansion of new FMDV variants in Western Asia?
2) Are West African buffalo persistently infected with FMDV and if so with which serotypes?
3) Does SAT 1 occur in West Africa (not recorded in that region since 1981)?
4) What role do African buffalo play in the maintenance of FMD in East African cattle?
5) Which FMD viruses occur in Chad and the Central African Republic?
6) Is FMDV SAT 3 widespread in buffalo herds outside of Southern Africa as serology suggests?
7) Are there any reservoirs of FMDV type C (which has not caused an outbreak since 2004)?
LIMITED TRANSMISSION OF FOOT-AND-MOUTH DISEASE VIRUS FROM INFECTED SHEEP TO NAÏVE CALVES

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Introduction:
Interspecies transmission of foot-and-mouth disease virus (FMDV) is not clear. FMDV infected sheep might be a high risk in the transmission of FMDV to cattle because FMD clinical signs in sheep are sometimes unapparent and, they can become FMDV carriers. We therefore have set up experiments to quantify FMDV transmission from sheep to cattle.

Materials and methods:
Two FMDV inoculated sheep (N=20) were housed together with 1 naïve calf (N=10) for 31 days. Sheep and calves were inspected daily for clinical signs. OPF swabs, urine, faeces and blood samples were tested by virus titration. Serum samples were tested by NS-ELISA and VNT. Probang samples were tested by RT-PCR. R nought (R\text{0}), the basic reproduction number, was estimated.

Results:
All sheep tested positive by NS-ELISA, VNT and virus titration from OPF samples. Sheep that tested positive in blood showed moderate to severe clinical signs. Nine sheep became FMDV carriers. Four calves had evidence of infection based on NS-ELISA and VNT results; only 1 of those 4 calves shed virus and showed clear clinical signs, 1 other calf became FMDV positive in probang samples and showed an indication of a FMD lesion. R\text{0} was 2.8.

Discussion:
FMDV can be transmitted from sheep to calves (with R\text{0} above 1). Subclinical infection was not expected in calves and might be due to a low dose of virus to which the calves were exposed. This study shows that transmission of FMDV from sheep to cattle is probably limited.
FMDV INFECTION IN VACCINATED AND NON-VACCINATED SHEEP: TRANSMISSION TO CONTACT ANIMALS AND DIAGNOSTIC ASPECTS

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Introduction:
As FMDV infections might go unnoticed in both non-vaccinated and vaccinated sheep, knowledge about transmission of FMDV and adequate strategies for detection of infection in this species are very important.

Materials and methods:
Transmission of FMDV was studied using 5 groups of 4 non-vaccinated sheep and 5 groups of 4 vaccinated sheep. In each group 2 sheep were inoculated with FMDV strain Asia 1/TUR/2000. FMDV infection was monitored in all sheep. Based on the final size of the experiment the reproduction number was estimated. In addition the results of different laboratory tests were compared to select the best possible strategy to identify infected sheep.

Results:
After infection, in both vaccinated and non-vaccinated sheep, limited or no clinical signs were observed. Viraemia was detected, for a short period only, in some of the infected non-vaccinated and in none of the infected vaccinated sheep. However, FMD virus could be isolated from OPF swab samples, from the majority of the infected animals, for the whole duration of the study (21 days) and virus excretion was estimated to be as long as 52 days for the non-vaccinated and 32 days in the vaccinated sheep. Despite this long duration of virus excretion, the reproduction number remained relatively low, being 1.14 in the non-vaccinated and 0 in the vaccinated sheep.

In the non-vaccinated population, transmission to contact sheep occurred mainly in the first week after infection, coinciding with the period in which the largest amount of virus was excreted in the environment which suggests a time-dependent infectiousness.

For detection of infection, detection of vesicles had both a low sensitivity as well as a low specificity. Viraemia was detected in 71% of the infected non-vaccinated sheep, but not in the infected vaccinated sheep. On average, viraemia lasted for only 2.1 days. VI of the OPF swabs lasted in 16 of the 27 infected sheep till 21 days post inoculation. Serologically, all but one of the sheep that were positive by VI in OPF swabs were detected using the NS-ELISA although the percentage inhibition of the NS-ELISA were variable.

Discussion:
The results indicate that previous used models to determine transmission rate for FMDV that assume the same infectivity over the whole infectious period might not be correct to use in sheep. The VI from OPF-swabs show that these samples are more likely providing a positive result for virus detection compared to vesicular material and/or plasma samples.
TRANSMISSION OF FMDV FROM INFECTED BUFFALO (Bubalus bubalis) TO VACCINATED AND NAÏVE BUFFALO AND CATTLE

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Introduction:
India holds the world’s largest cattle and buffalo population and according to 2003 animal census, 98 million buffaloes in India constitute 56% of the total population of buffalo in world. However, no detailed study has been carried out yet relating to the role of Indian buffalo in FMD epidemiology, transmission and immune response. This report describes the transmission of FMDV infection from infected Indian buffalo to naïve and vaccinated cattle and buffalo.

Materials and methods:
Monovalent FMD vaccine incorporating O/IND/R2/75 (7µg payload) FMDV inactivated antigen was formulated with Montanide ISA 206 (Seppic, France) as a water-in-oil-in-water (W/O/W) emulsion. Two groups of buffaloes (Gr1; n=6) and cattle calves (Gr2; n=6) were administered with 2.0 ml of formulated vaccine by intra-muscular route whereas another two groups of buffaloes (Gr3; n=6) and cattle (Gr4; n=6) were kept as unvaccinated. Donor buffaloes (n=12) were inoculated with 10^5 BID50 of O/HAS/34/05 FMDV through intradermalingual route. On 21 days of post-vaccination, one animal from each group (Gr1, 2, 3 & 4) were housed in an individual room along with two donor buffaloes that were inoculated with FMDV 24 hours before. The vaccinated and unvaccinated animals were separated from the donors after 5 days of direct contact challenge and housed with their original groups. Donor buffaloes were housed in two separate rooms (n=6 per room). Clinical signs and temperature were monitored for 15 days post challenge. Virological tests are being analyzed at IIL and Pirbright.

Results:
All the vaccinated cattle (100%) and four vaccinated buffaloes (66.6%) were protected from clinical disease. Two vaccinated buffaloes, six unvaccinated cattle and six unvaccinated buffaloes showed FMD clinical signs. All the donor buffaloes showed FMD lesion. All the vaccinated buffaloes and cattle showed medium to high neutralizing antibodies at the time of challenge.

Discussion:
The study indicates that FMDV could be transmitted from infected buffalo to naïve buffalo and cattle by direct contact. Though FMDV transmission was not possible from FMDV infected buffalo to vaccinated cattle, one third of vaccinated buffaloes were clinically infected with FMDV. This signifies the role of buffalo in FMDV transmission that may have an impact on future control strategy.
WITHIN HERD TRANSMISSION AND EVALUATION OF THE PERFORMANCE OF CLINICAL AND SEROLOGICAL DIAGNOSIS OF FOOT AND MOUTH DISEASE IN VACCINATED CATTLE

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Introduction:
In 2000 Bolivia started a foot and mouth disease (FMD) eradication programme based on a mass vaccination campaign, control movement and clinical and serological surveillance. Guidelines for the implementation of such surveillance activities had been established intuitively and although these proved useful, better scientific evidence was needed to refine them. The objectives of this study were to evaluate 1) the diagnostic performance of clinical inspection and serological tests for detection of FMD virus non-structural proteins (NSP), and 2) the possible within-herd transmission of virus in vaccinated cattle.

Material and Methods:
Data came from twenty three affected herds monitored during an epidemic of FMD type O in 2007. Clinical inspections were made regularly. All cattle from every herd were serum sampled one month after the last animal with clinical signs was detected. These samples were tested for the presence of antibodies against NSP of FMDV using the PANAFTOSA’s 3ABC-ELISA test and the EITB test. Data from clinical and serological diagnosis were analysed using a Bayesian model. Parameters such as the sensitivity $Se$ and specificity $Sp$ of the tests and the ‘true’ prevalence $p$ of FMD in the affected herds were estimated. The latter parameter was used to estimate the within herd reproduction ratio $R$ of the virus.

Results:
The $Se$ of clinical inspections, the 3ABC-ELISA and the EITB tests were estimated to be 0.36, 0.87 and 0.98 respectively. The various estimated $Sp$’s; were 0.88, 0.93 and 0.93 respectively. The within- herd prevalence of infected animals ranged from 0.06 to 0.91, and $R$ from 1.03 to 2.69.

Conclusion:
The estimates of test performance, prevalence and transmission parameters obtained in this study can be used to refine current surveillance guidelines and inform simulation models for surveillance and control of FMD in vaccinated cattle populations.
FOOT AND MOUTH DISEASE VIRUS (FMDV) IN THE AFRICAN BUFFALO 
(SYNCERUS CAFFER) IN KENYA

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Introduction:  
In sub-Saharan Africa, the African buffalo (Syncerus caffer) has been known as a maintenance host for the SAT serotypes of foot-and-mouth-disease virus (Thomson et al. 2003), while other serotypes have been mainly associated with livestock. However, some earlier studies in East Africa (Anderson et al. 1979, and Ayebazibwe et al. 2010) have provided evidence for the circulation of other serotypes in the African buffalo.

Materials and methods:  
Probang and serum samples were obtained from buffalos in 3 national parks (Maasai-Mara, Meru and Lewa). Sera were screened using Prio-check FMDV-NSP ELISA and Liquid-Phase Blocking ELISA (LPBE). Probang samples from NSP seropositive animals were initially screened using real-time Polymerase Chain Reaction (rRT-PCR) and positive samples will be cultivated on BHK cell cultures and tested using antigen detection ELISA.

Results:  
Overall, 52/67 (78%) buffalo serum samples tested positive for anti-FMDV antibodies in the NSP ELISA, distributed as follows; Maasai-Mara 35/39 (90%), Meru 12/23 (52%) and Lewa 5/5(100%). On LPBE, evidence of FMDV O was 9/68 (13.2%), A (2.9%), C (20.6%), SAT1 (52.9%), SAT2 (64.7%) and SAT3 (98.5%). However, these results will be verified by titration and VNT. Using rRT-PCR on the probang samples, 41% were positive (Ct values <32). The nature of the viruses is being determined.

Discussion:  
FMDV NSP assay indicated widespread infection of buffalo with FMDV. LPBE tests showed SAT viruses as most prevalent, consistent with previous studies in East Africa and that other serotypes may also be present (Ayebazibwe et al., 2010, Anderson et al.1979). This calls for further studies to establish the role of buffalo in the epidemiology of these serotypes. The RT-PCR positive probang samples suggest the circulation of virus in buffalo that may pose a threat to other animals (Vosloo et al.2002). Further virological assays and molecular analysis are on-going.
SEROPREVALENCE PROFILE OF FOOT-AND-MOUTH DISEASE IN WILDLIFE POPULATIONS OF WEST AND CENTRAL AFRICAN REGIONS WITH SPECIAL REFERENCE TO SYNCERUS CAFFER SUBSPECIES

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Introduction:
The role which West and Central African wildlife populations might play in the transmission dynamics of FMD is not known nor studies has been performed in order to assess the distribution and prevalence of FMD in wildlife species existing in those specific regions of Africa. This study reports the FMD seroprevalence profile extracted from samples collected from wildlife populations of West and Central African countries during the Pan-African Rinderpest Campaign and the subsequent programme for the Pan-African Control of Epizootics implemented between 1999 and 2003.

Materials and Methods:
Samples (n = 719) from 31 wild ruminants and pigs species were selected as representative of wildlife populations living in West and Central Africa from a largest collection stored at the CIRAD in Montpellier. The samples were initially screened for detecting antibody against the highly conserved NSP of the FMDV using the PrioCHECK® FMDV NS ELISA test kit. Subsequently, the NS ELISA reactive sera were further assessed using the SPCE in-house test developed at the IAH, thus enabling the characterisation of the specific antibody responses to 6 of the 7 FMDV serotypes (A, O, C, SAT 1, SAT 2 and SAT 3) and, therefore, the FMDV seroprevalence profile for each of the serotypes present in Africa at the time of the sampling. Results from the SPCE were confirmed selecting a random subsample of the resulting SPCE positive reactive sera by VNT, using the O1 Manisa, A22 Iraq, C Phi 784, SAT 1 105, SAT 2 Eritrea, SAT 3 309 FMDV strains. Statistical analyses were performed in R 2.15.1.

Results:
Two hundred and thirty samples out of 719 (31.99%) tested positive for NSP antibody against FMDV. FMDV positivity was found in 17 different wildlife species. Among the 196 buffalo samples 127 (64.80%) were scored as positive, where 68.45% and 50.00% of samples were estimated as positive for Nile Buffalo and West African Buffalo subspecies, respectively. No positivity was reported in African Forest Buffalo. SPCE test found buffalo as positive for A (44.09%), O (82.24%), C (66.11%), SAT 1 (73.16%), SAT 2 (81.89%) and SAT 3 (46.46%) serotypes, although highest O and SAT 3 prevalence were reported for the Central African region. Different patterns of reaction to the 6 serotypes were recorded, from sera only positive for a single serotype to multiple reactivities. Samples tested as positive for O, C, and SATs serotypes were confirmed by VNT.

Discussion:
The results confirm that FMDV is prevalent in wildlife ruminant species in both West and Central Africa regions and in particular in buffalo, also suggesting that multiple serotypes may be involved with type O, SAT 2 and SAT 1 being dominant. However, the extent to which findings of individual sera reactive to multiple serotypes is due to serological cross-reactivity or multiple infection is not entirely clear. Therefore, further work is needed to collect samples for virological examination to better understand the serotypes and strains circulating within wildlife populations of West and Central Africa and to confirm if FMDV serotypes normally present in domestic populations are really circulating among wild buffalo populations.
EPIDEMIOLOGICAL PATTERNS AND RISK FACTORS FOR FOOT-AND-MOUTH DISEASE EXPOSURE IN TRADITIONAL LIVESTOCK-KEEPING SYSTEMS OF NORTHERN TANZANIA

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Introduction:
Despite being one of the most important diseases affecting livestock production and trading globally, the study of foot-and-mouth disease (FMD) in traditional livestock-keeping systems in sub-Saharan Africa has received little attention. As a consequence we have a limited understanding of the epidemiology of the disease in these systems and of suitable control approaches. Critical questions relate to the importance of livestock- and wildlife-related factors in the maintenance and transmission of FMD around wildlife-protected areas.

Materials and methods:
A serological survey of FMD non-structural protein antibody in livestock and buffaloes, and household questionnaires were conducted in the Serengeti, Ngorongoro, Tarangire and Arusha ecosystems of northern Tanzania. Generalised linear mixed models were constructed with seropositivity as a binomial response variable, controlling for tribe/district, herd and animal age. The following predictors were screened using univariable analyses: species; production system (pastoralist/agro-pastoralist/rural smallholder); distance walked for grazing/watering; and wildlife-related predictors (distance to protected-area boundaries and frequency of interaction with wildlife).

Results:
Seroprevalence varied among ecosystems, with high levels of seropositivity in livestock in pastoralist (64% and 66% in Ngorongoro and Tarangire, respectively) and agro-pastoralist (67% in Serengeti) ecosystems, and lower rates in rural smallholder systems (34% in Arusha). Seroprevalence in buffaloes was high in Ngorongoro, Serengeti and Tarangire (90%, 69% and 96%, respectively) and lower in Arusha (48%). Age and herd were determined to be important factors to have controlled for in the model, and other significant predictors were species (higher seroprevalence in cattle) and production system (lower seroprevalence for smallholder systems). No grazing/watering and wildlife-related predictors were significant.

Discussion:
We demonstrated that FMD is very prevalent in traditional livestock-keeping systems across northern Tanzania and that non-wildlife-related risk factors are likely to be important. Exploring other predictors related to livestock management and movement will allow us to further investigate livestock-related risk factors.
RETROSPECTIVE SEROSURVEY OF FOOT AND MOUTH DISEASE (FMD) IN FREE RANGING DOMESTIC PIGS AND WILD SUIDS IN SUB-SAHARAN AFRICAN COUNTRIES

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Introduction:
Africa is one of the major reservoirs for FMD. However, despite the accumulative information available about FMDV infection in ruminants and predominant serotypes, data about the incidence of the disease in free ranging domestic pigs and wild suids is still lacking, and the role these animals play in the spread and maintenance of the disease in endemic areas is barely known.

In order to get information on existing gaps, encouraged by EU (SANCO) and FAO, synergies were promoted to integrate also other Reference Laboratories for animal diseases in FMD studies. Thus, a retrospective serosurvey was performed using field serum samples collected during ASF surveillance programs, between 2004 and 2011 from sub-Saharan regions, thanks to the African Swine Fever (ASF) EU Reference Laboratory (CISA-INIA) through a bilateral cooperation with ILRI and the Ministries of Agriculture of the respective African countries. The objective of this work was therefore to evaluate the presence of FMDV antibodies in suids from a range of sub-Saharan African regions traditionally considered endemic for FMD, in most cases with continuous presence of more than one serotype.

Materials and methods:
Test sera. 869 domestic pig field sera were analyzed comprising: i) 618 from Kenya, ii) 96 from Tanzania, iii) 94 from Uganda and iv) 61 from Cameroon. Characteristic FMD clinical signs were not present in pigs sampled. In addition, 77 sera from African wild suids from Kenya were included. FMD Tests. FMDV recombinant 3ABC non-structural protein (NSP)-based ELISAs and immunoblotting (IB) tests were used for antibody detection and confirmation, respectively.

Results:
Antibodies to FMDV NSP were absent in most (98%) free ranging pigs and all (100%) wild suid samples examined. Most sampling sites remained negative, and few positives were only observed in five (out of 18) sampling areas.

Discussion:
The low FMDV seropositivity rate observed in this pilot study in pigs, in FMD-endemic areas in Africa, suggests that this species does not play a relevant role as a reservoir host in the epidemiology of FMD in these areas.
RISKS ASSOCIATED WITH UNOFFICIAL LIVESTOCK MOVEMENTS IN THE GREATER MEKONG REGION

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Introduction: Increasing demand for livestock and livestock products, particularly cattle and beef by Vietnam and China stimulate movement of livestock from adjacent Mekong countries. Official movement channels may be too costly or otherwise inaccessible to small traders, who make informal arrangements to move livestock across extensive international borders.

Materials and methods: Understanding of livestock movements was obtained from livestock traders in Cambodia and Laos. Information was gained by both structures questionnaires and open interviews. Traders recognised the importance of movement of live animals in the spread of disease, and were cooperative in their approach. Details of movement patterns, volumes of trade, and unofficial (i.e. uncontrolled) movements were provided by traders. Traders requested further information on diseases and disease spread. Booklets, posters and digital stories were drafted, trialled, and finally produced in quantity for distribution.

Results: Maps of trading pathways were produced, along with volumes of livestock moved, for both pigs and cattle. While most of these pathways related to official movements, unofficial movement pathways were detailed by traders. The risks from these pathways arise from the movement of stock that are infectious, and the potential for healthy stock to interact (e.g. on the sides of paths) with animals carrying disease, and therefore moving the disease to another location. The volume of this trade is unknown, but believed to be substantial. Drivers of this unofficial or informal trade are the costs (in terms of time and money) from official processes.

Discussion: Risks for the spread of FMD (and other transboundary animal diseases) through informal and uncontrolled movement of livestock in the greater Mekong region are tangible, but difficult to quantify. These movements pose a substantial threat to the health of livestock at destinations, often in adjacent countries. Reducing the risks without increasing regulatory impositions is challenging, but may be effective through the use of culturally appropriate information and educational materials targeted at relevant groups.
RISK FACTORS FOR FOOT AND MOUTH DISEASE IN BEEF CATTLE HERDS IN ISRAEL

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Introduction:
Epidemics of foot and mouth disease (FMD) occur frequently in Israel and are mostly caused by viruses of serotype O. Due to this reason vaccination of cattle, sheep and pigs in Israel are mandatory. Beef cattle herds are involved in many of the outbreaks and may serve as important driver for virus spread. It is therefore of high importance to understand the risk factors and mode of spread in beef cattle herds.

Materials and Methods:
During 2011, a large epidemic occurred in Israel, which involved 26 localities that included 30 beef herds, 11 feedlots, 4 dairy herds and 8 sheep flocks. We analyzed the risk factors for the appearance of clinical signs in adult cattle in beef herds by comparing 25 affected herds (at least 1 case in an adult cow) and 26 non-affected herds. In order to analyze the pattern of virus spread we compared the distance between each affected herd and the most adjacent herd affected prior to it and compared this figure between feedlots and beef herds.

Results:
In multivariate analysis two risk factors were discovered; abundance of calves under the age of 6 months in the herd (adjusted odds ratio (OR)=10.6 (CI95%=1.7-64.7) and the elapse of more than 6 months from last vaccination (OR=8 (1.2-53.2)). The average distance of affected feedlots from most adjacent prior affected herd was 19.9 Km (range 2-43.7 Km) while for beef herds this distance averaged only 3.2 Km (range 0.2-8.9 km) (p=0.01).

Discussion:
We conclude that the dispersion of FMD virus in beef cattle differs significantly from the dispersion to feedlots. In beef herds the virus spreads by direct contact between grazing herds and amplified by the abundance of young non-vaccinated calves, while spread to feedlot is mainly associated with long distance transportation of animals (mostly calves). These findings have important implications for the control of FMD.
RISK MAPPING OF FOOT-AND-MOUTH DISEASE PREVALENCE IN CENTRAL ASIAN COUNTRIES

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Introduction:
Seroprevalence data are becoming increasingly important to assess risks and monitor progress within the framework of the Progressive Control Pathway for FMD (PCP-FMD) control. The concurrent establishment of regional roadmaps can provide an opportunity for harmonizing serosurvey designs and thus enhancing the precision of the estimates at regional scale. In the perspective of the PCP-FMD development, a clear picture of the country and regional status and the burden of FMD seroprevalence are therefore essential to estimate. In this context, and especially in those countries at the initial stage of the PCP, the analysis of serological data is then one of the key tools to make a preliminary assessment of the epidemiological status and the level of risk of FMD infection according to different metadata, such as age-groups, farming systems, geographical zones, or to monitor progress of specific control activities, such as vaccination, aimed at reducing incidence of FMD, and subsequently prevalence, in specific production systems or zones. The data discussed in this paper refers to large ruminants populations kept at village level (subsistence farming system).

Materials and Methods:
Harmonised cross-sectional surveys, using a two-stage cluster sampling design, were conducted in 2011 under the GTFS/INT/907/ITA project to estimate the seroprevalence and geographical distribution of FMD in Central Asian countries. A total of 11873 sera samples from Afghanistan (n = 3243), Pakistan (n = 3782), Tajikistan (n = 2112), Turkmenistan (n = 976) and Uzbekistan (n = 1760) were screened using the 3ABC trapping-ELISA test kit (IZSLER, Brescia, Italy) for detecting Abs against FMDV NSPs. All serum samples were collected from individuals (either cattle or buffalo) between 6 and 18 months of age. Descriptive and univariate analyses were performed using Stata SE 12.1 survey procedures. A Generalised Linear Model (Logit Link) was computed to identify potential risk factors associated with the observed prevalence, such as specie, age, farming system, vaccination, susceptible status and other variables extracted from spatial queries. Results obtained from the model were introduced in a geospatial analysis environment using ArcGIS 10 to produce risk maps of FMD prevalence distribution.

Results:
The FMD overall prevalence at regional level was found to be 24.48% (4692/11874). Different patterns of FMD seroprevalence were reported across the Central Asian countries, where 46.81%, 39.74%, 47.83%, 41.93% and 14.35% of samples were scored as positive for Afghanistan, Pakistan, Tajikistan, Turkmenistan and Uzbekistan, respectively. Computed OR were high for sheep (4.143) and buffalo (3.276) species, beef (1.717), genetic (3.090) and transhumant (4.418) farming systems, unvaccinated (1.718) and susceptible (2.354) animal sampled. Individuals sampled between 6 and 12 months and >12 months after vaccination were found at higher risk, OR 2.655 and 2.113, respectively. Spatial regional clusters defining hot-spots of FMD prevalence were reported in areas bordering between Badakhshan and Takhar provinces of Afghanistan and southern districts of the Kathlon province in Tajikistan. Within countries, risk areas were identified in Herat and Kandahar provinces of Afghanistan, and in Mary province of Turkmenistan.

Discussion:
The PCP and regional roadmaps are essential frameworks to describe the progress at national and regional level of activities aimed at improving FMD control. Moving higher to a global prospective the harmonisation of data collection and analysis should be considered as a key component of the global strategy. As an essential requisite to move forward the pathway for FMD control, the PCP indicates that, especially in early stages, serological surveys could complement other field activities to gain a better understanding of the patterns of FMD risk at different levels of the livestock farming and marketing structure or identify spatial clustering that may require joint control efforts between neighbouring countries. The vast majority of the large ruminants population of the beneficiary countries of the FAO GTFS/INT/907/ITA is kept at village level (subsistence farming system). The overall estimates may provide an insight into the level of exposure at population level which in the long run (moving higher into the PCP, typically stage 3) may be useful to assess the expected decrease of the load virus.

Appliance of science in the progressive control of FMD
Open session of the EuFMD, Jerez de la Frontera, Spain. 29-31 October 2012
PREVALENCE AND RISK FACTORS FOR FMD-NSP-ANTIBODIES IN COW AND BUFFALO CALVES, AND SMALL RUMINANTS IN EGYPT

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Introduction:
In 2009 a pilot sero-survey was conducted in five out of 26 governorates in Egypt. This was followed up (ECTAD/FAO project (MTF/INT/003/EEC) by a nationwide sero-survey on FMD-NSP antibodies in young ruminants (cattle and buffalos) 6-18 months of age and non-vaccinated small ruminants between May-December 2011 to learn about the current FMD situation.

Materials and methods:
A two-stage sample design was implemented. Governorates were assigned to five regions, reflecting regional differences in husbandry systems and geographical features. Within regions, a proportional number of villages were randomly selected. Within villages, a fixed number of 14 animals were sampled. Information on animal characteristics, management practices in the household and the situation of the village were collected through a questionnaire. Samples were tested for NSP antibodies (PRIOCHECK® FMDV NS). For the analysis, a total of 5299 animals from 310 different villages in 165 districts in 26 governorates were included. Large and small ruminants were sampled with some overlap in 298 and 82 villages, respectively.

Results:
For large ruminants, 78% of villages and 19% of animals tested positive for NSP antibodies, similar to results from the pilot sero-survey in 2009. For small ruminants these numbers were 54% and 11% respectively. In 98 (31.6%) villages, local veterinarians had seen signs of clinical FMD in the 12 months prior to the sampling. Except for the Western region, there was no difference in the proportion of infected animals in the other regions. Risk factors for FMD infection in calves were related to contacts with other villages, whereas for small ruminants risk factors were related to factors present within the village.

Discussion:
In both sero-surveys, we found a higher level of FMD infection in buffalos compared with cattle. The reason for this finding is unclear and requires more research. Animal movements between villages appeared to be main drivers of FMD virus circulation. The role of small ruminants seemed to be one of dispersion of FMD within a village rather than between villages. Control measures should focus on restricting (or conditioning) animal movements, with the primary focus on large distance movements, and vaccination may best be focussed on large ruminants rather than small ruminants.
DETERMINING THE LEVEL OF VACCINE-INDUCED VERSUS FIELD-VIRUS INDUCED ANTIBODIES IN LIVESTOCK IN WEST-AZERBAIJAN, I.R. IRAN

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Introduction: A recent sero-survey in West-Azarbijan, Iran was conducted to determine the level of FMD infection in young-stock (calves 6-24 months) using NSP ELISA. As routine vaccination campaigns are performed 3 times a year, vaccinating all livestock of 4 months and above, some of these calves may have received one or multiple doses of FMD vaccine. It is known that the locally-produced vaccine (Razi Institute, Iran) is impure and for that reason, increased NSP-Ab titres may not solely reflect FMD infection. This study has compared NSP-Ab titres to SP-Ab titres for FMD serotype A, O and Asia 1 in an effort to distinguish between FMD field-virus versus vaccine induced antibodies.

Materials and methods: Six-hundred of 8349 original samples were randomly selected to be tested for SP-Ab for FMD serotype A, O and Asia 1. Of these samples, information was known on ‘NSP titre (percentage inhibition)’, ‘age in months’, ‘estimated number of vaccinations received’, ‘booster vaccination received’, ‘location had observed clinical FMD’, ‘village’ and ‘district’. NSP testing with the Prionics ELISA was performed in WAZB and was re-evaluated in Brescia using the IZSLER ELISA. SP testing was performed in Brescia.

The assumption was that livestock vaccinated (with Razi’s trivalent vaccine) would develop SP-Ab against all FMD serotypes, whereas livestock infected with FMD field virus and not vaccinated would develop SP-Ab mainly reacting against a single FMD serotype. This assumption is tested using vaccination and clinical FMD outbreak history.

Results: The overall agreement between the two NSP-ELISA was high (Kappa-value 0.85) confirming the field results from WAZB with those from the reference laboratory in Brescia. Interpretation of SP-Ab titres in relation to NSP titers are not available yet, as SP test results were only available as of the end of July 2012.

Discussion: In the discussion, interpretation of NSP Ab test results will be related to the SP Ab titres for three different FMD serotypes. It is envisaged that this study will shed light on what advantages and disadvantages lie with the use of NSP-Ab ELISA in situations where an impure vaccine is used. This is highly relevant as in many countries in PCP-FMD Stages 0-2, impure vaccines are used, while outcomes of PCP-FMD stage 1 and 2 emphasize the need for sero-surveys to develop and monitor a risk-based FMD control strategy.
SPATIO-TEMPORAL ORIGIN AND TRANSMISSION OF THE FOOT-AND-MOUTH DISEASE VIRUS OUTBREAKS IN BURGAS REGION (BULGARIA) IN 2011

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Introduction:

On the 5th January 2011, Bulgaria notified the presence of FMD virus (FMDV) in a wild boar with vesicular lesions on the feet, losing the foot-and-mouth disease (FMD)-free-without-vaccination status maintained since 1996. This animal had been hunted close to the border with Turkish Thrace, a FMD free-with-vaccination region since 2010. Subsequent VP1 sequence analysis of the virus classified it within the ANT-10 sub-lineage of O/ME-SA/PanAsia-2, recently spread throughout the Middle East from Iran. Moreover, the Bulgarian sequence differed by only one nucleotide from sequences of contemporary FMDVs obtained from seven different provinces in Anatolia (Turkey), where the disease is endemic. The detection of FMDV in wild boar was followed by two waves 47 days and 30 Km apart including 11 outbreaks in livestock within the same region (Burgas). This study used full genome sequencing, field data and computational phylogenetics to reconstruct the spatiotemporal origin and transmission of the virus.

Materials and methods:

Nineteen full genome FMDV sequences were generated and analysed by statistical parsimony methods (TCS v1.21) and Bayesian Markov chain Monte Carlo inference (BEAST v1.6.2), including eight representative viruses from all of the virus-positive outbreaks of the disease in Bulgaria and 11 closely-related contemporary viruses from Anatolia and Israel.

Results:

All Bulgarian sequences shared a single putative common ancestor closely related to the virus from the wild boar. The next closest virus was a FMDV collected during 2010 in Bursa (Anatolia, Turkey). Within Bulgaria, two discrete genetic clusters were detected that corresponded to the two waves of outbreaks.

Discussion: The data disclosed undetected infection, in livestock and/or in wildlife (wild boar), and link the two different waves of outbreaks within the region, excluding multiple introductions of the virus. Field and laboratory data support the outcome of the analysis. This study highlights how these analyses can be used as an effective on-the-spot tool to support and help direct epidemiological investigations of field outbreaks.
MOLECULAR EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE VIRUS IN THE AFRICAN BUFFALOES IN SOUTHERN AFRICA

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Background:
Foot-and-mouth disease (FMD) is endemic in most countries in Southern Africa. African buffaloes (Syncerus caffer) are known to play a significant role in the transmission and dynamics of FMD virus (FMDV) in wildlife-livestock interface areas. The aim of this study was to investigate the serotype and determine the genetic relationships of FMDV recovered from animals in Tanzania, Zambia and Mozambique, and compare them with viruses detected from elsewhere in the sub-Saharan region.

Methods:
A total of 150 probang samples collected in 2010 from Cattle and buffaloes in Katavi (Tanzania), Lochninvar (Zambia) and Morromeu (Mozambique) National Parks were used in this study. The presence of FMDV was determined by laboratory methods such as VI, antigen ELISA and real-time RT-PCR. Phylogenies of VP1 sequences were determined by the Neighbor-joining method.

Results:
The overall FMDV genome detection rate was 6.7% (n=10), with SAT1 being the most frequent serotype (60%; n=6) isolated in cattle and buffaloes in Tanzania, Zambia and Mozambique followed by SAT 3 (30%; n=3) and SAT 2 (10%; n=13). Genotyping showed that type SAT 1 viruses fell into either the TOPOTYPE 1 (NWZ) or UNASSIGNED topotypes, type SAT 2 into the AFRICA topotype I and type SAT 3’s into topotype IV (SEZ).

Discussion:
This study reveals that serotypes SAT 1-3 are maintained in cattle and buffaloes in livestock-wildlife interface areas in Katavi, Lochinvar and Morromeu National Parks. Phylogenetic analysis of FMDV isolates from Tanzania, Zambia and Mozambique showed that they are genetically related to lineages and topotypes from Africa. This information contributes to the understanding of the epidemiology of FMD in Southern Africa. In Tanzania, Zambia and Mozambique, lack of consistent surveillance systems and animal movement controls make it difficult to determine the exact source of FMD and transmission dynamics of FMDV. Further studies are needed to elucidate the complex epidemiology of FMD in Africa.
EFFECTIVENESS OF VACCINATION PROGRAMMES

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Vaccination programmes can be highly effective in control of animal and human diseases, but they are not simple. There is large literature on the evaluation of human vaccines and vaccination programmes, much of which is relevant to veterinary vaccines and programmes. Importantly, a basic terminology is now generally agreed: vaccine potency – is a lab measure of the contents of a vaccine (eg antigen units); vaccine efficacy – is the reduction in risk of target disease in vaccinated compared to similarly exposed unvaccinated individuals, under ideal conditions (eg randomised trials); vaccine effectiveness – is the reduction in risk of target disease in vaccinated compared to similarly exposed unvaccinated individuals, under field programme conditions; vaccination programme effectiveness = Impact – is the reduction in disease or economic burden in a population, attributable to a vaccination programme.

The effectiveness of a vaccine is typically dependent on several factors, including: the quality of the vaccine itself, the cold chain and quality of administration, the number of doses, age, time since last dose, and intensity of exposure. The effectiveness of a programme is dependent upon both the effectiveness of the vaccine and the vaccine “coverage” in the population (eg by dose, age and area). Both vaccine and vaccination programme effectiveness will differ between populations and over time. Their evaluation is essential for proper management of a programme, and requires rigorous collection and analysis of appropriate data. Routine monitoring of vaccine coverage and disease incidence may provide a crude indicator of programme effectiveness, but critical investigation often reveals problems requiring changes in strategy.

Whooping cough (pertussis, attributed to Bordetella pertussis) vaccine is recommended in all populations of the world, typically three doses in infancy supplemented in some countries by a booster at 18 months and/or at 4 or more years. Many wealthy countries shifted from whole cell to acellular vaccine ten years ago. Recent increases in pertussis in several of these countries have revealed disease concentrated in older children and teenagers, reflecting that immunity from acellular vaccines wanes with time. This observation has led to calls for additional doses and/or shifting to a more immunogenic vaccine. The increased transmission has in turn meant a recent increase in infections and severe illness in very young infants – as a consequence of which The UK has just (October 2012) introduced a booster dose in pregnant women to enhance passive maternal immunity to very young infants.

Polio has been target of a global eradication programme, since 1988. The strategy has emphasised high coverage of oral (live attenuated virus) vaccines in infants, supplemented in tropical countries by repeated campaigns eg to all under age 5. Studies have revealed that these vaccines are far less effective in low-hygiene tropical settings than in northern Europe (eg 15 % versus 80 % per dose) apparently because of competition by other enteroviruses. The eradication programme has compensated for this by mounting repeated campaigns in particular populations - eg in UP and Bihar states of northern India, campaigns to all children under age 5 occur monthly. This carries immense cost (but India has now been 22 months without a case of polio from wild virus). These are typical examples of monitoring of vaccine coverage, performance and impact, and consequent programmatic responses, based upon a combination of routine surveillance and specially targeted field studies.

A variety of different FMD vaccines are now used, with different schedules, in different countries of the world. Rigorous field monitoring of vaccine coverage and effectiveness should be an integral part of programmes in order to identify problems and inform adjustments to optimise impact and cost effectiveness.
FMD ASIA-1 VACCINE EFFECTIVENESS IN TURKEY

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Introduction:
 VACCINE EFFECTIVENESS (VE) is the reduction in incidence in vaccinated animals compared to similarly exposed unvaccinated animals in the field \([1-R_v/R_n], \) where \(R_v\) and \(R_n\) are incidence rates in vaccinated and unvaccinated animals respectively. It may be affected by vaccine quality, vaccine matching, number of doses, time since last dose, vaccine delivery, exposure intensity and prior infection.

In 2011, Foot and Mouth Disease (FMD) Asia-1 serotype was detected in Turkey for the first time in nearly ten years. Unlike serotypes A and O, livestock lacked immunity from prior Asia-1 exposure and most had not been vaccinated against this serotype.

Materials and methods:
 In January 2012, an FMD Asia-1 outbreak was investigated in Afyon, Turkey to assess a new vaccine based on the 2011 Turkish FMD Asia-1 field strain (TUR 2011) first used in October 2011. No animals had received more than one dose of this vaccine. The two outbreak villages consisted of many small herds of cattle housed at the time of the outbreak. 229 cattle were selected by systematic sampling. Vaccination and FMD status were ascertained from the owner. FMD status was also assessed by clinical examination and serology.

Results:
 Cumulative incidence of clinical FMD was \(9/63=14\%\) and \(53/101=52\%\) among cattle with and without prior Asia-1 vaccination, respectively. Crude VE of the Asia-1 TUR 2011 vaccine was 73\% [95\% confidence interval (CI)=54\%-84\%]. After controlling for age and sex this protective effect was still present. However, the vaccine did not appear to prevent infection assessed via serology; VE=4\% [95\%CI=\(-42\%\) to \(35\%\)]. Only 44\% of examined animals had been vaccinated during the last vaccination campaign.

Discussion:
 Caution should be taken when extrapolating the results of a single, retrospective, observational study. However, the new vaccine appeared to be effective at preventing clinical disease but not infection under these circumstances.
AN INVESTIGATION OF VACCINATION EFFECTIVENESS IN TWO CAMBODIAN VILLAGES FACING AN OUTBREAK OF FOOT-AND-MOUTH-DISEASE

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Introduction:
An outbreak of Foot-and-Mouth Disease (FMD) in Cambodia in 2010 provided an opportunity to investigate the effectiveness of FMD vaccination in the face of an FMD outbreak. A limited vaccination program using donated FMD vaccine was managed by local veterinary authorities in Kampong Cham province. Because the vaccination program wasn’t supervised by the central veterinary authorities and didn’t take place in the controlled environment of a research project, the results provide a ‘real world’ indication of vaccination effectiveness in Cambodia.

Materials and Methods:
The Australian Centre for International Agricultural Research (ACIAR) investigated livestock movements and the spread of FMD in Cambodia in collaboration with the Cambodian Department of Animal Health and Production (DAHP). Research was conducted in seven villages in Cambodia’s Kampong Cham province which were affected by the 2010 FMD outbreak, two of which had received a quantity of donated FMD vaccine to use as a protective measure against the advancing FMD outbreak. Information was obtained from the records of village animal health workers (VAHWs) and district and provincial veterinary authorities, and from interviews with VAHWs, villagers, village chiefs, and district and provincial veterinary officers.

Results:
In Chrey Vien village there was a statistically significant difference (p = 0.000565) between the proportion of vaccinated (51.43%) and unvaccinated (71.52%) animals showing signs of FMD. In Tropeang Ampil village, the difference in FMD attack rates between vaccinated (57.2%) and unvaccinated (61.54%) animals was not statistically significant at the 5% significance level (p = 0.524).

Discussion:
Likely reasons for the poor results included inadequate vaccination coverage to produce herd immunity, uncontrolled movement of sick animals during the outbreak, overwhelming infection challenge created by roadside tethering and communal grazing of livestock, and weaknesses in planning and carrying out the vaccination process. Implications for the management of donated FMD vaccine in Cambodia are discussed.
THE FIELD EFFECTIVENESS OF INACTIVATED VACCINE FOR PREVENTION OF FOOT AND MOUTH DISEASE

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Introduction:
Inactivated foot and mouth disease (FMD) vaccines are used in many countries either to prevent or to control outbreaks. In non-endemic countries, high potency vaccines are used during outbreaks for emergency vaccination in order to prevent virus spread, while in endemic countries vaccines are used for routine vaccination. Despite their wide use there is little data on the field effectiveness of inactivated FMD vaccines. Epidemics of FMD occur frequently in Israel and are mostly caused by viruses of serotype O. Therefore, cattle, sheep and pigs in Israel are routinely vaccinated with a high potency vaccine (≥6PD₅₀), which may be used also for emergency vaccination during outbreaks.

Materials and Methods:
During 2011 a large outbreak of FMD, caused by a virus of serotype O, occurred in Israel and affected many herds of cattle and sheep. We investigated one of these outbreaks, which took place in a feedlot and an adjacent dairy herd. Comparison of morbidity and antibodies to NSP enabled the assessment of the effectiveness of various vaccine regimes and reactive vaccination.

Results:
Infection prevalence reached 96% in calves that received two doses of vaccine at least three months prior to the outbreak. Almost 100% of these calves showed clinical signs compatible with FMD. Heifer calves vaccinated 3-5 times, 7 months prior to the outbreak showed 100% infection and 18% showed clinical signs. As opposed to these groups, animals vaccinated as low as only once but up to two weeks before the outbreak, were almost 100% protected from clinical disease and to a lesser extent, protected from FMD virus infection.

Discussion:
Reactive vaccination was highly effective for prevention of clinical and sub-clinical infection and should be encouraged during outbreaks. However, routine vaccination with the same vaccine provided only limited protection due to poor longevity of the immune response.
FOOT AND MOUTH DISEASE: VACCINE IMPACT AND PROGRESSIVE CONTROL IN INDIA

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Introduction:
Foot and mouth disease is a highly contagious and trans-boundary disease for all cloven footed animals including wild animals. The disease is globally recognized for huge economic loss including export barrier and a major threat to food security. Economic loss due to reduction in milk, meat and wool production and reduction in draught power of animals affecting Indian GDP. Thus national immunization programme is launched to control and eventually eradicate the disease from India. Foot and mouth disease vaccine is an important tool in controlling the disease through vaccination which will provide self gainful employment to the rural folks.

Materials and Methods:
Foot and mouth disease vaccine was developed on production scale adopting Dr. Frankel’s method for using in Europe for a prophylactic vaccination programme. Subsequently, significant development of FMDV antigen production in bioreactor system using cell culture system. They exploited the ability of BHK21 cells to grow in deep suspension cultures in large scale Bioreactor under control conditions widely used in India.

Results:
The quality vaccine produced and delivered at grass root level under cold chain in India following FAO/OIE pathway. Vaccine manufacturers produce trivalent O, A and Asia-1 serotype vaccine and try to cover 528 million domestic animals; cattle 199m, goat 140.5m and sheep 71.5m. Due to resource constraint, this is planned in phased manner. The population is at risk due to less coverage of population. Direct loss is more than 05 billion US$ per year.

Discussion:
FMD control programme is covered 221 districts across India. South American model will be a guiding principle for the progressive control of FMD in India and other endemic nations.
A HIGH THROUGHPUT LIQUID PHASE BLOCKING ELISA FOR QUANTITATIVE ESTIMATION OF ANTIBODY TITERS AGAINST STRUCTURAL PROTEINS OF FOOT-AND-MOUTH DISEASE VIRUS

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Introduction: Currently FMD sero-surveillance is mainly performed by a LPB-ELISA. With the present format of ELISA, only 11 samples can be titrated on a plate for one serotype. With implementation of FMD control programme in India, the number of sera to be tested is increasing. The current assay format is cumbersome and expensive with respect to large scale testing. To circumvent this drawback, antibody responses were titered by an alternate format of ELISA utilizing interpolation of the titers by linear regression method.

Materials and methods: Reference serum standards were prepared by diluting the bovine vaccinated serum with fetal bovine serum (FBS) so as to get a range of antibody titres. The standards were lyophilized and evaluated for thermo-stability. LPBE assay with single dilution of test serum (1:64) and serial two-fold dilution of eight reference standards (1:32 to 1:128) was standardized. Titer in terms of 50% inhibition in OD of the test serum was interpolated by linear regression of the reference standards.

Results: The lyophilized sera with titers between 1.5 and 2.4 were stable up to 1 year. The coefficient of regression (r) between calculated and the reference titers were >0.9. The optimized assay had high correlation with the conventional LPBE (≥0.97). The assay was validated by testing vaccinated (n=1360) and bovine sera collected at random (n=3060).

Discussion: The limiting factor for estimation of titers by linear regression is the reference sera panel as minor changes in the titer of the sera affects the calculated titers. The problem was addressed by diluting the bovine vaccinated sera with FBS to obtain large volume of sera with range of titers (between 1.5 and 2.4). Once prepared, the reference sera panel was lyophilized and could be used for up to 1 year. In this format, 34 sera on one plate can be tested. This high throughput assay will not only save time and man power but also the costly reagents.
NEW ELISAs FOR FMD DIAGNOSIS

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Robust and simple diagnostic kits are essential complements of the measures for disease prevention and control; for FMD, their availability is particularly crucial in endemic countries adopting the Progressive Control Pathway. However, the availability of such kits for FMD diagnosis has been historically very limited, also due to the restrictions in handling of FMD viruses and to a not easy accessibility to samples intended for assay validation.

To overcome this shortcoming, one of the objectives of an EU-funded project (FMD-Disconvac) was the development of ready-to-use ELISA kits for the detection of FMD viruses and anti-FMDV antibodies.

The antigen detection ELISA is still an effective tool for the rapid confirmation of FMD on epithelium vesicle samples. Therefore, simplified sandwich ELISAs were designed using a panel of serotype-specific monoclonal antibodies (MAbs), coated onto ELISA plates as catching antibodies, and conjugated cross-reactive MAbs as tracers. One kit was designed for detection and serotyping of O, A, Asia1 and C-type FMD viruses, while another one was tailored for African countries, thus including diagnosis of serotypes O, A, SAT1 and SAT2. Antigen capture is performed thanks to the broad intra-typic reactivity of the selected catching MAbs; in addition, a pan-FMDV test, detecting any isolate of types O, A, C, Asia1 as well as some isolates of the SAT serotypes, is included in the kits.

The test is fast (2.5 hours) and simple: microplates are supplied pre-coated with catching MAbs and with positive controls already adsorbed onto plates, whilst the operator makes use of one or at most two immunological reagents, corresponding to detector conjugates. The diagnostic performances of the new kits were shown to be similar or better than those of the more complex polyclonal double-sandwich ELISA and the kits have been appreciated by users in West Eurasia, Middle East and Africa.

FMD serology is mainly based on ELISA assays, both for NSP and SP-serotype-specific antibody detection. In order to complete the spectrum of diagnostic kits for FMD serology, a set of three user-friendly and stable ELISA kits for the measurement of antibodies to FMDV serotypes O, A, Asia1 was developed. The reaction, that originated from in-house Solid-Phase Competitive ELISAs, requires only two incubation steps: incubation of test sera in plates, which are supplied pre-sensitized with FMD viruses trapped by specific MAbs, followed by addition of a homologous conjugated MAb to measure inhibition of binding caused by positive sera. Internal (IZSLER, Italy) and external (WRL, UK) validation confirmed adequate sensitivity of the test, irrespective of the antigenic FMDV variants that elicited antibodies; specificity was shown to range from 99.7% to 100% for the three ELISAs. The development of similar kits for SAT1 and SAT2-specific antibody is in progress.
FMD AND SVD COMBINED PROFICIENCY TEST STUDIES 2011

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Introduction:
The aim of the exercise was to complete a proficiency testing study for virology and serology diagnosis for FMD and SVD during 2011 to enable a clear picture of the performance of tests, the scale of activities and the state of QA accreditation from participating laboratories of Member States and other regions. The particular tests requested were not specified, instead labs were invited to select tests and interpret the results as if the samples were from FMD suspected cases.

Materials and Methods:
The study consisted of four panels, for virology and serology diagnosis. Each panel had a different hypothetical case. Laboratories were invited to participate, and samples were dispatched on request along with a set of instructions and a template for results. The laboratories were asked to answer which samples were FMDV or SVDV positive or negative in which tests, and an overall interpretation for each sample and case. They were also asked to give details about each test they used to enable analysis of possible causes of discrepancies.

Results:
Fifty four out of the fifty six laboratories that samples were sent out to have responded with their results. The results were collated, analysed and reported. Many of the results from the labs corresponded well, however there were some discrepancies, due to sensitivity or specificity issues, or lack of the specific tests.

Discussion:
Some labs did not send the results back as requested, and others had samples damaged due to delays in transfer times. The overall performance from the labs will be reviewed and presented at the meeting.
NEW ROLES FOR “AUXILIARY LABS” IN THE DIAGNOSIS OF FMD?

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According to currently EU legislation, the laboratory diagnosis of suspect cases of FMD involving handling of materials from infected holdings requires a laboratory listed in Annex XII of Council Directive 2003/85/EC which has to meet the FAO EuFMD “Minimum Standards”. The same applies to any in-vitro research with infective FMDV. It has turned out that in most countries not all the diagnostic tasks in the framework of FMD control can be carried out in such listed FMD laboratories, because there are too few of them available and these labs are usually research facilities with a limited sample throughput. Therefore regional or “auxiliary laboratories” have become part of many contingency plans. They are dealt with in No. 13 of Annex XV of Council Directive 2003/85/EC and in Annex II of the “Minimum Standards”. In the later regulation, it is stated that such “auxiliary laboratories” only have to meet very limited containment requirements, i.e. quarantine for staff in respect to susceptible animals and autoclaving of waste, but no negative pressure is required and makeshift shower facilities are acceptable. According to Annex II of the “Minimum Standards”, in an outbreak situation, such auxiliary labs can process samples from holdings without clinical signs (serological samples in the framework of disease surveillance) as well as samples from holdings with clinical signs (PCR tests to detect virus), the later kind after inactivation on the premise. In contrast to the expectation when the “Minimum Standards” were drafted, currently still no validated protocol exists for the inactivation of FMD samples on the premise and there are doubts whether this really is the best approach to the problem. While it is possible to inactivate virus in swabs by putting them into a buffer containing guanidinium-isothiocyanate, this procedure may not always completely inactivate the virus in larger solid pieces of tissue and it is not feasible for serum samples one would like to test also for antibodies. Therefore, during the 2010 FMD epidemic in Bulgaria, the EU has tolerated that also non-inactivated samples from holdings with clinical signs were examined in a Bulgarian laboratory not meeting the requirements of the “Minimum Standards”. It can now be concluded that this approach worked very well. The Bulgarian lab quickly produced valuable results on the FMD situation in the country which were crucial for the control of the disease and there is no reason to assume that the activities of the lab posed any inappropriate risk to the environment. The alternative to tolerating the examination of Bulgarian field samples in this laboratory would have been to send all suspect samples to a foreign laboratory, which in particular in times of crisis, is a logistical and communication nightmare and would have substantially increased the turn-over time while reducing the throughput. While originally, “auxiliary laboratories” were mainly considered as a supplement to a listed FMD lab within a country, it now appears that we have to reconsider their role. In accordance with Article 66 of Council Directive 2003/85/EC, the EU FVO carried out a series of audits on the bio-risk management systems applied at laboratories authorized to handle live FMD virus between 2009 and 2012. One of the findings was that some laboratories need considerable investment, mainly into their effluent treatment and air handling plants. Unfortunately, not all member states are able to afford such an investment. Furthermore, it appears that for economic reasons not even all the European countries with a high livestock density will maintain a FMD laboratory meeting the “Minimum Standards”. However, for effective and swift disease control, it is crucial that official vets as well as the national crisis centres can contact a diagnostic laboratory with staff that is familiar with national legislation and conditions without a language barrier at any time. An absolute requirement to send any samples for FMD testing to a foreign laboratory would considerably raise the psychological threshold for sending any samples at all. Furthermore, in case of an FMD outbreak in a country with a high livestock density and export volume, any major European FMD laboratory which theoretically may be able to help will soon be stressed to the limit by the examination of suspect samples from its own country, even if the country is not (yet) affected.

Therefore suggestions are made for modifications of No. 13 of Annex XV of Council Directive 2003/85/EC and Annex II of the “Minimum Standards” for “auxiliary laboratories”. While we have to insist that “auxiliary labs” not meeting the “Minimum Standards” must not handle live FMDV in “peace times” and only examine suspect samples send in by the national veterinary service by methods that don’t require live FMDV as reagents, small risk-based adaptions of the legal documents would put something that is already done on a sound regulatory basis and actually help to decrease and control the risk posed by FMD.
LABORATORY CAPACITY FOR DIAGNOSIS OF FOOT-AND-MOUTH DISEASE IN EASTERN AFRICA: IMPLICATION ON PROGRESSIVE CONTROL PATHWAY

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**Introduction:**
Accurate diagnosis is pertinent to any disease control programme. If Eastern Africa is to work towards control of foot and mouth disease (FMD) using the progressive control pathway (PCP) tool, the capacity of National Reference Laboratories (NRLs) should match this task. We assessed the laboratory capacity of 14 FMD NRLs under Eastern Africa Regional Laboratory Network and its implications on the PCP.

**Materials and methods:**
Semi-structured questionnaires and retrospective data from World Reference Laboratory for FMD (WRLFMD) annual reports and Genbank\(^\circledR\) for the period 2006-2010 were used.

**Results:**
The questionnaire response rate was 13/14 (93%). Only Kenya and Ethiopia had laboratories at biosecurity level 3 and had serotyped causal FMDV in the study years. Twelve NRLs used serological techniques and 3/12 had added molecular techniques, which were the tests requested from collaborating laboratories by most NRLs. Six out of thirteen did not submit samples to WRLFMD for free typing while those that submitted were inconsistent. Only 4/13 NRLs participated in proficiency testing for FMD and 7/13 had quality management systems (QMS) which were still deficient thus, none of the NRLs had achieved accreditation for FMD diagnosis.

**Discussion:**
The high dependence on serological techniques, known to show cross reactions (Mackay et al., 2001), and failure or inconsistency in submitting outbreak samples to WRLFMD greatly contribute to the obscured regional FMDV overview. Furthermore, the deficient QMS creates doubt about accuracy and reliability of test results (De Clercq et al., 2008) and may lead to use of inappropriate or multivalent vaccines, which increase the cost of vaccines and reduce the number of animals vaccinated. Therefore, for Eastern Africa to progress on the PCP it is necessary to: implement regional control measures, improve the serological tests and laboratory capacity of the NRLs, and establish a regional reference laboratory to enforce QMS and molecular diagnosis.

Appliance of science in the progressive control of FMD
Open session of the EuFMD, Jerez de la Frontera, Spain. 29-31 October 2012
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EVALUATION OF FTA® CARDS AS A LABORATORY AND FIELD SAMPLING DEVICE FOR THE DETECTION AND SEROTYPING OF FOOT AND MOUTH DISEASE VIRUS

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Introduction:
Foot-and-mouth disease virus (FMDV) samples transported to the laboratory from far and inaccessible areas for serodiagnosis pose a major problem in a tropical country like India, where there is extreme temperature fluctuation. The present study evaluated the usefulness of FTA® Classic cards for the collection, shipment, storage and identification of the FMDV genome by RT-PCR, real-time RT-PCR, RT-LAMP and sequencing.

Materials and methods:
The impression smears were made on the FTA® cards directly from the tongue and foot epithelial samples obtained from experimentally infected animals used for FMD vaccine potency studies and naturally affected animals in the field. The impregnated cards were transported to various destinations across India at ambient temperature during summer and winter months by post or courier and stored for at least three days at each destination. The temperature and relative humidity were recorded. The impregnated cards were received at laboratory after 45 days of transport, storage at different places in India. The cards on receipt at Hyderabad were stored at 20-25ºC till testing. One set of FTA card samples and 10% virus suspension were directly exposed to the environmental temperature for about 30 days at Hyderabad. The cards were processed as reported earlier (Muthukrishnan et al., 2008).

Results:
The Maximum and minimum temperature was 21-45 ºC during transport and 25-44 ºC at Hyderabad. The RT-PCR, RT-LAMP and qRT-PCR could detect all the samples of serotype O, A and Asia 1 from the transport study and exposed to environmental temperature. FMDV 1D (VP1), IRES and 3D region was amplified and sequenced using RNA from the FTA cards. However, the virus suspension sample which was exposed to sunlight and/or stored at 20-25ºC was positive for 5 days.

Discussion:
The stability of the viral RNA, the absence of infectivity and ease of processing the sample for molecular methods make the FTA® cards an ideal alternative for transport of FMDV genome for identification and serotyping. The method can be used routinely for FMDV research.
DEVELOPMENT OF RNA TRANSFECTION METHOD FOR RESCUE OF FMD VIRUS IN SUSCEPTIBLE CELL

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Introduction:

Foot and mouth disease (FMD) virus isolation is done by serial passaging of infected tissue material in cell culture, where generally 10-30% revival can be achieved. In this study, RNA transfection protocol in BHK-21 cells was developed and applied to rescue live FMD virus from suspected clinical material obtained from cattle, buffalo, pig and sheep. From a sizable number of samples unfit for virus isolation in cell culture, virus could be isolated in this method.

Materials and methods:

Method was optimized to rescue FMD virus from total RNA preparations extracted from clinical samples using commercial kits. Optimal concentrations of isolated RNA and transfection reagent was determined and used to transfect BHK-21 cells for maximum recovery of virus.

Results:

Cytopathic effect could be observed at a minimum concentration of 50 ng RNA per 10⁵ cells in a 24 well plate. Supernatants from such cultures were subsequently given serial passages in BHK-21 cells in order to isolate the virus. A total of 144 RNA preparations, positive in mPCR, were individually transfected in BHK 21 cells, of which 100 could be revived (76 serotype O, 7 serotype A and 17 serotype Asia1), whereas only 19 samples could be revived by conventional cell culture isolation.

Discussion:

FMD viruses of serotype O, A and Asia1 were successfully rescued from the RNA samples (revival rate of 69.5% by transfection as against 13.1% by conventional virus isolation method). The optimized transfection method helped in diagnosis in difficult clinical samples showing usefulness of RNA transfection technique in isolation of the virus. Nucleotide sequence of the capsid coding region of the viruses isolated by transfection and conventional method were 100% identical, indicating authenticity of this method for isolation of virus for diagnosis.
OPEN-FMD: A POSSIBLE RESOURCE FOR AUTOMATIC AND CURATED NOMENCLATURES AND TOOLS FOR THE FMD COMMUNITY

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Sequencing viral genomes has become routine and relatively inexpensive resulting in an exponential increase in viral sequences deposited in genetic databases. GenBank/EMBL/DDBJ is the major public repository database but sequences can only be modified by the submitters, making it impossible to curate data to correct missing or wrong annotations. Creating specialized virus-specific databases in which data are annotated and standardized to provide high quality sequences can overcome this problem. Such databases have been created for influenza, human immunodeficiency virus, dengue, etc. These databases offer tools and interfaces useful to the scientific community and adapted to each virus.

OpenFluDB was created in 2009 for the influenza community. It uses an influenza-specific data model and a set of automatic tools that allow efficient data processing, data sanity surveillance and curation. Several analysis tools are incorporated in this database, including Sequence Similarity Maps (which support large scale visualization of virus evolution, complementary to phylogenetic trees), multiple sequence alignments and phylogenetic tools. More recently, joint efforts by FAO and SIB led to the creation of a genetic module within the FAO EMPRES-i global animal health database. This module links epidemiological data related to influenza outbreaks present in EMPRES-i with genetic data, present in OpenfluDB. Integration of viral characteristics into an animal disease database provides a unique tool to improve knowledge in disease epidemiology and ecology and highlights the need for curation efforts in terms of improving the reusability of such knowledge.

FMDV is a major animal disease able to infect several hosts and which genome has high genetic diversity. Like Influenza, the knowledge on FMDV could be improved with a public database streamlining FMDV needs and able to link genomic sequences to serotype/genotype, epidemiological, and outbreaks data. This sequence resource could provide dedicated tools like genotype/serotype prediction or recombination analysis. A curated public database would offer the best set of sequence and tools for phylogenetic analysis.
DEVELOPMENT AND EVALUATION OF A REAL-TIME REVERSE TRANSCRIPTION-LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR RAPID SEROTYPING OF FOOT-AND-MOUTH DISEASE VIRUS

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Introduction:
Laboratory diagnosis of FMDV is mainly carried out by virus isolation in cell culture and genome detection by reverse transcriptase PCR (RT-PCR) or by real-time RT-PCR (qRT-PCR). The purpose of the present study is to develop and evaluate FMDV strain-specific (O, A and Asia 1) RT-LAMP assays for the rapid detection and serotyping of FMDV in field.

Materials and methods:
FMDV used in the RT-LAMP assays were derived from clinical samples i.e. tongue and foot epithelium collected from clinical cases of FMD in the field as well as from the experimentally infected cattle and buffaloes at Indian Immunologicals. The partial P1 sequence of O/TNN/24/84, FMDV A/HAH/14/00 and Asia 1/WBN/117/85 were used for designing strain-specific RT-LAMP primers. The RT-LAMP was carried out in a final reaction volume of 25 µl using a Loopamp RNA amplification kit.

Results:
The RT-LAMP assay was found to be $10^3$ to $10^5$ fold more sensitive in comparison with RT-PCR, with a detection limit ranging from $10^{-3}$ to $10^{-5}$ TCID$_{50}$ of virus samples of all three serotypes. The RT-LAMP assay and qRT-PCR could detect 100 percent of clinical samples of three serotypes, whereas the RT-PCR detected 69.7% of type O, 58.1% of type A and 60.0% of Asia 1 samples. The assay conditions with absence of cross reactivity within the three serotypes of FMDV and FMDV negative samples were established.

Discussion:
In comparison with the performance of the RT-PCR; the RT-LAMP appears to be more sensitive, rapid and specific, with the potential for use as a point-of-care (POC) test, especially in developing countries.
NOTES
DIAGNOSTIC PERFORMANCE OF AN IMMUNOCHROMATOGRAPHIC LATERAL-FLOW STRIP TEST USING GENERIC RAPIDASSAY DEVICE FOR DETECTION AND SEROTYPING OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPES O OR ASIA 1 IN CLINICAL SAMPLES

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Introduction:
The development and laboratory validation of a rapid immunochromatographic lateral-flow strip test (ILFST) were described for the pen-side diagnosis of foot-and-mouth disease (FMD). However, in general it performed less well in term of the diagnostic sensitivity. To address this issue, we used a generic RapidAssay Device (gRAD) to develop a new generation of FMDV serotype O-specific ILFST (O_ILFST) and Asia 1 (Asia 1 ILFST). In the present study, both ILFSTs were validated with field vesicular epithelia samples.

Materials and methods:
A total of 96 and 126 vesicular epithelia samples, which were submitted to the FAO World Reference Laboratory for FMD (WRL for FMD) from 2008 to 2012, were used to evaluate O_ILFST and Asia 1 ILFST, respectively. An ILFST consists of a serotype independent monoclonal antibody (Mab) conjugated to colloidal gold particles as the detection antibody and serotype specific Mabs biotinylated as the capture antibody. The test reagent was mixed with the sample before it was applied to the device. The biotinylated capture antibody is immobilized at the test line.

Results:
The overall sensitivity of O_ILFST was similar to antigen detection ELISA (Ag-ELISA) (95.45 versus 96.97%) while the sensitivity of Asia 1_ILFST was higher than Ag-ELISA (64.29 versus 52.38%). When compared to Ag-ELISA, O_ILFST was 100% specific and 98.44% sensitive while Asia 1_ILFST was 100% specific and 122.73% sensitive. All of either non-O or non-Asia 1 and no virus detected samples (n = 30 for O_ILFST and n = 84 for Asia 1 ILFST) were scored negative. In comparison to RT-PCR, the performance of O or Asia 1_ILFST had a sensitivity of 95.45 and 64.29%, respectively. All samples scored negative by RT-PCR were also scored negative by both ILFSTs.

Discussion:
The results indicate that both O_ILFST and Asia 1_ILFST were highly sensitive and specific in detecting and serotyping FMDV type O or Asia 1 present in clinical samples, respectively, and would be useful tools to rapidly detect FMDV O or Asia 1 in the field as well as at a diagnostic laboratory.
DEVELOPMENT AND EVALUATION OF A ONE-STEP DUPLEX REAL TIME RT-PCR FOR DIAGNOSIS OF FOOT AND MOUTH DISEASE

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Introduction: FMD is a highly contagious and economically devastating viral trans-boundary disease of cloven-hooved animals. This study reports the development of a one-step duplex rtRT-PCR method for FMD virus detection and typing of the three serotypes O, A and Asia1.

Material & Methods: RT-PCRs were developed by using primer sets targeting 3D gene, IRES, VP1 gene and β-actin. FMDV artificially spiked samples were prepared by making 10 fold serial dilutions of the strains OMnisa, A22 or Asia1 in FMDV-free bovine tongue epithelial tissue suspension. Each dilution was subjected to virus isolation (VI), Antigen Capture ELISA (Ag-ELISA), Lateral flow device test (LFD, Svanodip), the pan-FMDV and typing O, A or Asia1 one step duplex rtRT-PCRs as well as a conventional two step simplex rtRT-PCR. All rtRT-PCR protocols were evaluated with 19 FMDV-positive samples from West Africa.

Results: Both pan-FMDV rtRT-PCRs were more sensitive than the Ag-ELISA and LFD test. VI and all rtRT-PCRs displayed similar sensitivity. The 3D/β-actin one-step was the most sensitive within molecular methods. The typing rtRT-PCR was slightly less sensitive than the pan-FMDV one-step rtRT-PCR. When using the field samples, the one-step pan-FMDV rtRT-PCRs were more sensitive than the two-step method. Four samples found negative for IRES by a two-step rtRT-PCR were positive using the one-step method. Typing protocols failed to detect the 19 field samples.

Discussion & conclusion: The one-step duplex rtRT-PCR method is more sensitive than other tested methods recommended for FMDV detection. Simultaneous detection of FMDV and β-actin within the same reaction allows the exclusion of false negatives that may result from improper extraction or degradation of the RNA, and permits normalization of the results. New primers and probes specific to WA lineages should be designed and evaluated. Thus, multiplex one-step rtRT-PCRs could be of interest for FMD diagnostic laboratories and provide an improvement for rapid detection of FMDV.
THE DEVELOPMENT AND EVALUATION OF A SAT-ADAPTED 3ABC DIVA TEST FOR FOOT-AND-MOUTH DISEASE VIRUS IN THE SOUTHERN AFRICA CONTEXT

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Introduction:

FMD is controlled in Southern Africa essentially through vaccination, restriction of animal movement and frequent animal inspections in the controlled areas. The use of serological tests that can distinguish between vaccinated and infected animals (DIVA) is of utmost importance to gain FMD-free status following an outbreak. The genetic heterogeneity of the FMDV 3ABC-coding region of the SAT type viruses indicates that the current tests may not be sensitive in the southern African sub-region. Thus, developing a 3ABC ELISA using SAT-type antigens might exhibit a higher level of sensitivity towards the SAT types.

Materials and methods:

A truncated peptide of a SAT-type 3ABC (tr3ABC) was successfully replaced in the type O recombinant 3ABC antigen of the IZSLER DIVA ELISA format (IZSLER-3ABC-ELISA). Validation of the assay included testing the SAT-adapted ELISA (SAT-3ABC-ELISA) against a total of 1946 bovine sera: naive (n=601), FMDV experimentally infected (n=215), and FMDV vaccinated and sampled during a SAT 1 outbreak (n=1130). Samples were tested in parallel with the IZSLER-3ABC-ELISA and the commercial Priocheck kit (Priocheck-ELISA). Results were compared using Cochran’s Q and McNemar’s tests and a Bayesian latent class analysis was performed to estimate sensitivity and specificity within vaccinated cattle group.

Results:

Specificity of the three ELISAs within the naïve cattle group was similar i.e 99.3% (Priocheck-ELISA), 98.0% (IZSLER-3ABC-ELISA) and 96.5% (SAT-3ABC-ELISA). The sensitivity within the experimentally infected group varied significantly with Priocheck-ELISA (93.3%) > IZSLER-3ABC-ELISA (85.6%) > SAT-3ABC-ELISA (76.7%). However, within the field vaccinated group exposed to-SAT1 infection, the sensitivity for the SAT-3ABC-ELISA was the highest (89.3%) followed by IZSLER-3ABC-ELISA (72.2%) and Priocheck-ELISA (69.3%). Additionally, the specificity was >96% for all assays within this group of cattle.

Discussion:

The accuracy of SAT-3ABC-ELISA was comparable to the commercial Priocheck kit and the IE assay. Thus, the SAT-3ABC-ELISA ELISA is a viable alternative to the more expensive commercial kits.
DETECTION, ISOLATION, AND TYPING OF FOOT AND MOUTH DISEASE VIRUS FROM ORAL SWAB SAMPLES COLLECTED FROM BALOCHISTAN PROVINCE OF PAKISTAN

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Introduction:

Foot And Mouth Disease is endemic in Pakistan, Afghanistan and Iran. Balochistan province of Pakistan has importance because of common borders with Afghanistan and Iran. Sample collection for the diagnosis of the disease is very difficult in this region due to less developed means of transportation, lengthy and difficult to travel routes. This study reports isolation and characterization of FMDV from suspected animals in Balochistan.

Materials and Methods:

Oral swab samples collected in buffer with glycerol (50:50) were tested for virus isolation and detection through conventional and real time RT-PCR. Positive samples were further characterized for the serotype by Ag-ELISA (IZSLER) and RT-PCR. Full sequences of VP1 were determined and used for Phylogenetic analysis.

Results:

Out of 35 samples 8 were positive for virus isolation, 23 were positive by RT-PCR but negative for virus isolation. 21 samples were of serotype A and 9 of serotype Asia1. Dual infection with serotype A and Asia was also detected. Two lineages of serotype A were identified. Phylogenetic analysis showed a cross relation to the strains circulating in the region.

Discussion:

We report the first isolation and characterization of FMDV from Balochistan. Two serotypes A and Asia1 were identified. This study revealed the presence of two different strains of type A. One of them is closely related to the strains recently identified in Kazakhstan (2012) and Kyrgyzstan (2011) and is different from other type A and has not been reported previously in Pakistan. Serotype Asia is closely related to the strains circulating in the region. This study provides new insight in the epidemiology of FMDV in Pakistan by characterization for the first time the strain circulating in Balochistan province. In addition, methodologies used for the detection and characterization of FMDV from oral swabs can assist with rapid and accurate disease diagnosis.
PARALLEL SESSION
THE CELLULAR INNATE IMMUNE RESPONSE DURING ACUTE INFECTION OF CATTLE AND SWINE WITH FOOT-AND-MOUTH DISEASE VIRUS (FMDV)

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**Introduction:**
Early, innate immune responses to viral pathogens are mediated by multiple cell types. For example, dendritic cells (DCs) are efficient at secreting type I interferons that block viral infections from spreading. In addition, viral infections can be controlled by activation of natural killer (NK) cells to lyse infected cells in an antigen independent manner, thus aborting production of new virus. More recent data has demonstrated that a prominent class of circulating lymphocytes in livestock, found in lower numbers in humans, the gamma delta T cell (γδ T cell), can be transiently activated to “NK-like” killing of virus infected cells.

**Results:**
We have previously reported that the interferon response of multiple subsets of dendritic cells is blocked following infection of swine with FMDV. In most cases the lost interferon response recovers rapidly, but the response of Langerhans cells (of the skin) requires much more time, more than 35 days, to recover. We have also reported swine NK cells can be activated *in vitro* to killing of FMDV infected cells culturing blood cells with the porcine cytokine, IL-15. However, NK cells isolated from pigs during acute infection with FMDV are not activated to killing *in vivo* and have lost the response to IL-15 stimulation to activate NK killing *in vitro*.

Similar analysis of cattle showed NK cells from the blood of naïve cattle required cytokine activation to induce virus infected cell killing, as in the pigs. However, during acute FMDV infection, activated NK cells were isolated directly from the blood, exhibiting NK activity without additional *in vitro* activation with exogenous cytokines. Activation of NK cells was not observed following vaccination. In addition, we have analyzed the function of γδ T cells during acute FMDV infection of cattle and show these cells are activated to “NK-like” killing *in vivo*, when subsequently measured *in vitro*.

**Discussion:**
These results indicate that cellular innate responses of cattle are effective at reducing FMDV spread *in vivo* during the acute infection and before induction of the adaptive immune response. By comparison, swine innate responses are transiently blocked by infection with FMDV. These data may indicate why swine shed more virus than cattle following infection, and as a result, amplify outbreaks of FMDV.
ADAPTIVE IMMUNE RESPONSES IN THE RESPIRATORY TRACT OF FMD-VACCINATED CATTLE AFTER ORONASAL INFECTION

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Introduction:
FMDV aerosol-infection in naïve cattle induced a rapid and vigorous genuine local antibody secretion, showing a time-course and isotype profile consistent with an efficient T-independent antibody response. Here, we report the kinetics of appearance of specific antibody-secreting cells (ASC) in lymphoid organs along the respiratory tract induced in vaccinated cattle following aerogenous administration of FMDV.

Materials and methods:
Animals (n=16) were intramuscularly vaccinated in the neck with a high-payload O1 Campos FMD oil vaccine, and FMDV-specific ASC were studied at 7 and 29 days post-vaccination (dpv) and 1 to 6 days post-ornonasal homologous challenge (at 30 dpv, n=2 each time). Mononuclear cells were obtained from prescapular (PSL) and mandibular lymph nodes (ML), pharyngeal tonsil, lateral and medial retropharyngeal lymph nodes (MRL), tracheobronchial lymph nodes (TBL) and spleen, and studied using a FMDV-ASC ELISPOT previously developed.

Results:
Antibody responses induced by vaccination were found in the PSL draining the inoculation site and followed a maturation process throughout 29 dpv. Nevertheless, a modest though consistent IgM-mediated stimulation was detected at 29 dpv in all mucosal-related lymphoid tissues. None of the animals showed clinical symptoms after infection, and mucosal responses remained low at 2-3 dpi. However, class-switch was observed in the most stimulated organs, TBL and MRL, where IgG1-ASC were detected at 3 and 4 dpi. Naïve-infected animals showed responses starting at 4 dpi, being almost exclusively IgM-ASC and class-switch to IgG1 was only observed from 6 dpi in ML. The early class-switch in vaccinated-infected cattle augmented at 5 and 6 dpi to include all analyzed tissues.

Discussion:
Systemic vaccination may induce a basal stimulation in lymphoid tissues associated to the cattle respiratory tract, showing an IgM-mediated pattern even at late times post-vaccination (29 dpv). Further oronasal infection did not produce clinical symptoms in none of the animals, while promoting an early class switch and rapid response compatible to a secondary response.
CHARACTERIZATION OF OPSONIZING ANTIBODIES AGAINST FMD VIRUS

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Introduction:
Cattle challenge tests for vaccine selection and matching are ethically problematic, costly and have a poor statistical power. Thus, reliable in vitro correlates of vaccine-induced protection are required. Antibodies do not only mediate antiviral immunity through direct virus neutralization but also through virus opsonization and subsequent Fc receptor mediated uptake by cells of the immune system.

Materials and Methods:
To determine the level and reactivity of opsonizing antibodies we have established a bovine FcyRII expressing murine RAW264.7 cell line. These cells are resistant to FMDV infection unless the virus is opsonized by serum or monoclonal antibodies (mAbs). In the presence of antibodies, FMDV infection results in measurable cytopathogenicity. A collection of cattle sera and neutralizing and non-neutralizing mAbs was tested against a panel of FMDV isolates from different serotype.

Results:
Opsonizing activity of immune but not naïve serum and mAbs was observed against homologous and heterologous strains. Cross-reactivity was broad, even seen against different serotypes. Often opsonization was still measurable with around 10 times lower serum dilutions as those required for neutralization. MAbs were also able to opsonize in the absence of neutralization. The degree of amino acid conservation required was lower for opsonization when compared to neutralization, when a mAb against the linear site A epitope was tested. Interestingly, a better correlation to the vaccine dose was observed with opsonizing antibody levels when compared to neutralizing titers.

Discussion:
The results indicate that low avidity antibodies can opsonized but future research is required to investigate the possible presence of non-neutralizing but opsonizing epitopes on the FMDV capsid. We are currently testing more serum samples to determine the value of opsonizing antibody quantification as a correlate of vaccine-induced protection.
WHOLE 140S FMDV PARTICLES ARE NEEDED TO ELICIT SPECIFIC CELLULAR IMMUNITY IN VIVO AND TO STIMULATE RECALL RESPONSES IN VITRO

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Introduction:
Cross-reactive IFN-$\gamma$ responses against foot-and-mouth disease virus (FMDV) have been described by vaccinating cattle with one strain and studying recall responses against other strains \textit{in vitro}, stimulating bovine whole-blood culture with FMDV antigens. Here we studied the relevance of viral integrity in the homo- and heterotypic induction of cell-mediated immunity (CMI).

Materials and methods:
In a first experiment, blood from 8 multi-vaccinated cattle was stimulated with sucrose-purified 140S particles (inactivated virus, O1Campos and A24 strains), or 12S subunits obtained by heat treatment. IFN-$\gamma$ was quantified using a commercial ELISA. In a second experiment IFN-$\gamma$ was quantified in plasma from \textit{in vitro}-stimulated blood of O1 Campos (n=16) and A24 (n=10) monovalent-vaccinated animals.

Results:
Stimulation of blood from multi-vaccinated cattle with 12S particles significantly decreased the ability of both viruses to induce the anamnestic production of IFN-$\gamma$ ($p < 0.05$ with respect to O1 Campos 140S, $p<0.01$ for A24). This decrease was not due to loss of T-cell epitopes because capsid proteins were intact in the 12S subunits when evaluated by antigen ELISA and SDS-PAGE. The kinetics of destabilization was different for both strains. After 24 h at 37°C, 54% of the 140S- A24 particles were preserved but only 20% of O1Campos particles were left. These intrinsic characteristics of each strain were verified testing blood cultures from cattle immunized with monovalent O1campos and A24 vaccines. Levels of IFN-$\gamma$ detected by stimulation with A24 were significantly higher than those with O1Campos for both vaccines ($p < 0.05$).

Discussion:
This work demonstrates that whole-intact 140S FMDV particles are essential to elicit specific CMI \textit{in vivo} and to stimulate recall responses \textit{in vitro}. Our data also support the use of 140S particles for \textit{in vitro} stimulation of whole blood prior to IFN-$\gamma$ ELISA assessment in vaccinated cattle.
INTRASEROTYPE CHIMERIC FOOT-AND-MOUTH DISEASE VACCINE ANTIGEN ELICIT PROTECTION IN CATTLE

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Introduction:
FMD is controlled in southern Africa essentially through vaccination, restriction of animal movement and frequent animal inspections in the controlled areas. FMD vaccine candidates should be closely related to emerging strains and induce long-lived immune responses with broad immunological cross-reactivity for appropriate protection against FMD viruses (FMDV) from different topotypes. Reverse genetics is a powerful tool in the design of vaccine seed viruses with improved capsid stability and broad antigenic spectrum. Here, we described the design of a chimeric SAT type FMDV to produce conventional, chemically inactivated vaccine antigen as an alternative control measure for FMD.

Materials and methods:
The chimeric construct, pŽIM14SAT2, was engineered by replacing the external capsid-coding region (1B-1D/2A) of a SAT2 genome-length clone, pSAT2, with that of a field isolate, ZIM/14/90. Viable chimeric virus, vŽIM14SAT2, was recovered from transfected BHK-21 cells and chemically-inactivated vaccine antigen prepared. Chimera and parental antigens were formulated as double oil emulsions and used to immunise cattle.

Results:
The vŽIM14SAT2 chimeric virus and parental isolate did not differ in their ability to infect and replicated in cultured cells or in the capsid stability in various biophysical environments. Virus neutralisation assays showed a similar neutralisation profiles against selected reference sera. Vaccine antigen prepared from the chimera and parental antigens induced similar antibody responses in cattle. The majority of cattle vaccinated with the chimeric vaccine produced neutralising antibodies and were protected against homologous FMDV challenge.

Discussion:
Structurally-improved chimera antigens address the concerns relating to the genetic and antigenic diversity of SAT type viruses and longevity of antibody responses. The duration of antibody responses have been linked to stability of the antigen in the early 1980’s, a feature that can be improved using reverse-genetics. These results provide support that chimeric vaccines containing the external capsid of field isolates can be successfully produced and induces protective immune responses in cattle.
RATIONAL DEVELOPMENT OF FMD VIRUS VACCINES

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Abstract:
Current FMD virus vaccines are highly effective at inducing protective immunity in cattle. A single low microgram dose in adjuvant can generate protection from disease (though not necessarily infection) within 4-5 days. Vaccination is currently reliant on the use of inactivated virus produced in large-bioreactors in high containment facilities; their set-up and running costs, limit the global production capacity. Storage and supply are further constrained by the poor vaccine stability at ambient temperatures. Thus, on several grounds the current vaccine manufacturing situation is unsatisfactory and developments that increase the options available for FMD vaccine production are urgently required.

We have performed proof-of-principle experiments for a vaccine produced from non-infectious cultures. The implementation of methods to produce non-infectious FMDV capsids as vaccines, outside of high containment facilities, would significantly lower costs, improve production capacity and eliminate the risks associated with infectious virus during vaccine production. Also, the absence of non-structural proteins from the vaccine antigen means companion DIVA diagnostic tests will provide greater certainty of discriminating between vaccinated and infected animals.

In addition, our initial work has demonstrated that a non-infectious source of virus capsids allows sequence manipulation to address the issue of antigen stability. X-ray crystallography shows the mutant and wild-type capsids to be essentially the same structure as virus. Implementation of improvements in vaccine stability would reduce the quantity of antigen required per vaccine dose, mainly by reducing losses during production and improving the shelf life of the formulated product. Cattle vaccinated with wild-type and stabilised recombinant capsids showed sustained virus neutralisation titres and protection from challenge 34 weeks after immunization.

In summary, combined with new tests to facilitate pre-clinical/ pre-transmission diagnosis, these new rapidly deployable recombinant vaccines support a “vaccine to live policy”.
DEVELOPMENT OF A BOVINE ENTEROVIRUS–BASED VECTOR THAT EXPRESSES MULTI-EPI TOPES OF FOOT-AND-MOUTH DISEASE VIRUS

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Introduction:
Currently, a wide range of virus families is under intensive development as vaccine vectors for either human or veterinary use, including some that are replication competent but also many that are specifically attenuated. In this study, a synthesized DNA encoding multi-epitopes of FMDV that containing both of B and T cell epitopes was inserted into a recombinant infectious bovine enterovirus (BEV) vector at the VP1/2A junction.

Materials and methods:
The DNA sequences encoding multi-epitopes of FMDV O/Andong/SKR/2010 strain or O Manisa strain were synthesized and inserted into BEV vector. The plasmids were linearized and transfected into BHK/T7-9 cells that stably expressing T7 RNA polymerase. Viruses were rescued and passaged twice on fresh MDBK monolayer cells. Plaque assay was performed using the rescued viruses together with their parental. For detection of inserted epitopes, western blot and indirect immunofluorescence assay (IFA) were routinely performed.

Results:
There were no significant differences in the growth kinetics and plaque morphologies between transfectant viruses and their parental virus. Furthermore, the expressed multi-epitopes were successfully detected by using IFA and western-blot.

Discussion:
In this study, we have successfully demonstrated a process of using BEV as viral vector to express the FMDV multi-epitopes and this may be a good candidate for a live viral vaccine vector. We are currently preparing animal experiments to determine the immunogenicity and protective efficacy by using the recombinant viruses derived from this study.
DEVELOPMENT AND EVALUATION OF AN ADENOVIRUS VECTOR BASED INTRANASAL FMDV CAPSID VACCINE IN MICE FOR INCREASING IMMUNE RESPONSES

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Introduction:
An important aspect of FMDV is the establishment of the asymptomatic persistent infection in ruminants following recovery from the disease. Traditional FMD vaccines do not induce sterile immunity and may allow viral replication at epithelial surface giving rise to the carrier state in some vaccinated animals following live virus challenge. Since the naso-pharynx is the major portal of entry in FMDV infection and is the site of FMDV persistence, induction of potent adaptive immune responses at this site by local delivery system may generate pre-existing mucosal antibody that could prevent the initial viral colonisation. Therefore, the main aim of this study is to develop and evaluate a viral vector based intranasal FMDV empty capsid vaccine in mice before testing it in the target animals.

Materials and methods:
The A22 FMDV P1-2A and 3C genes were cloned into defective human adenovirus vector where FMDV P1-2A-3C genes were placed under the tetracycline operator 2 (TetO2) sites. Human 293T-Rex cells were used to generate the recombinant adenovirus whereas HEK293A cells were used for FMDV empty capsid expression. The sucrose gradient fractions of the infected cells were also stained for the presence of FMDV empty capsids using a transmission electron microscope. Blood samples from two A22 FMDV (inactivated antigen) vaccinated cattle and 2 unvaccinated cattle were used for preparation of MoDCs, proliferation of PBMCs and interferon-gamma production. Finally this rAdVFMD empty capsid vaccine has been used as intranasal vaccine in 40 mice to evaluate the local as well as systemic immune responses.

Results:
We have developed a replication deficient human adenovirus expressing FMDV empty capsids (rAdVFMD). This recombinant AdVFMD virus was able to infect bovine respiratory epithelial cells and monocyte-derived dendritic cells (MoDc). Further, they were able to activate FMDV-specific bovine memory T cells ex vivo. Furthermore, rAdVFMD virus has been used as intranasal vaccine in mice that induced a high titer of IgG1, IgG2a and IgG2b immune responses in serum and a high IgA titer in lung wash.

Discussion:
This pilot study envisages the efficacy of rAdVFMD intranasal vaccine in mice and has potential to generate immune responses in cattle that could be evaluated after vaccination and subsequent challenge with FMDV.
ALTERNATIVE FMD VACCINE POTENCY TESTS BASED ON SEROLOGY AND PAYLOAD

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Objective: This study addresses alternatives to the standard European in vivo vaccine potency test for cattle. Alternative serology-based vaccine potency tests have been published. Now the effect of antigen payload on vaccine potency is investigated.

Materials and methods: A single batch of the FMD O⁰ı Manisa reference strain was used to produce 5 batches of monovalent double-oil emulsion vaccine with different antigen payloads. Each vaccine batch was tested in triplicate in a standard PD₅₀ test. Cattle serum samples were collected at 21 days post vaccination and antibody titers were determined. The in vivo observations and serological data were analysed and logistic regression models were constructed for assessment of alternative vaccine potency.

Results: The challenge trials resulted in overall PD₅₀’s of 8.8, 4.3, 10.5, 20.1 and 17.7 for the respective antigen payloads of 25%, 50% 100%, 200% and 400%. Different logistic regression models were compared for their ability to differentiate between protected and unprotected animals. The model including all parameters (antigen payload, vaccine dose and antibody titer) had a sensitivity and specificity of 76% and 90%, respectively. For the models based solely on antibody titer or antigen payload the sensitivities were 79% and 47% and the specificities were 80% and 81%, respectively. For models created with the subset of data containing only animals that received the full 2ml vaccine dose the model combining antigen payload and antibody titer performed best (sensitivity: 97%, specificity: 100%), however differences with the model correlating only antibody titer to in vivo protection were small (sensitivity: 93%, specificity: 100%) and the model based solely on antigen payload had a lower sensitivity of 80%.

Discussion: Vaccine potency can be assessed with alternative tests to the in vivo test. However the confidence of the estimated potency will be influenced by the characteristics of the chosen alternative vaccine potency test.
IMPROVING CHALLENGE-FREE FMD VACCINE BATCH ACCEPTANCE

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Introduction:
The OIE Terrestrial Manual and the European Pharmacopoeia (EP) still describe live challenge experiments for FMDV vaccine potency tests. However, the EP allows for other validated tests, and specifically serological tests if a “satisfactory pass level” has been determined. While much work exists on individual assays, we extend this work by examining the existing challenge tests to determine their quality in practice, and by combining and comparing different assays too assess the best approach to replace them.

Materials and methods:
We collated data from live animal work carried out at Pirbright as well as in Argentina and Russia for a total of 1763 challenges of vaccinated animals, 701 of which have associated recent serology from Pirbright, and 655 have both Virus Neutralisation (VN) and Liquid Phase Blocking ELISA (LPBE) results. These latter experiments used 14 different vaccines challenged by 10 viruses of 5 different serotypes. A Bayesian framework was developed in R and JAGS to analyse the factors that determine the serology-protection relationship, and we constructed a model predicting protection from serology.

Results:
We showed that existing challenge tests for vaccine batch acceptance have high false positive rates (up to 31\%) and moderate false negative rates (up to 11\%), and investigated other equally good tests involving fewer animals. We then determined that VN and LPBE have contrasting advantages as serological assays, and that combining the two tests therefore gave a significantly improved ability to correctly predict protection.

Discussion:
We show that the quality of \textit{in vivo} challenge experiments is lower than has been assumed, and its replacement with \textit{ex vivo} assays is therefore feasible without lowering standards, though immunogenicity and potentially further batch trials must be carried out \textit{in vivo} until the relationship is established; however, combining different assays improved predictive power, allowing higher accuracy to be achieved faster.
RELATION BETWEEN ANTIBODY RESPONSE AND PROTECTION IN FMD VACCINE DEPENDS ON VACCINE QUALITY

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Introduction:
The relation between antibody response and protection after foot-and-mouth disease (FMD) vaccination has been studied extensively. All these studies show that in both cattle and pigs there is a significant relation between antibody response and protection. But there is evidence that the relation between antibody level and protection is batch dependent. In the current study we analysed whether the batch difference can be explained by the properties of the vaccine used.

Materials and methods:
Virus neutralisation test titres of 447 cattle sera from challenge experiments with 10 different FMDV strains within serotype A, O and Asia 1 were available as well the result of homologous challenge. The data were analysed by logistic regression using a forward stepwise procedure. In which we used protection at challenge as the result variable. As explanatory variables we used: the logarithm of the antibody titre, vaccine strain, vaccine batch, the amount of injected antigen, the amount of antigen in a full dose, the logarithm of the dose, the injected volume and, a combined variable using the vaccine strain and the antigen dose.

Results:
The logarithm of the antibody titre showed to be the best single predictor for protection. Vaccine strain, the amount of antigen in the full dose, the logarithm of the used dose and the interaction between antibody titre and strain, in this consecutively order, became part of the final model that described protection.

Discussion:
The results show that antibody titre is a good predictor of protection after FMDV vaccination. The relation between antibody response and protection is not the same, however, for each vaccine strain, the amount antigen and vaccine. The interaction between antibody titre and strain means that a common slope for the relation between antibody response and protection is not valid.

It can be concluded that the relation between antibody response and protection can differ between different vaccine producers, but it can be used as a release criterion by vaccine producers that have set their criteria based on challenge tests for their own GMP controlled vaccines. For consumers that buy vaccine, the vaccine that induces the highest antibody titre against the circulating virus is the best choice.
CROSS PROTECTION AGAINST CURRENT ASIA 1 FIELD ISOLATES IS PROVIDED BY A HIGH POTENCY ASIA 1 SHAMIR VACCINE

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Introduction:
A newly emerging FMDV Asia 1 virus, which has shown little to no antigenic match with the common Asia 1 Shamir vaccine strain following laboratory testing at the World Reference Laboratory for FMD (WRLFMD®), IAH, Pirbright, was identified in Pakistan in 2009 and has become widespread throughout pool 3 appearing in Afghanistan, Bahrain, Iran and Turkey during 2011/2012. It was a matter of urgency that the vaccine held in the EU vaccine antigen bank should be tested for efficacy against these current field strains. To investigate if a high potency Asia 1 Shamir emergency vaccine could protect animals from challenge with a current Asia 1 field isolate, a vaccine potency experiment was conducted by WRLFMD® and the results are presented in this study.

Materials and methods:
The Asia 1 Shamir vaccine potency test were carried out following the standard procedure defined in the Monograph of the European Pharmacopoeia. Vaccinates were challenged with FMDV isolate Asia 1 TUR 49/2011 at 21 days post single vaccination.

Results:
The vaccinated animals were protected from this severe heterologous challenge according to the PD50 obtained. The antibody responses post vaccination were examined using various tests including virus neutralisation test, liquid phase blocking ELISA and solid phase competition ELISA. The correlations between the r1 values obtained and observed protection were also investigated.

Discussion:
It has previously been demonstrated that high potency FMD type A vaccines can induce protection against heterologous virus challenge even when low r1 values against the vaccine strain are recorded (Haas et al. 2008). Results of this study demonstrate that a similar protective effect with the use of high potency FMD Asia 1 Shamir vaccine has been induced in vaccinates.
NOTES
TESTING THE EFFICACY OF O1 MANISA HIGH POTENCY VACCINE AGAINST CHALLENGE WITH O/VIETNAM/2010 (O MYA98 TOPOTYPE) IN PIGS

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Introduction: The efficacy of a high potency O1 Manisa vaccine from the Australian antigen bank was tested in pigs 4 and 7 days post vaccination. In addition, unvaccinated pigs were kept in close contact to determine whether vaccination decreases virus excretion to prevent spread of disease.

Materials and Methods: Five pigs were vaccinated 4 and 7 days prior to challenge with a pig adapted O/Vietnam/2010 (O Mya98 topotype) strain via the heel bulb route. They were kept in the same room with 5 more unvaccinated pigs that were separated by a waist high steel wall. The same experiment was also performed with unvaccinated controls. Clinical scores were noted daily and virus levels measured using quantitative real-time PCR. In addition, the serological responses to non-structural and structural proteins were determined using ELISA.

Results: All the unvaccinated control pigs were successfully infected and disease was transmitted to the in contact pigs at 13 days post challenge (DPC). Virus RNA was detected in saliva and nasal secretions of the challenged pigs between 2-10 DPC in contrast to the in contact pigs where virus RNA was detected only in the saliva. In both vaccinated groups, four out of five challenged animals were protected and none of the in-contact pigs were infected. Virus RNA was detected between 2-8 DPC in the nasal secretions in animals challenged 4 days post vaccination (DPV) whereas RNA could be detected only between 2-4 DPC in the pigs vaccinated 7 days prior to challenge. The unvaccinated pigs excreted more virus when compared to the vaccine groups (P<0.001). The animals challenged at 7 DPV excreted little or no virus compared to the animals challenged 4 DPV (P<0.05). Two of the five pigs challenged 7 DPV had detectable antibodies to the structural proteins while none of the ppigs challenged 4 DPV showed any detectable antibodies. However, 5 DPC both groups showed antibodies.

Discussion: The high potent O1 Manisa vaccine successfully protected pigs from infection as early as 4 DPV when challenged with O VIT 2010 (Mya 98 topotype). The vaccinated animals shed insufficient virus to infect the contact animals.
ANTIBODY TITRES IN FMD TYPE A STRAINS: COMPARISON OF METHODOLOGIES TO PREDICT CROSS-PROTECTION

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Introduction:
The result of an FMD vaccination programme will depend on the quality and suitability of the chosen vaccine. Antigenic matching is usually studied by in vitro analysis to determine the suitability of the vaccine strain. In this study we will compare serological methods to determine the differences between methodologies.

Materials and Methods:
Ten different FMDV serotype A strains were made into aluminium hydroxide saponin adjuvanted vaccine, 2 ml per dose, containing 10 µg of antigen and was used to vaccinate cattle (5 animals for each strain). Sera, 21 days post-vaccination, were tested by virus neutralisation, neutralisation index and liquid phase blocking ELISA to measure antibody titre. Five statistical methods of analyses were used namely, mean titres, r1 values, scaled titre, cluster analysis, and principal component analysis. A dendrogram was constructed from the VP1 gene sequence of the 10 FMDV serotype A strains using BEAST method to determine genetic relationship.

Results:
Not all homologous vaccines showed the highest titres in the three serological test methods against their homologous strain, resulting in r1 values much greater than 1. When titres were scaled then in all homologous titres the highest results are observed and the r1 values based in scaled titres became lower. Clustering based on Euclidean distance and principal component analysis produced different clusters of serotype A on the strains and vaccine sera when the different serological tests were used. The PCA analysis of the three serological test methods are not strongly related and show distant relations and inconsistent in the three test methods. Genetic clustering showed groups of African, Asian and the European virus(es) which was not similar to any of the antigenic clusters found.

Discussion:
In both neutralisation assays the differences between titres were greater than in the ELISA indicating that neutralisation assays have a better discrimination capacity. Scaled titres can reduce variations between tests and reduce very high r1 results. Nevertheless, neither using scaled titres nor the titres observed resulted in a consistent clustering which was similar between various serological tests and the clustering was not consistent with the genetic clustering that could be related to the continent of origin. The current research cannot determine which technique is the best, as the results should be compared with cross-protection results.
CROSS-PROTECTION BETWEEN STRAINS OF SEROTYPE A

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Introduction:
The most accurate way to assess the match between a particular combination of vaccine and field strain is by carrying out a vaccine potency test. Heterologous challenge trials were carried out in the framework of the FMD DISCONVAC project in order to check whether existing vaccines still cover newly arising field strains and also in order to establish a correlation between protection and VNT.

Material and Methods:
DOE vaccines formulated according to the specifications for vaccine bank vaccines from stored antigens (9 µg/dose) were received from Merial, Pirbright, UK. Healthy, unvaccinated German cattle, 6-12 months old, were purchased locally and kept in high security stables of the Friedrich-Loeffler-Institute, Riems. Starting with low passage cell culture virus received from the WRL for FMD, IAH, Pirbirght, challenge virus was prepared by cattle inoculation and tested for the ability to induce typical clinical signs. Sera were tested in homologous and heterologous virus neutralisation tests (VNT) according to the OIE manual using BHK21 CT cells. Vaccination and challenge were carried out according to the FMD monograph of the European Pharmacopoeia and PD50 values were calculated by the Spearman-Karber method.

Results:
As A/TUR/1/06 shows a very high sequence homology in the VP1 of 99.22 to 99.84% to other early isolates of the A Iran 05 group it was considered suitable for a quasi-homologous challenge of animals vaccinated with A IRN05 vaccine (exact vaccine parent strain confidential). The trial resulted in a PD50 value of 18.2. However, as seen before with early isolates of the A Iran 05 group, the podal lesions in the control animals were weak with just 2 -3 small vesicles. A second challenge experiment with A Iran05 vaccinated animals using A IRN/29/09 virus (95.77 % homology in the VP1 sequence to A Iran 1/05, r=0.42) resulted in a PD50 value of 8. A third challenge experiment with A Iran05 vaccinated animals using A ISR/11/09 virus (95.77 % homology in the VP1 sequence to A Iran 1/05, r=0.22) resulted in a PD50 value of 6. In the latter two experiments, there were typical podal lesions in non-protected animals.

As many vaccine banks including the European vaccine bank contain A22 IRQ 24/64 antigen which, if administered as a high potency vaccine, gives a broad coverage, it was also tested if such a vaccine would protect against A Iran 05 strains. Despite the sequence homology of A IRN/29/09 with A22 IRQ 24/64 being just 82.16% and the r-value 0.27, the PD50 value was determined as 8. However, the challenge trial with A ISR/11/09 (82.16% sequence homology and r-value of 0.09) resulted in a PD50 value below 2.

Discussion:
It was confirmed that high potency FMDV type A vaccines can induce a broad protection and that the A Iran 05 vaccine is a valuable component of European vaccine banks. In a previous series of trials, in 6 out of the 8 heterologous challenge experiments, high potency type A vaccines still conferred a protection of at least 6 PD50. However, as the A22 vaccine could not protect against ISR/11/09, heterologous protection within serotype A has limits which were also indicated by the very low serological r-value.
NOTES
ANTIGENIC CARTOGRAPHY FOR ANALYSIS OF ANTIGENIC VARIATIONS IN FMD VIRUS

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**Introduction:**
Antigenic cartography is a contemporary approach for analysis of antigenic variations based on immunological datasets using particular class of algorithms and it simplifies immunological data interpretation through intuitive antigenic maps. As this method readily allows monitoring of antigenic differences among vaccine and circulating strains, it would be of immense importance in antigenic analysis of similar viral pathogens like FMD virus strains. Though comparison of ‘r’ (relationship) values obtained in LPBE and 2D-MNT assays are frequently in use for such purpose, antigenic cartography can improve interpretation of immunological assay datasets.

**Materials and methods:**
Forty FMD virus serotype O isolates covering different circulating genetic lineages were selected for the study. The LPBE and 2D-MNT data of all these 40 with 6 different serum panel antibodies were used to construct antigenic cartograms/antigenic maps with help of ATIVS, a web interface of NHRI (http://influenza.nhri.org.tw/ATIVS). Antigenic coverage analysis was carried out by drawing the probable protection lines for each of the reference serum using ‘Radius of protection’ which considers the maximum reactivity of a particular antiserum with any antigen and minimum reactivity needed for optimal protection in antigenic units.

**Results:**
Four of the six representative serum panels showed adequate coverage against the circulating field isolates and rest two showed limited coverage. The results of coverage analysis were congruent with the international guidelines of vaccine strain selection for FMD. The LPBE data set was found to be more suitable for antigenic analysis of FMD virus serotype O isolates using antigenic cartography approach though 2D-MNT is more reliable for assessing the protective titres as it relies on neutralisation of virus infectivity in cell culture system.

**Discussion:**
Approach of antigenic cartography in antigenic analysis of FMDV is suitable and appropriate which can be used not only for vaccine strain selection but also for better understanding of antigenic evolution and spectrum when a large number of field isolates are analysed with convalescent serum from different field outbreaks and different mono-specific BVS against viruses of different circulating lineages.
EVIDENCE OF FURTHER NEUTRALISATION AFTER REMOVAL OF FIVE NEUTRALISING ANTIGENIC SITES IN SEROTYPE O FMDV

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Introduction:
Foot-and-mouth disease virus (FMDV) capsid displays various epitopes on its outer surface. Five neutralising antigenic sites have been identified in serotype O FMDV using murine monoclonal antibodies (mAb). In addition, there is evidence of the existence of other, yet unidentified epitopes, which are believed to play a role in antibody-mediated protection. However, the relative importance of different epitopes in FMD vaccine induced-protection has not been ascertained to date. The aim of this work is to define viral determinants of antibody-mediated protection in FMDV which may aid to the development of novel broadly cross-reactive vaccines.

Materials and methods:
Critical amino acid residues of the five neutralising antigenic sites of a O1-Kaufbeuren cDNA clone were mutated using standard site-directed mutagenesis techniques (O1K-5 site mutant, 5M). In addition amino acid residues at three other positions either individually or in combination were also mutated to less immunogenic amino acids. In-vitro transcribed RNA was electroporated to BHK cells to recover live virus.

Results:
Viable recombinant viruses were recovered from all the nine constructs. All the viruses did not bind to mAbs specific for each neutralising antigenic sites. Growth kinetics and phenotypic characteristics of the mutant viruses were found to be similar to the parent virus. Results of preliminary virus neutralization test (VNT) exhibited approximately 60% reduction in VNT titre by 5M virus compared to the parent virus (O1K-WT) using guinea pig serum raised against O1K-WT virus. Viruses with additional mutations in the capsid showed further reduction (up to 20%) in VNT titre.

Discussion:
This preliminary result indicates that more amino acids, in addition to the already identified five neutralising antigenic sites could be involved in the process of neutralisation in case of serotype O FMDV. Work is on-going in our lab to study the contribution of each antigenic site in the process of antibody mediated-protection.
DETERMINING THE EPITOPE DOMINANCE ON THE CAPSID OF A SAT2 FOOT- AND-MOUTH DISEASE VIRUS BY MUTATIONAL ANALYSIS

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Introduction:
The majority of foot-and-mouth disease virus (FMDV)-neutralizing antibodies are directed against epitopes located in the three surface-exposed capsid proteins of the virus, of which the flexible βG-βH loop in VP1 is important. In the case of SAT2 viruses, studies involving monoclonal antibody resistant mutants revealed at least three antigenic sites for the FMDV SAT2 serotype. We have carried out extensive studies on the antigenic dominance of known epitopes and structurally exposed regions, using an epitope-swapping approach in an infectious cDNA clone of a SAT2 virus.

Materials and methods:
Amino acid sequences of the capsid proteins of SAT2 field viruses were analyzed to identify putative antigenic sites on the surface of SAT2 virions. Ten structurally exposed loops were identified and mutated. The putative epitopic structures of SAT2/KNP/19/89 were replaced in the pSAT2 plasmid, derived from SAT2/ZIM/7/83. The overall antigenic distance of the epitope-swapped mutant and parental viruses were examined by virus neutralization assays using convalescent antisera raised against the parental viruses.

Results:
The neutralization profile of four epitope-replaced viruses revealed higher neutralization titres with anti-SAT2/ZIM/7/83 sera than obtained with the parental virus. No significant differences in the neutralization titres were observed for the remainder of the epitope-replaced mutants compared to SAT2/ZIM/7/83. Two VP1 mutants revealed a significant increase in neutralizing titre when measured against the SAT2/KNP/19/89 serum. Interestingly, the most dramatic change in the antigenicity of the virus was observed for the mutation in the N-terminal part of the βG-βH loop of VP1.

Discussion:
Our results indicate that the TQQS→ETPV mutation in the βG-βH loop of VP1 caused a significant increase in the neutralization titre against SAT2/ZIM/7/83 serum in vitro, ascertaining it as a dominant reactor to anti-SAT2 sera. In addition, increased neutralization was observed for two of the VP1 mutated viruses (AFA→TFN and HNN→NKG) towards SAT2/KNP/19/89, causing an antigenic shift from SAT2/ZIM/7/83 to SAT2/KNP/19/89.
STUDY OF ANTIGENIC SITE VARIATION IN FMD VIRUS SEROTYPE O GROWN UNDER VACCINAL SERUM ANTIBODIES IN VITRO

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Introduction:
Foot and mouth disease virus is constantly evolving under neutralizing antibody pressure in either naturally infected or vaccinated animal population of disease endemic India. So to understand the dynamics of evolution of antigenic sites this simulation study was carried out.

Materials and methods:
Isolation and characterization of FMDV antibody-resistant populations after serial passages of 3 strains of FMDV serotype O (INDR2/1975, IND 120/2002, IND271/2001) in BHK21 cells in the presence of subneutralizing level of bovine vaccinal sera (BVS) was carried out.

Result:
The partial neutralization escape variants showed many characteristic changes like increased resistance to neutralization by BVS, reduction in plaque size, selection of amino acid substitutions on loops and termini of capsid protein. Fixation of aa substitutions were observed at critical residues of all established antigenic sites of type O [144 (site 1), 45 and 48 (site 3), 72 and 132 (Site 2)] except site 4 and 5. Besides, substitutions were also observed in proximity to the identified residues within antigenic sites of serotype O or other serotypes which could be significant in terms of neutralizing antibody binding and immune escape [41 and 51 (B-C loop); 133, 140 and 143 (G-H loop); 201, 204 and 209 (C termini) of VP1; 71 and 75 (B-C loop); 131 (EF loop); 179 (G-H loop) and 219 (C termini) of VP3].

Discussion:
In majority of the virus-serum combinations, site 3 was seen to be substituted. Variants could be identified with substitutions at site 2 or 3 only but site 1 variants were always accompanied by substitutions elsewhere in the capsid. Changes observed in VP3 were always associated with substitutions at VP1 suggesting a minor role for such sites which act in synergy with other sites towards the neutralization escape phenotype. Presence of substitutions at the same locations as identified in this study in the Indian field isolates supports the importance of these sites.
MODELLING INTO POLICY: HOW CAN AN ‘INTELLIGENT CUSTOMER’ ENSURE APPROPRIATE USE OF EVIDENCE?

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Introduction:
Epidemiological modelling may be used to aid the development of animal health policy, to support disease surveillance activities, and to evaluate existing or new intervention strategies. In order to use model outputs effectively, the decision maker has to consider a range of issues. In some areas, there is an increasing reliance on models across Government but few people who understand outputs.

Materials and methods:
A Quantitative Modelling (QM) Intelligent Customer Function (ICF) has been established using expertise from a range of relevant disciplines to assist policy makers with using the full value of epidemiological modelling in policy development. The group is made up of a mathematical modeller, epidemiologists and an economist. The ICF translates the requirements from customers/users and the technical outputs of the modellers; provides institutional memory to policy makers; and provides technical understanding to commission and/or interpret the outputs.

Results:
During the past couple of years the ICF has liaised effectively with both policy makers and expert mathematical modellers. ICF involvement has ensured that the modelling commissioned has been relevant and has contributed to the evidence informing policy.

Discussion:
ICF has provided: expert challenge during the modelling process; veterinary epidemiology and risk assessment challenge and validation of model assumptions and preliminary results (i.e. do they match what we believe to be going on in the field). We believe that this is a valuable approach ensuring that model outputs are used appropriately in policy development, without each official engaged in a modelling project being required to develop sufficient technical understanding to commission and/or interpret the outputs, and to prevent modelling outputs being ignored through lack of time and capacity to make use of them. An ICF should also lead to significant cost savings by working with officials commissioning work to prevent unnecessary modelling work.
SCALING UP FROM ONE-TO-ONE ANIMAL TRANSMISSION EXPERIMENTS TO EPIDEMIOLOGICAL MODELS OF NATIONAL OUTBREAKS

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Introduction:
Simulation models of FMD spread can make a valuable contribution in formulating control policies and are used widely around the world. Existing regional models are based upon disease transmission between livestock premises, without explicitly describing the disease dynamics or within farms. Here data from one-to-one transmission experiments in cattle (Science 332, 726: 2011) are scaled up to inform herd-based models of disease, which are then integrated into regional models of spread in Great Britain (GB).

Materials and methods:
Estimates of the latent, incubation and infectious period for FMDV infected cattle were derived from transmission experiments at IAH Pirbright using a Bayesian framework. These were integrated into a stochastic compartmental model for within-herd transmission to determine the level of infectiousness of premises and the potential for detection based upon clinical signs. Herd dynamics were incorporated into a model for transmission between holdings in GB with critical parameters estimated using approximate Bayesian computation methods applied to data for the 2001 outbreak. Importantly, this framework allows uncertainty at one scale to be incorporated at other scales.

Results and discussion:
Analysis of transmission between individual animals provides new insights into the relationship between infectiousness and clinical signs, and on the duration of infectiousness. Herd models indicate the potential for detecting disease well in advance of the farm reaching peak infectiousness in all but the smallest herds. Fitting of regional models to previous outbreaks allows us to evaluate quantitatively the benefits of incorporating multi-scale dynamics into disease spread models and has demonstrated the consequences of the animal-level results for spread at a national level.
NOTES
EPIDEMILOGICAL MODEL FOR OUTBREAKS OF FMD IN TWO DIFFERENT AUSTRIAN REGIONS

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3 Federal (National) Reference Laboratory for Foot-and-mouth Disease, Austrian Agency for Health and Food Safety (AGES), Robert Koch-Gasse 17, A-2340 Mödling, Austria.
4 Department 8 – Veterinary Department Regional Government of Styria, Friedrichgasse 9, A-8010 Graz

Introduction:
The study took information from real time tests (PICORNA 04, PICORNA 09) in two Austrian regions. Region I is defined with an area of 39.541 km² and a total of 43.973 farms, region II is defined with an area of 15.249 km² and a total of 15.805 farms. We compared following scenarios:
1. Culling of animals in detected farms;
2. Culling of animals in detected farms in combination with a culling of animals within a perimeter of 0.5 kilometers;
3. Culling of animals in detected farms in combination with immunization within a perimeter of 0.5 to 10 kilometers
4. Culling of animals in detected farms in combination with immunization within a perimeter of 0.5 to 10 kilometers and a culling of animals within a perimeter of 0.5 kilometers;
5. Controlled collection of milk in combination with a culling of animals in the detected businesses.

Material and Method:
The epidemiological model was created with Interspread Plus® version 2.1.14. The monitoring parameters based on studies by Sanson (2006a, 2006b) and Martinez-Lopez (2010). The aerogenic spread of the virus was modeled on the basis of regional weather data for the period between 2006 and 2010, taking into account the transmission probabilities defined by Yoon et al. (2006).

Results:
The maximum outbreak duration in the basic scenario in region I was 153 days, in region II 166 days. Depending on the subordinate control measures we were able to reduce the maximum outbreak duration to as little as 124 days, i.e. for approximately 25%. A reduction of the median outbreak duration of approximately 10% was observed.

Discussion:
The spread of FMD must be seen in connection with the respective region and its organization. Due to the geographical conditions, test approaches based on Cellular Automatons may be beneficial in Austria.
MULTI-CRITERIA DECISION ANALYSIS FOR EVALUATING CONTROL OPTIONS DURING FMD OUTBREAKS

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Introduction:
Multi-criteria decision analysis (MCDA) helps providing structure to decision-making that involves different stakeholder groups with conflicting criteria. Decision options on FMD control often receive criticism for stakeholders with conflicting values.

Objectives:
The aim of this study was to evaluate the value of MCDA when deciding on control options during FMD outbreaks.

Materials & Methods:
MCDA methodology comprises three major parts: problem structuring, value trade-off and sensitivity analysis. Problem structuring starts with describing the best option for eliminating the population at risk using 1) preventive depopulation, 2) vaccination-to-live, 3) or vaccination-to-dead. The objective when controlling FMD control should be minimizing economic losses, maximizing social values as animal welfare, and maximizing political values as the reputation of the decision maker. All these objectives can now be expressed in terms of attributes. The different decision options will have different impact on the values for the different attributes. It is, of course, difficult to compare these values as they are all expressed on a different scale. Therefore a Simple Multi-Attribute Rating Technique (SMART) is used to bring all attributes to the same scale between 0 and 100. Stakeholders were elicited for weighting the importance of changing from worst to best for an attribute. All these weights were averaged and standardized for all stakeholders. In an additive value model the standardized values were multiplied with the scaled values for each of the attributed and summed for each of the decision options. The decision option with the lowest value was identified as the best supported by the stakeholders.

Results:
Attribute values differed between the different control options. As a final result, applying preventive depopulation, appeared the best-supported control option.

Discussion:
MCDA adds transparency to decision making for animal disease control as it focuses on inputs that make a difference and highlights areas of dispute.
NOTES
META-ANALYSIS ON THE EFFICACY OF FOOT-AND-MOUTH DISEASE EMERGENCY VACCINATION

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Introduction:
The objective of this study was to provide a summary quantification of the efficacy of foot-and-mouth disease (FMD) emergency vaccination based on a systematic review and a meta-analysis of available literature. The outcomes of the current systematic review can be used in simulation exercises to assess the economic consequence of FMD emergency vaccination, in order to advise the veterinary authorities on the optimum strategies to control FMD outbreaks.

Materials and methods:
Peer-reviewed, symposium, and unpublished studies were considered in the analysis. Clinical protection and virological protection against FMD were used as parameters of the efficacy of emergency vaccination. Clinical protection was estimated based on the appearance of clinical signs, while virological protection was estimated based on the outcomes of laboratory tests used to diagnose FMD. A meta-analysis relative risk was calculated per protection parameter. The effect of publication bias was examined.

Results:
Table 1 shows that vaccinated cattle, swine and sheep with an FMD emergency vaccine would be significantly protected from FMD clinical signs and infection compared to unvaccinated animals. For example, vaccinated cattle would have, on average, 0.71 times lower risk of infection compared to unvaccinated cattle. Fortunately, no publication bias was identified in the different analyses.

Table 1. Relative risk with 95% confidence interval of clinical and virological protection

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Clinical protection</th>
<th>Virological protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>0.13 (0.09-0.18)</td>
<td>0.71 (0.59-0.85)</td>
</tr>
<tr>
<td>Swine</td>
<td>0.48 (0.36-0.65)</td>
<td>0.67 (0.51-0.87)</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.31 (0.18-0.53)</td>
<td>0.59 (0.44-0.80)</td>
</tr>
</tbody>
</table>

Discussion:
Emergency vaccination seems to protect cattle against the clinical disease better than swine and sheep. Nonetheless, the virological protection seems to be similar as the confidence limits for the different species overlap.
EVALUATING VACCINATION FOR FMD CONTROL — AN INTERNATIONAL STUDY

Garner MG\textsuperscript{1}, Roche SE\textsuperscript{1}, Gauntlett F, Sanson RL, Stevenson MA, Forde-Folle K, Birch C, Dube C, Cook C, and Rawdon T

\textsuperscript{1}Department of Agriculture, Fisheries and Forestry, ACT, Australia.

Introduction:

Vaccination is increasingly being recognised as an important tool for managing foot-and-mouth disease (FMD) outbreaks. However, there is considerable uncertainty as to when and how vaccination should be optimally used, and how vaccinated animals should be managed. This is of particular relevance to countries previously free from FMD. The study is an international collaboration between modellers from Australia, New Zealand, Canada, the United States (QUADs) and the United Kingdom. The primary objectives are to identify the conditions under which vaccination may offer benefits in controlling an FMD outbreak, and identify the key features and parameters that influence the effectiveness of vaccination.

Methods:

The study uses four modelling platforms from each country to compare a range of vaccination strategies with a standard control (no-vaccination) approach. Data from the 2010 UK FMD exercise, Exercise Silver Birch, is used as the basis for the study. Key factors under investigation include the vaccination approach (suppressive versus protective vaccination), timing of when vaccination is introduced, species vaccinated, vaccination priorities and deployment methods.

Results:

Initial calibration studies (without controls) showed some differences between model outputs that could be explained by different approaches to representing FMD transmission. Notwithstanding, preliminary results suggest similar relativities in effectiveness of control measures between models. Vaccination is likely to be most effective in situations where disease is widespread, high rates of spread are expected or there are resource shortages.

Conclusions:

The study provides important knowledge on operational issues associated with using vaccines for FMD control. The multi-country modeling approach adds credibility to the use of simulation models in disease control decision making, as well as highlighting important technical issues in model implementation. The findings will be used to develop and support more robust and acceptable policies for FMD control.
A NEW APPROACH TO THE OLDEST DISEASE
DEVELOPING AN ANTIVIRAL DRUG STRATEGY FOR THE CONTAINMENT OF FOOT-AND-MOUTH DISEASE OUTBREAKS

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Introduction:
Conventional FMD control measures have focused on culling and/or (emergency) vaccination. In contrast to human medicine (e.g. Tamiflu®), stockpiling of small molecule antiviral drugs to combat veterinary epizootic diseases has received little attention. Nonetheless, antiviral drugs are logistically easy to apply in emergency situations (by mixing with feed), act directly on virus replication thereby reducing virus excretion/transmission, and can be tailored to be stable and serotype-independent.

Materials and methods:
Owing to a unique collaboration, 50,000 molecules have been screened in vitro for anti-FMDV activity using a semi-robotised system. Hit molecules were retested to confirm selectivity and pan-serotype activity. Subsequently, chemical and pharmaceutical hit-to-lead optimisation was directed towards improving potency (EC\textsubscript{50} below µM-range, ≥3 log\textsubscript{10} reduction in virus yield and RNA reduction assays) and oral bio-availability (>10-20%).

Results:
Three chemically different classes of molecules with sub-µM anti-FMDV activity have been identified. All are selective, potent and broad-spectrum inhibitors of the FMDV replication. Their mechanism of action and barrier towards resistance are being studied. Additional optimisation is on-going to increase bio-availability. Safety and efficacy of promising lead molecules will be investigated in surrogate FMDV infection models [SCID mice and guinea pigs (De Vleeschauwer et al.)].

Discussion:
Even though the project is in the early stages of the drug development trajectory, significant progress has been made. Based on our experience with classical swine fever, another epizootic disease, the antiviral drug strategy for disease outbreak containment can be an epidemiological and economic alternative to culling and vaccination policies.
THE PYRAZINECARBOXAMIDE DERIVATIVE T-1105 OFFERS PROTECTION AGAINST O₁ MANISA VIRUS INFECTION IN GUINEA PIGS

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Introduction:
We are optimizing the in vitro anti-FMD activity of different classes of small molecules. Early antiviral lead compounds will be evaluated in guinea pigs (GP). Preparatory, the activity of T-1105, a molecule with known anti-FMD activity (Furuta et al., 2008), was assessed in FMDV-infected GP.

Materials and methods:
Dunkin Hartley GP were administered with T-1105 (200mg/kg po, twice daily) for 5 consecutive days. Two independent experiments were performed using a total of 16 treated and 8 untreated animals. One hour after the first administration, all animals were inoculated with GP-adapted O₁ Manisa. Eight treated and all untreated animals were euthanized at 4dpi, the remaining at 10 dpi. Clinical signs were recorded daily. Serum, collected at 2 and 4 dpi was examined with real-time RT-PCR. RT-PCR data from the first experiment only are shown (n=8).

Results:
Serum concentrations peaked at ~140µM 2 hours after oral administration of 200mg/kg (in vitro EC₅₀=25µM).
All untreated control animals showed severe generalized infection. Mean Cp-values in serum at 2 and 4 dpi were 24.39±8.78 and 30.55±4.15, respectively. Disease severity was markedly less pronounced in treated animals. At 2 dpi, 16/16 treated animals had moderate lesions at the inoculation site and 5/16 slight reddening at the other footpads. At 4 dpi, 4/16 and 1/16 animals developed vesicular lesions at the inoculation site and mouth, respectively. At 2dpi, 4/8 animals tested RNA positive in serum (mean Cp-value of positives 37.00±5.36). At 4dpi, 3/8 animals tested RNA positive in serum, of which 1 that was positive at 2 dpi (mean Cp-value of positives 34.56±7.47). Viral RNA load in serum, oral swabs and various organs is currently being quantified.

Discussion:
T-1105 offered substantial clinical and virological protection against O₁ Manisa infection in GPs. The utility of the GP model for the preliminary evaluation of anti-FMD drugs is confirmed.
SIMULATED EFFECTS OF INTRODUCING EMERGENCY VACCINATION OR DEPOPULATION DURING FMD OUTBREAKS IN DENMARK

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² Department of Informatics and Mathematical Modelling, Technical University of Denmark
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Abstract:
The purpose of this study was to explore the effects of modifying FMD control 14 days following detection of the first infected herd in a simulated FMD epidemic in Denmark. The spread of FMD was simulated using an adapted version of the well described DADS simulation model, called DTU-DADS, using Danish herd locations and movements data. The epidemics were initiated in 1000 randomly chosen cattle herds located in cattle-dense areas.

The basic scenario consisted of: the minimum EU control measures, culling of forward-traced herds and a 3-day national standstill on animal movements. Alternative scenarios included depopulation, suppressive or preventive vaccination within 1 km.

The results show that there may be positive effects of applying additional control measures on the size, duration and costs of the epidemics. The median duration decreased from 56 days in the basic scenario to 45-47 days in the vaccination scenarios, and to 40 days in the depopulation scenarios. Furthermore, the number of infected herds decreased, but with fewer infected herds in the protective vaccination scenario. The total costs of an epidemic, including export losses, changed from €562 million in the basic scenario to €515, €535 and €610 million in the depopulation, suppressive and protective vaccination scenarios, respectively.

These results suggest that vaccination will often be a more expensive strategy in a country with a large export, like Denmark. Furthermore, the simulated results show that from an economic point of view depopulation in zones is often preferable.
MODELLING THE SPREAD OF FMD IN ENDEMIC REGIONS

Tildesley, M.J. & Keeling, M.J.

Abstract:
The outbreak of FMD in the UK in 2001 was one of the first occasions when mathematical models were used to inform policy during the course of a veterinary epidemic. During 2001 several groups developed a range of models that were able to predict the spatiotemporal pattern of disease spread and the impact of control strategies with a high level of success [1-3] and highlighted the role that models could play in shaping policy. Since then, the infection data from the 2001 epidemic has enabled research to be carried out to predict optimal culling and vaccination strategies both for the 2001 epidemic itself and for any future FMD epidemic in the UK and elsewhere [4-7]. In addition, modelling has now become an integral part of planning again many novel and invading infections.

In this paper we will utilise the Keeling model [2,4-7] to review the impact of control policies such as culling and vaccination upon future outbreaks of FMD in currently disease-free regions. We will then discuss the applicability of such models to control of FMD in endemic regions. In particular we contrast the epidemic and endemic scenarios and focus on the data requirements, the difficulties of multiple FMD strains, the costs associated with control and the aims of any control strategy. Such mathematical models could help inform policy decisions in endemic FMD regions to improve the welfare of livestock, reduce disease burden and ultimately improve the livelihood of livestock owners.

References:
ASSESSING AND COMPARING CONTROL STRATEGIES FOR FMD IN ENDMIC COUNTRIES: ADAPTATION OF THE NORTH AMERICAN ANIMAL DISEASE SPREAD MODEL (NAADSM)

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¹ Animal Population Health Institute, Colorado State University, Fort Collins, Colorado, USA

Introduction:
The use of modeling and simulation to assess control and mitigation strategies for the spread of FMD is a task achievable through the use of existing software packages given reliable and available observations and data. In countries where FMD is endemic, and where animal husbandry practices differ from Western practices, existing software packages require modifications in order to reflect these differences. The Republic of Turkey will be used as an example for FMD endemic countries due to the availability of data and the introduction of NAADSM to the local officers.

Materials and Methods:
The NAADSM simulation modeling framework was presented to the Ministry of Agriculture and Rural Affairs (MoA) at the FMD (ŞAP) institute in the Republic of Turkey for potential application. MoA representatives and experts in FMD research in Turkey evaluated the software with respect to their needs and their practices.

Results:
The NAADSM framework was identified as being a starting point for a useful tool for evaluating mitigation and control strategies of FMD in endemic areas. Modifications and additions were identified, which when applied to NAADSM would enable its full use for the animal husbandry practices of Turkey, as well as for areas of endemic FMD. Some of these modifications are being made to NAADSM at the time of this writing.

Discussion:
Coding modifications, and their associated complexity, to the NAADSM framework were identified in an EuFMD report concluding this workshop in the Republic of Turkey. The identification of these alterations is a major step forward in enabling NAADSM for use by member countries for assessing and comparing mitigation and control strategies for both endemic and non-endemic areas. Such modifications, when complete, will provide standardized and normalized data and research results for application across all member countries.

Appliance of science in the progressive control of FMD
Open session of the EuFMD, Jerez de la Frontera, Spain. 29-31 October 2012
GEOGRAPHICALLY-GROUNDDED, COST-BENEFIT BASED CONTROL POLICIES: BUILT AS EQUAL CIRCLES OR CONSIDERING LOCAL CONNECTING NETWORKS?

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Introduction:
Epidemic control policy (ECP) typically consists of zones that include cases, within which some intervention is implemented. Utilizing cost-benefit estimates, here two ECPs, which considered or not considered geographical data on local connectivity, were evaluated:

Materials and methods:
Assuming that the ‘benefit’ of an ECP could be expressed as FMD case density (i.e., the greater the number of FMD cases controlled per sq km, the greater the efficacy of a policy), while ‘costs’ could be estimated as the area to be covered, both the FMD case density and its associated area (expressed as sq km) were compared in: (1) an ECP designed as equal-radius circles (ER), which were centered on the location of FMD cases; and (2) polygons that included cases and also considered the local (road-mediated) connecting network (CN).

Such evaluation was implemented in a geo-referenced scenario that closely reproduced the geographic conditions of an actual epidemic.

Results:
The hypothesis that equal-radius circles control an equal or similar number of cases/circle, was negated: the number of FMD cases/sq km differed up to 4 times among ER circles. Consequently, this policy showed a low case density. In contrast, the CN policy displayed higher benefits (higher case density) and lower costs (smaller area to be controlled): it revealed a 23.8% greater number of FMD cases/sq km and required 20% less area to be protected than the ER policy.

Discussion:
Epidemic control policy is likely to be both less costly and more beneficial when the local geography is considered, including data on common connecting structures, such as road, railroad, and/or river networks. Because connecting networks can be measured even before epidemics take place, anticipatory studies are recommended so that, in the event an epidemic occurs, usable information is available to promptly implement locally adjusted, cost-benefit based interventions.
COSTS AND BENEFITS OF FMD VACCINATION PRACTISES BY COMMERCIAL DAIRY FAMRS IN CENTRAL ETHIOPIA

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Wageningen University, ashufey80@yahoo.com

Abstract:
Foot and mouth disease (FMD) is a highly infectious and economically devastating disease of cloven-footed animals and is characterized by fever and vesicular eruptions in the mouth, on the feet and teats. FMD has an enormous impact on livestock productions, which include reduced milk yields, abortions, perinatal mortality, lameness, loss of weight in growing animals and premature cull. Control of FMD using stamping out policy is impossible in developing countries; however, vaccination with good quality vaccines can help to prevent losses in livestock production. In Ethiopia FMD is one of the most important livestock diseases. Despite this fact, there is no national FMD prevention and control strategy except a prophylactive vaccination that is practised by some dairy herds containing exotic animals. Moreover, the economic return from FMD vaccination is not known. Therefore, the objective of this study was to assess the costs and benefits of FMD vaccination practices by dairy farms. The costs and benefits of FMD vaccination under three scenarios, namely no vaccination (baseline), reactive vaccination during an outbreak and preventive vaccination (twice a year), have been compared. Information regarding reduced morbidity, mortality, milk loss, abortion rate, culling rate due to FMD outbreak and vaccination expenditures have been collected from literature, local and international FMD experts and farm records. Three types of farms, which have not used FMD vaccine, those who have been vaccinating their animals during an outbreak and those who have been regularly vaccinating their cows in the last five years, have been selected and their farm records have been referred. The generated data have been analysed using stochastic Monte Carlo simulation model that accounts variability and uncertainty of the values of the variables.

This research is expected to estimate the costs and benefits of different FMD vaccination practises in commercial dairy farms in Ethiopia. The output of this study can be an input to design FMD control and prevention strategies at dairy subsector level or at national level. Also, knowing the returns from different FMD vaccination strategies will encourage farmers to adopt the more beneficial one.
GFRA: STATE OF THE ALLIANCE OVERVIEW

F.F. Maree

Foot-and-mouth disease (FMD) remains a threat to agriculture and human prosperity worldwide. Currently, sophisticated global networks of reference laboratories exist to track the emergence and spread of FMD viruses (FMDV). Based on this, intelligence control measures are tailored to local needs.

Improved control measures for FMD in the future will depend on the development of new tools derived from basic research programmes. The efficient development of product streams to meet the needs of control programmes will require co-ordination of these research efforts to ensure that the most important gaps in our control strategies are being addressed and duplication is avoided. Considerable effort has been made in recent years by the Global Foot-and-mouth disease Research Alliance (GFRA) to raise the profile of FMD basic research and co-ordinate research programmes. The alliance has grown significantly over the last two years. The success in attracting funding and raising awareness of the necessity to coordinate research programmes has equally grown. These successful achievements may be looked upon with philosophical resignation as being the fruit that is now being harvested as a result of growing partner interaction and knowledge exchange within the GFRA. It is highly likely that participants of the GFRA consortium have recognized the capabilities and expertise of co-participants in fields that are of mutual strategic or commercial interest in the research area of FMD.

Currently, the GFRA is represented by 32 public and private institutions, which are distributed in five continents. At least three types of memberships to the GFRA exist. As an example, the FAO is an associate member of the GFRA. Many research activities within the GFRA partnership are performed as collaborative efforts between two or more GFRA partners. GFRA programs will continue to expand the alliance globally and will actively reach out to new areas of the world that have a stake in the progressive control and eradication of FMD. The vision and mission of the GFRA are concentrated in (i) coordinating a global alliance of scientists producing scientific evidence and innovation on FMD research, and (ii) establishing and sustaining global research partnerships in order to generate scientific knowledge and discover the tools to successfully prevent, control and eradicate FMD.
ROLE OF BUFFALO IN THE MAINTENANCE OF FOOT AND MOUTH DISEASE VIRUS

Nicholas Juleff\textsuperscript{a}, Francois Maree\textsuperscript{b}, Roy Bengis\textsuperscript{c}, Lin-Mari de Klerk-Lorist\textsuperscript{c}, Bryan Charleston\textsuperscript{a}
\textsuperscript{a}The Pirbright Institute, Ash Rd, Woking, Surrey, GU24 0NF, UK. \textsuperscript{b}TADP ARC-Onderstepoort Veterinary Institute, Onderstepoort 0110, South Africa. \textsuperscript{c}Office of the State Veterinarian, Skukuza, 1350, Kruger National Park, South Africa.

Foot-and-mouth disease (FMD) is the most contagious transboundary animal disease affecting cloven hoofed animals. FMD virus (FMDV, family Picornaviridae) serotypes A and O have the widest global distribution, occurring in Africa, Asia and South America. Types SAT 1, 2 and 3 are currently restricted to Africa only and Asia 1 to Asia; the capacity to invade free areas is common to all types and periodically SATs are introduced into the Near East, and Asia-1 into western and eastern parts of Eurasia. New strains of viruses arise continually and persistently infected African buffalo (Syncerus caffer), the primary reservoir for FMDV SAT serotypes, constantly generate genetic and antigenic variants.

Clinical FMD in African buffalo is mild and in most cases naturally-infected buffalo do not develop obvious signs of FMD. However, buffalo are considered to be more efficient carriers of FMDV. Studies under field conditions have reported virus recovery from individuals for 5 years and from an isolated buffalo population, which varied from 30 to 100 animals, for 24 years indicating that FMDV can perpetuate long-term in buffalo without re-introduction from neighboring populations. However, the frequency and titre of virus recovered can decrease over time and there are reports of carrier buffalo under experimental conditions clearing virus over a 15 month period. Field studies support the experimental observations as it appears that a significant number of animals fail to maintain persistent infection for prolonged periods because the proportion of persistently infected animals falls after reaching a peak in the 1-3 year age-group. These findings may be more consistent with delayed virus clearance, rather than a classical persistent host infection.

Although transmission by carrier buffalo has been demonstrated, most attempts at affecting transmission between persistently infected and susceptible buffalo or cattle under experimental conditions have been unsuccessful. Our recent attempts to affect transmission from carrier buffalo, challenged simultaneously with SAT 1-3, support the apparent inefficiency of transmission by carrier buffalo under experimental conditions.

We have shown that FMDV particles are maintained in the light zone of germinal centres in lymphoid tissue following primary infection of naïve cattle, sheep, pigs and African buffalo. Retention of intact FMDV particles on the follicular dendritic cell (FDC) network provides an ideal mechanism for maintaining a highly cytopathic and lytic virus-like FMDV extracellularly. It is important to note that the presence of antibodies and the formation of FMDV immune complexes allows for a shift in the tropism of FMDV to target and productively infect immune cells that express Fc-receptors, for example; dendritic cells and macrophages. FMDV immune complexes held on the surface of FDCs may infect lymphoid cells that come into contact with the FDC network, supporting intermittent virus replication cycles, despite the presence of high titres of neutralising antibodies. This mechanism may in part explain the observation of dexamethasone treatment leading to reduced virus shedding, as corticosteroids are known to inhibit lymphocyte migration and induce involution of lymphatic tissue. Indeed, given viral retention on the FDC network, one would expect decreased shedding in immunosuppressed carrier hosts, and increased shedding in hosts experiencing any immune activation that enhances lymphocyte migration through lymph nodes.
FMD VIRUS ECOLOGY: COLLABORATIVE STUDIES
L. Rodríguez
Available upon request

DEVELOPMENT OF A SAFE ANTIGENIC MARKER FMD VACCINE PLATFORM
E. Rieder
Available upon request

TOP PRIORITIES FOR RESEARCH
D. Paton
Available upon request
NOTES
ESTIMATING THE INCIDENCE OF FOOT AND MOUTH DISEASE

M. McLaws¹, C. Bartels¹, T. Knight-Jones¹, ²

¹European Commission for the Control of Foot and Mouth Disease (EuFMD)
²Institute for Animal Health, Pirbright, UK

Introduction:
Estimating the incidence of foot and mouth disease (FMD) is a key activity in the progressive control pathway (PCP), and serves diverse purposes as countries progress along the pathway. Methods available to measure FMD incidence include counts of reported outbreaks, serological surveys, participatory approaches and active surveillance for clinical disease. In this paper, we will compare results from these methods, and consider which are most appropriate for different purposes.

Materials and Methods:
The results from serological surveys and numbers of reported outbreaks from representative countries with available data are analyzed and described. The results from active clinical surveillance for FMD are also presented. Clinical incidence amongst animals that seroconverted during outbreaks has been used to estimate clinical incidence from sero-surveillance data.

Results:
Preliminary results indicate that there is a high degree of subclinical infection and/or under-reporting of clinical outbreaks; in both Egypt and Iran approximately 80% of villages sampled had serological evidence of recent FMD infection yet clinical signs had been observed in less than 20% of these villages. The number of outbreaks detected by active, clinical surveillance closely mirrors the pattern of results detected by passive reporting.

Discussion:
While serological surveys are the most unbiased method to determine the incidence of FMD, passive surveillance also has an important role to play. The implications of these analyses for different stages of the PCP-FMD will be discussed.
RISK FACTORS FOR TRANSMISSION OF FOOT-AND-MOUTH DISEASE DURING AN OUTBREAK IN SOUTHERN ENGLAND IN 2007

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Introduction:
In summer of 2007, South England had a small outbreak of FMD. Only eight farms were infected and it was not apparent how the virus spread and what farmers could do to protect themselves. A case control study was conducted during the epidemic to assess risk factors for infection and investigate the relative impact of transmission routes between farms.

Materials and methods:
All, but one case farm and 22 control farms were interviewed about practices and occurrences on their premises up to and during the outbreak. Infected premises were separated into primary and secondary according to whether infection was most likely to originate from the source or from another premise. Data was analysed using comparative statistics to identify risk. Three composite variables: hygiene risk, environmental risk and boundary risk were generated and relative importance of each was assessed using multivariable regression analyses.

Results:
Primary IPs were more likely to have a larger proportion of young stock than the other farms, whereas secondary IPs were more likely to have outdoor calvings during the epidemic. Lack of hygiene and good biosecurity appeared to be a stronger risk factor (OR=6.2, p=0.03) during this outbreak than environmental exposure or risk from routine movements into the livestock areas.

Discussion:
The study provided an insight into practices, which may help the farmers protect themselves during an outbreak. It also provided evidence that despite the presence of airborne spread, biosecurity and good hygiene still remains the most important mean of protection for farmers during an outbreak. This evidence could play an essential role in maintaining motivation for good biosecurity during future outbreaks.
THE USE OF ORAL FLUIDS FOR PRE-CLINICAL DIAGNOSIS AND MONITORING IN AN FMD EMERGENCY: CURRENT RESEARCH AND FUTURE DIRECTIONS

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Introduction:
Rapid detection of foot-and-mouth disease (FMD) virus is fundamental to the rapid control, elimination and eradication of the disease in an FMD emergency. Current methods for detecting FMD virus for pre-clinical diagnosis and monitoring depends on blood and vesicular epithelium/vesicular fluid samples collected from animals, which can be a time consuming task associated with significant stress for people and animals. It has been established that oral swabs collected from pigs and cattle infected with FMD virus reflect levels of FMD virus detected in serum/blood, in addition to a longer detection window compared to blood. Recently, an increasing number of studies also showed that a variety of other pig viruses were shed in oral fluid. These studies suggest that oral fluid can be tested for the presence of viral pathogens via virus isolation or polymerase chain reaction (PCR). The purpose of this paper was to summarise the current knowledge and gaps about the potentials of using oral-fluid specimen for pre-clinical diagnosis and monitoring of FMD virus in an FMD emergency.

Methods:
A review was conducted using search terms including virus isolation and RT-PCR assays for detection of FMD virus in swabs. In addition, our recent study using strip test for detection of FMD virus in swabs was also included.

Results:
Pigs infected with different isolates of FMD virus showed that FMD virus or viral RNA could be detected in oral swabs by virus isolation or RT-PCR 1 to 2 days post inoculation (dpi)/direct contact (dpc), and even be detected for several days from contact exposed pigs before showing clinical signs. A similar pattern was observed with bovine oral swabs collected from cattle infected with FMD virus. A comparison of matched samples from individual pigs and cattle showed that oral swabs were equal to serum for the detection of FMD virus the first week of infection. In addition, the viral RNA could be detected in oral swabs by RT-PCR up to 9 dpi/12 dpc in pigs and up to 18 dpi/dpc in cattle, by which time no virus was detectable in blood (i.e., viremia had ceased). During this period, more oral samples were scored positive by RT-PCR than virus isolation. It was noted that in those studies oral samples were collected from individual animals using non-harmonised sampling methods. In addition to RT-PCR or virus isolation-based assays, adaptation of a penside test to oral fluid specimens is an important development. Our preliminary study with FMD virus serotype O-specific immunochromatographic lateral-flow strip test (ILFST) showed that oral fluid could be tested for presence of viral antigen using ILFST, but further optimization is required for sampling and for optimal sensitivity of ILFST.

Conclusion:
Pre-clinical viral presence and a longer detection window support that oral fluid-based testing could facilitate rapid detection of FMD. However, there are still some technical issues to be resolved before its full potential diagnostic value could be reached, including inconsistent oral fluid and elution buffer volume, variable virus recoveries and as yet inadequate sensitivity for oral fluid testing by ILFST.
NOTES
FOOT-AND-MOUTH DISEASE VIRUS TRANSBOUNDARY MOVEMENTS BETWEEN SUB-SAHARAN AFRICA, NORTH AFRICA AND THE MIDDLE EAST

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Introduction: Foot-and-mouth disease (FMD) viruses are frequently geographically restricted, both at the serotype and topotype level, and thus transboundary spread of virus can often be traced using VP1 sequence information. We have examined the recent and historical spread of FMD viruses between sub-Saharan Africa, North Africa and the Middle East to assess current and future threats.

Materials and methods: FMD viruses, isolated in cell cultures from clinical samples received by the FAO World Reference Laboratory for FMD, were subjected to RT-PCR of the VP1-coding region using previously described methods. The resultant amplicons were sequenced using an ABI 3730 Automated Sequencer. VP1 sequences were assembled using SeqMan Pro 10 (DNASTar Inc.) and phylogenetic trees were constructed using MEGA 5.05 software.


Discussion: Until recently the spread of FMD viruses into North Africa from sub-Saharan countries has been a rare event. However, in 2012, examples of trans-Saharan FMD virus movements have been identified involving three serotypes, O, A and SAT 2 (three lineages). In the same period movement of FMD virus type SAT 2 from East Africa to Bahrain was also detected. The apparent increase in FMD virus diversity in Egypt and Libya may be the result of recent political and social changes in Arab world leading to increased trade across the Sahara. Close monitoring of FMDV movements in this region are vital to identify the possible origins of infection.
MAXIMISING EFFICIENCY WITH A SURVEILLANCE STRATEGY FOR FOOT-
AND-MOUTH DISEASE DURING AN OUTBREAK IN A PREVIOUSLY FMD-FREE
COUNTRY.
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Introduction:
New Zealand has never had an outbreak of foot-and-mouth disease, but preparedness planning for responding to an incursion is a high priority for New Zealand’s Ministry for Primary Industries. As part of FMD preparedness in New Zealand, a sampling and diagnostic surveillance strategy was developed to guide rapid and efficient detection of infected farms, through to proof-of-disease-freedom surveillance.

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

Methods:
Factors assessed in development of the strategy include:

- the available diagnostic tests for FMD in the country, and the sensitivity and specificity of these and parallel and series combinations thereof;
- the practicalities of obtaining certain sample types and numbers, and of laboratory resource requirements;
- the design prevalences and desired confidence in results for the surveillance programme;
- a generalised time-line of virus and antibody presence in an individual animal;
- the surveillance category of a property (for example: an at-risk place with epidemiological links to an infected place, or a property within a surveillance zone around infected places, or a report case), the probable time since exposure to virus, and whether clinical signs are present or not; and
- the species, and number of animals and management groups present on a farm.

Results:
Simple table-format presentation of the sampling guidelines, along with a pre-specified laboratory submission form, enable the field veterinarian to easily determine the appropriate samples to collect and for the laboratory to conduct testing based on established priority. Hence, the need for decision-making is streamlined or removed, with the aim of improving efficiency.

Discussion:
Other aims of the strategy, beyond the speed of implementation of surveillance, includes collecting appropriate samples to describe the epidemiology of the outbreak, which may be used both real-time and retrospectively, and to provide guidelines for the farm-level sampling and testing for a proof-of-freedom claim.
FMD CONTROL IN THE SOUTHERN CAUCASUS REGION: A BATTLE AGAINST WINDMILLS?

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Abstract:

Given the current Foot-and-mouth disease (FMD) freedom in most of Europe the possibility of future outbreaks is threatening. Thus FMD control in the southern Caucasus region (Armenia, Azerbaijan and Georgia) and neighbouring countries has been supported by the European Commission and FAO since several years. Sero surveys for the detection of antibodies against non-structural proteins (NSP) of the FMD virus were conducted in the cattle and sheep populations of the southern Caucasus in 2010 and 2012.

Our survey results were used to estimate the level of virus circulation, to describe the geographical distribution of NSP antibodies, to evaluate the success of the vaccination campaigns, and to identify and quantify risk factors for positive NSP antibody outcomes. Results indicated continued exposure of ruminants to FMD virus in the region despite several years of FMD control. Temporal patterns of FMD infection in the Caucasus region prior to the surveys correlated with the occurrence of major FMD epidemics in Iran and Turkey. In 2010 mean animal level NSP antibody prevalence ranged between 3 to 22 % in cattle and between 3 to 36 % in sheep for the three countries monitored in the region. Within countries large spatial variation was noted and between village NSP antibody prevalence ranged between 0 to 97% and between 0 to 69%, for both species respectively. Associations of the NSP antibody status with spatial, FMD control and animal husbandry risk factors were demonstrated.

Our findings highlighted that FMD control strategies will increasingly need to take into account measures such as zoning, improved biosecurity as well as targeted vaccinations and surveillance for high-risk sub-populations. Further, regional cooperation is essential for controlling this highly contagious disease in small countries.
DEVELOPMENT OF A LONG (2012 – 2022) TERM ROADMAP FOR THE PROGRESSIVE CONTROL OF FOOT-AND MOUTH DISEASE IN EASTERN AFRICA: LESSONS AND CHALLENGES

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In March 2012, chief veterinary officers from the Eastern African countries met in Nairobi-Kenya to development a long term road map for progressive FMD control. The workshop resulted into classification of all Eastern African countries in one of the PCP stages and updating regional roadmap until 2022.

However, the classification was based on subjective evaluation of secondary and heterogeneous data derived from studies carried out using different methods, based on different time periods, relating to different production systems in different agro-ecological zones. In particular data compiled from passive surveillance reports rather than national or zonal surveys of randomly selected samples of the cattle population. Consequently, the knowledge and the epidemiology of the disease are not sufficient to provide a scientific basis for designing a national strategy.

A comparison to a similar process for the SADC region revealed that the Eastern African countries lack a favourable institutional environment to drive the PCP-process. The PCP process is further constrained by underfunding, weak implementation capacity for policies, regulations and standards, lack of harmonised regional efforts to prevent and control animal diseases, lack of adequate incentives for a demand driven approach to comprehensive FMD control (therefore the veterinary services, farming communities and society at large cannot sustain a national programme against FMD), failure by countries to clearly identify the socio-economic benefits of FMD control to the public, and farmers’ attitude towards FMD.

Therefore there is a need for a more critical and objective evaluation process, and reformulation of the PCP to be more adaptive to local epidemiological conditions. A successful roadmap will depend on the ability and speed at which countries shall address fundamental issues identified by the OIE PVS and Gap analysis reports, such as vet-governance, provision of favourable policies and legal framework. However, adequate incentives for a demand driven approach to a comprehensive FMD control, and farmers’ attitude towards FMD, are probably the main key factors.
TRACKING PROGRESS ALONG THE JOINT FAO/OIE PROGRESSIVE CONTROL PATHWAY FOR FMD

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Introduction:
The Joint FAO/OIE Progressive Control Pathway for FMD Control (PCP-FMD) was developed by FAO, EuFMD and OIE as a tool to assist FMD endemic countries to develop risk-based, sustainable and feasible strategies to progressively reduce the impact of FMD and circulation of FMD virus. The PCP-FMD consists of 5 Stages, and as it becomes more widely used there is a need for a procedure to identify the appropriate Stage for each participating country.

Discussion:
The primary objective of the assessment is to track progress along the PCP-FMD globally; important secondary objectives are to provide useful feedback to the country, to enhance understanding about the PCP-FMD and to identify priority areas to target technical and/or financial support. The assessment procedure balances a number of criteria: to be consistent across diverse regions and situations yet acknowledge that outcomes may be achieved through a variety of means; to be evidence-based yet user-friendly and not too arduous; and to be transparent yet respect confidentiality.

A checklist has been developed consisting of yes-no questions that can be easily answered by one or more people familiar with livestock husbandry and the FMD situation in a particular country. Each Stage is defined by a series of Outcomes, and the assessment gauges the level of achievement for each Outcome. There are required and recommended achievements; in order to complete a Stage a country must fulfill all required elements while the recommended achievements indicate how to improve the quality or thoroughness of the work.
COMPARATIVE ANALYSIS OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPE A POSITIVE SELECTION IN PAKISTAN, AFGHANISTAN, IRAN AND TURKEY

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Introduction:
One of the most challenging aspects of foot-and-mouth disease (FMD) control is the understanding of the virus genetic variability, and the forces that impact such changes in virus evolution. Selection of specific sites within the FMD virus (FMDV) capsid structure may vary depending on the values observed of certain environmental factors, which are heterogeneous through different endemic settings. Serotype A FMDV is of special interest because of the variable nature of the virus, posing a major difficulty for selecting appropriate vaccine antigens in specific areas.

Materials and Methods:
FMDV serotype O VP1 sequences from Pakistan and Afghanistan (n=37, sampled between 07/2008-09/2009), Iran (n=38, sampled between 04/2005-10/2007) and Turkey (n=57 sampled between 11/2005-06/2008). Sequences were analyzed separately for each region for detection of evidence of positive selection by exploring the differences between synonymous (dS) and non-synonymous changes (dN). Three different statistical methods were used to identify individual codons under selection. Mean values of dN/dS (MdN/dS) were computed for each dataset. A site was considered under positive selection if evidence was detected by at least 1 statistical method.

Results:
The values of MdN/dS were similar for viruses from Iran and Turkey (1.60 and 1.61, respectively), whereas it was relatively higher (1.84) for Pakistan/Afghanistan sequences. The individual codon analysis yielded 18 positively selected sites for Iran sequences, 5 sites in Turkey and 3 in Pakistan/Afghanistan. One site (141), located close to the RGD motif, was the only codon consistently found under positive selection for all three datasets. Within Turkey and Iran sequences, additionally codons (45, 149, 149, and 196) were consistently under positive selection.

Discussion:
Characteristics of the animal population in different areas may have a different impact in selecting FMDV. These results will help to develop effective prevention and control strategies in the region.
PHYLODYNAMIC RECONSTRUCTION OF TYPE O CATHAY TOPOTYPE FOOT- AND-MOUTH DISEASE VIRUS EPIDEMICS IN PHILIPPINES BETWEEN 1994 AND 2005

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Introduction:
Reconstructing the evolutionary history, demographic signal and spatial diffusion processes from virus sequences provides fundamental understanding of the epidemiological dynamics underlying epidemics. Employing a Bayesian phylogenetic analysis framework, we examine the phylodynamics and spatio-temporal dispersion of the last FMDV epidemics reported in Philippines between 1994 and 2005 when FMD was eradicated.

Materials and Methods:
A dataset comprising 112 VP1 sequences obtained from type O CATHAY topotype FMDV isolates collected between 1994 and 2005 from Philippines was analysed. Bayesian phylogenetic analysis was performed in BEAST 1.7.3 using the Random Local Clock model and demography of FMDV transmission reconstructed using the Bayesian Skyline and Skyride models. Spatial patterns of FMDV dispersal were computed through a probabilistic discrete asymmetrical diffusion model using a Continuous-Time Markov Chain (CTMC) process, adopting a Bayesian Stochastic Search Variable Selection (BSSVS) procedure to select among all possible migration pathways. Furthermore, the relationship between Philippines lineages and other FMDV type O CATHAY topotype isolated within the Southeast Asia region was assessed including into the analysis further 183 VP1 sequences collected from Hong Kong and Taiwan and retrieved from both GeneBank and the WRL-FMD databases.

Results:
The introduction date of FMDV into Philippines was calculated to be 1993.939 (95%HPD 1993.087 to 1994.632), where the Time of the Most Recent Common Ancestor (TMRCA) was estimated at 1993.576 (95%HPD 1992.635 to 1994.409). Reconstructed FMDV phylodynamic describes an oscillatory demographic history leading to three phases: after an initial exponential increase until late 1997, a sudden and short period of decline was observed, which can be expressed as a population bottleneck event. A second fast exponential epidemic phase beginning in middle 1998 and lasted up to the first months of 1999 reached a plateau until late 2002, when the Philippines experienced a steady decline in FMD epidemics until the effective eradication. The Philippines lineages were found as descend from a common ancestor shared with the Hong Kong isolates (1957.331, 95%HPD 1793.303 to 1965.674), whereas the Taiwanese cluster seems to be directly descend from the MRCA of the CATHAY topotype (1955.969, 95%HPD 1591.075 to 1965.463). The three country-tree-clusters were described as monophyletic. No other FMDV introduction or escape was ascribed to the Philippines CATHAY FMDV epidemic history.

Discussion:
Phylogenetic models have been developed with the aim of capture genetic signatures for reconstructing demographical dynamics and transmission routes of viral system in either epidemic or endemic settings. However, the evolutionary history of viruses is only fully comprehended by also considering its geographical context, the spatial component, and the host population structure in which the pathogen acts. Direct knowledge of the tree of infections is likely needed in addition to sequence data for the accurate inference of prevalence from sequence data. Therefore, further studies are needed to investigate the potential of integrating epidemiological and genetic data for fully resolve the transmission dynamics of viral dispersal.
FMDV GENOTYPING OF AUSTRIAN STRAINS ISOLATED FROM 1965 TO 1981

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Introduction:  
Austria recorded its last Foot-and-mouth disease (FMD) outbreak in the year 1981. In total 29 samples from positive animals from different farms were collected between 1965 and 1981. During that time the diagnostic tools were limited to virus neutralization and AG-ELISA. Within the last years DNA genotyping has become more important. In order to compare the sensitivity and specificity of the above mentioned diagnostic methods with methods from molecular biology, we tested 29 formerly serotyped positive samples by DNA genotyping assay.

Materials and methods:  
We performed FMDV genotyping as described by Shin et al. (2003) with slight modifications. We used the commercial kits Qiagen® (QIAamp® Viral RNA Mini kit and RNeasy® Mini kit) for nucleic acid extraction and the one-step RT-PCR kit SuperScript® III One-step RT-PCR System with Platinum® Taq from Invitrogen® for reverse transcription and PCR amplification. In addition to the formerly positive found samples, we tested 107 negative samples from epithelium and serum collected in Austria. In order to test the specificity of the molecular method, 16 other viruses (BTV-1, EHDV, SVDV, 2 serotypes of Vesicular Stomatitis, PPRV, RPV, Sheeppox virus, BVDV-1, BDV, ASFV, PEV, Vaccinia virus, Hepatitis-A virus, BPSV and AHSV) were included in this study.

Results:  
All positive samples were formerly serotyped as C and O with the methods virus neutralization and AG-ELISA. The results of the RT-PCR genotyping were compared with the above mentioned methods and resulted in 100% accordance, both sensitivity and specificity.

Discussion:  
FMDV genotyping is not commonly used in diagnostic laboratories. This study demonstrates that the modified FMDV genotyping assay by Shin et al (2003) is capable for detecting the FMDV serotypes C and O, respectively. In this study we could demonstrate that DNA genotyping can be used as a confirmatory assay to the AG-ELISA.
PHYLOGENETIC AND ANTIGENIC CHARACTERISTICS OF TYPE O FMDV ISOLATES RESPONSIBLE FOR OUTBREAKS IN RUSSIA AND NEIGHBOURING COUNTRIES IN 2010-2012

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Introduction:
The FGBI “ARRIAH” is the OIE Regional Reference Laboratory for foot and mouth disease (FMD). Examination of antigenic and genetic characteristics of FMD virus isolates circulating in Russia and neighbouring countries is one of the Laboratory tasks. The isolates are phylogenetically characterized to identify the origin of the isolated viruses and their antigenic properties are determined to select the most appropriate strains of FMDV for use as vaccines to control disease (D.J. Paton et al, 2005).

Material and Methods:
Twelve isolates of type O FMD virus submitted to the FGBI “ARRIAH” from Russia, Mongolia, Kazakhstan, Tajikistan, Kyrgyzstan and South Ossetia were selected for study. FMD virus was isolated from pathological materials in primary and continuous porcine kidney cell cultures.
VP1 gene was amplified with RT-PCR and automated sequencing of PCR products followed by phylogenetic analysis was performed.
The antigenic match between the isolates and vaccine O1 Manisa, O/Russia/2000 (O-PanAsia) and O/PanAsia2 strains was studied with cross microneutralization test using sera from cattle immunized with monovalent vaccines. The r1 value was calculated as ratio of serum titres against hetero- and homologous strains and interpreted according to the OIE recommendations: if r1 ≥ 0.3 the isolate was antigenically related to the vaccine strain, if r1 <0.3 the isolate was antigenically differed from the vaccine strain.

Results:
The phylogenetic analysis showed that the examined isolates belonged to different topotypes of type O FMDV.
The isolates responsible for FMD outbreaks in Russia and Mongolia in 2010-2012 belonged to the South-East Asia (SEA) topotype that had not been registered in the Russian Federation earlier. The vaccine O1 Manisa, O/Russia/2000 and O/PanAsia-2 strains covered all these isolates. The O/Kazakhstan/2010, O/Kazakhstan/May2011, O/Tadjikistan/2011, O/Kyrgyzstan/2011 and O/South Ossetia/2011 belonged to O-PanAsia-2 genetic lineage of the Middle East-South Asia (ME-SA) topotype. Those isolates demonstrated maximal level of antigenic relatedness to vaccine O-PanAsia-2 strain. Two isolates (O/South Ossetia/2011 and O/Tadjikistan/2011) differed from vaccine O1 Manisa and O/Russia/2000 strains in antigenic properties (r1 <0.3).
The isolates that had caused FMD outbreaks in Eastern Kazakhstan in 2011 and 2012 as well as in Russian Far East in 2012 belonged to O-PanAsia lineage and were genetically close to O/CHA/7/2011 (JF837375 in the GenBank). The isolate recovered in East Kazakhstan in 2011 was demonstrated by microneutralization test to be close antigenically related to all examined vaccine strains. Russian isolates collected in 2012 were antigenically related to O1 Manisa strain but differed from vaccine O/Russia/2000 and O-PanAsia-2 strains.

Discussion:
The study revealed significant genetic and antigenic diversity of FMD virus isolates collected in the territory of Russian Federation and neighbouring countries in 2010-2012.
SITUATION OF FOOT-AND-MOUTH DISEASE IN ETHIOPIA FROM 2010-2012

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Introduction:
FMD surveillance using both serological and outbreak investigations was performed in Ethiopia from 2010 to 2012 as a set of FMD progressive control programmes in the region. During this period 2282 sera samples from cattle, 3156 from small ruminants and 48 pooled clinical samples were collected and tested. This can help to understand the serotypes circulating in the country to improve the vaccine matching to the file isolate.

Materials and methods:
Sera samples were collected and tested against non-structural (NSP) of FMD virus by using 3ABC ELISA at National Animal Health Diagnostic and Investigation Center (NAHDIC). Epithelial tissue, oral and vascular lesion swab and whole blood were also collected from FMD outbreak areas of Ethiopia and submitted to WRLFMD for further characterization and serological matching to vaccine strains.

Results:
Out of the total 5438 serum samples examined, 13.9% (318 of 2282) of cattle and 2.5% (79 of 3156) of small ruminant were found to be positive for NSP of FMD virus. Characterization of the 48 pooled clinical samples from outbreak areas showed that FMDV O was the most prevalent recovered serotype during the survey period with all isolates belonging to the East Africa 3 (EA-3) topotype. The phylogenetic analysis of FMD type O viruses isolated from cattle in the Tigray Region showed that they were most closely related to the O type viruses from Sudan isolated during 2008, 1999, and 2004. While the isolates from Oromia, Amhara and Southern Nations, Nationalities, and People’s Region were closely related to FMD O type previously isolated in Ethiopia during 2009, 2010 and 2011. The FMD type O isolates O/ETH/1/2011 and O/ETH/7/2011 from Debre Ziet and Addis Ababa areas isolated from swine and cattle, respectively, were antigenically matched with O 4625, O Campos, O Manisa and O TUR/5/2009 vaccine strains, whereas the O/ETH/13/2011 and O/ETH/28/2011 field isolates from the same year from Adama and Shire (Tigray Region) was different from these vaccine strains which were unlikely to provide protection.

Discussion:
The FMD virus isolates currently from the Tigray Region in the North of Ethiopia were distinct from those previously present in other parts of Ethiopia suggesting a risk of introduction of the disease from neighbouring countries through pastoralist and animal trade movements. The presence of two antigenically distinct FMD type O viruses in Ethiopia should be noted for current vaccine used. Such as O vaccine from NVI (Ethiopia) and KEVIVAPI (Kenya) and Indian Immunologicals Company for FMD control strategies.
EMERGENCE OF NOVEL GENETIC LINEAGE OF SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUS IN INDIA

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Introduction:
In India, approximately 80% of the Foot-and-mouth disease (FMD) outbreaks are attributed to serotype O (Subramaniam et al., 2012). In this study, VP1 sequence analysis of 141 serotype O field outbreak viruses collected during 2009-2012 revealed emergence of a new genetic group named as Ind2011 lineage and continued dominance of Ind2001 lineage in the field since 2009.

Materials and Methods:
RNA extraction, Reverse transcription and PCR amplification of VP1 region was carried essentially as described earlier (Hemadri et al., 2002). Sequencing was performed on a AB1 3130 Genetic analyzer (Applied Biosystems). Phylogenetic analysis was conducted using MEGA 5.05 software (Tamura et al., 2011) employing the best fit nucleotide (nt) substitution model, TN93+G +I.

Result:
In the Maximum Likelihood tree, maximum number of isolates (n=95) clustered in Ind2001 lineage. Within Ind2001 lineage, two sub-lineages; Ind2001a and Ind2001b were detected. Decline in circulation of PanAsia lineage is noticed as only 10 outbreak strains clustered within this lineage. A new genetic group (Ind2011 lineage) with more than 9% nucleotide divergence from contemporary viruses circulating in India was evident.

Discussion:
The Ind2001 lineage that resurged in 2009 continued its dominance in the field during 2010 and 2011 as well. Within Ind2001 lineage very high genetic diversity was detected and with time the lineage has diversified leading to two sub-lineages. The newly emerged Ind2011 lineage is so far restricted to southern region of the country. Within the span of 5 months, the Ind2011 lineage has caused 19 outbreaks. Though, PanAsia virus was present in India since 1982, its dominance in the field was evident after a gap of 14 years in 1996. Similarly, Ind2001 lineage was identified as divergent strain from PanAsia in the year 2001; its predominance was noted in 2009. With the emergence of new genetic group, overall epidemiological picture of FMD in the region is expected to change in coming years and hence is required to be monitored continually.
PHYLOGEOGRAPHIC STUDY OF FOOT AND MOUTH DISEASE VIRUS
A/ARGENTINA/2001 STRAIN

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Introduction:
The serotype A Foot-and-Mouth Disease Virus (FMDV) epidemic that affected Argentina in 2001 had a significant impact on the local economy. More than 2000 outbreaks were reported that year, mostly produced by the A/Argentina/2001 strain. The purpose of the present work is to compare FMDV evolution at two different sampling scales: one at the local level (Mar Chiquita county, two months’ period) and the other at the country level (eleven months’ period).

Materials and methods:
We studied the complete genome of 34 samples isolated from all around Argentina (and two samples from Uruguay) (country level study), and 15 samples from Mar Chiquita (local level study). To study how genetic divergence could be predicted by geographic and/or temporal distance between infected herds, we used a non parametric multiple regression approach. Phylogenetic analyses were conducted using maximum likelihood approach.

Results:
The phylogenetic analyses suggested that there were two genetic clusters: one distributed mainly over central and northern Argentina, and the other distributed over central and eastern Argentina. The basal samples for both clusters were found in the central region. Thus, the central region contained both genetic clusters and their basal nodes. At the local level, neither geography nor time were predictors of genetic divergence. At the country level, only temporal distance between samples was statistically related to genetic divergence. We found a substitution rate of 0.75% genome divergence/year, which is slightly lower than the estimated rate for VP1 in a different sample set of the same virus outbreak (Perez et al., 2008).

Discussion:
Our results indicate that the central region of the country could have been the area of origin for these samples, and also that temporal distance could be a predictor of genetic divergence only at the country level.
STUDY ON FOOT-AND-MOUTH DISEASE VIRUS VACCINAL STRAIN SELECTION OF ETHIOPIAN ISOLATES

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Introduction:
The complex epidemiological situation of FMD in Ethiopia due to the circulation of multi-serotypes of the virus as well as roaming free cross borders between the neighboring countries and lack of control of animal movements urges the need of having appropriate vaccinal strains for vaccine based disease control strategies of the country.

Materials and methods:

Results:
The result showed that the field isolates are highly related to the candidate vaccine strains with their ‘r1’values above the cut-off point, except one Serotype A isolate.

Discussion:
For vaccine matching purposes, FMDV vaccine strain selection is based on indirect serological methods (r-values), on sequence data (Paton et al., 2005) or alternatively on the calculation of the relatedness between the field isolate and available vaccine strains using in vivo challenge tests (Brehm et al., 2008; Goris et al., 2008). This study was performed by the use of indirect in vitro serological method using two-dimensional virus neutralization test to assess serological or antigenic relations of the field isolates to antisera raised against the candidate vaccine strains. In general, the overall assessment of antigenic relations between the candidate vaccine strains and circulating field isolates tested showed better matching. Therefore these vaccine strains could be used for effective FMD control strategy in the country.
EVALUATION OF FMD TREND IN I.R.IRAN (2006-2011)

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Introduction:
2006 is an important point in FMD History of Iran as starting point of Collaboration with Eu_FMD (FAO projects) as a part of regional efforts to control and eradication of FMD in central Asia. Use of internet base reporting system linked to GIS (ADRS) for better understanding the evolution of Transboundary animal diseases specially FMD, as one of the most important endemic animal disease in the country, help to be able to follow the evolution of the disease during a period of years statistically and imagery. In this article we try to show the trend of FMD from 2006-2012 and evaluate the effects of the project on the control of this disease in Iran.

Materials and Methods:
Animal Disease Reporting System data used to analyze the trend and spatial distribution of FMD. Number of the outbreaks in a defined month used as a mean value for comparing with the overall calculated Mean values and Confidence interval of previous months as a way to be able to interpret the evolution of the outbreaks. Disease Trend Analysis used to compare the above analysis and the degree of movement of disease outbreak around the trend line.

Results:
It is showed that as a general feature of the disease, there are uncontrolled explosion of the outbreaks still occurring. Comparing the results of trend analysis and moving average plot do not show any change in the situation of disease. The calculated values and graphs significantly show the same pattern during the time. Except that spatial pattern of disease is going to be much more distributed in space and time.

Discussion:
In both cases (cattle and Sheep & Goat) the analysis of trend and moving average plot and distribution and density images shows a stable pattern. Means that disease does not decided to be self controlled and is scattered all over. In other words implemented measures (vaccination mainly, Biosecurity) and specifically management of parameters effective on control of disease (personnel, costs, training) really are not taken in to consideration. In this respect it is pertinent to say that not only scientific studies over the disease are helpful in control of it but also, these measures are key points in control processes. Moreover, Disease management is the only important key for controlling the disease. In this case decision making is the major one. Harmonized actions under supervision of an authorized person who could decide and apply the actions had the main role. Otherwise we learnt that we lost our time and money. Means "Zero" movement.

As a result, FMD control pathway (PCP) does not make any sense if there is not any plan for encouraging the countries to correct their decision making procedures and improving their thinking about management of the disease.
RELATIVE OCCURRENCE OF FMD OUTBREAKS AND THE STRATEGIES FOR CREATION OF DISEASE FREE ZONES IN UGANDA (JANUARY – JULY, 2012)

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Introduction:
Foot-and-mouth disease (FMD) is endemic in Uganda and most other African countries. To promote disease control and livestock trade, four disease control zones (DCZ’s) were mapped and earmarked to eventually become disease free zones. However, due to resource considerations only two zones (DCZ 1 and DCZ 2) are due for initial gazettement.

Materials and methods:
Occurrence of FMD outbreaks (January – July, 2012) was summarized from the reports at the Ministry of Agriculture Animal Industry and Fisheries, Uganda. Outbreaks were mapped relative to the location of the high cattle density “South West – North East” stretch along the country (the cattle corridor) and the DCZ’s.

Results:
FMD outbreaks by month were reported in 11 out of 111 districts; March (Kaberamaido, Alebtong and Amuria), April (Nakasongola and Adjumani), June (Nwoya, Isingiro, Ntungamo, Rakai, Kiruhura and Kyegegwa). With exception of Adjumani and Nwoya districts, affected districts lie along the cattle corridor and are closely related to disease control zones 1 and 2.

Discussion:
Most 6/11 (54.5%) FMD outbreaks occurred during the dry season month of June. Seasonal livestock migrations across national borders especially on the Southern border, and in some cases interaction with wildlife in the national parks probably play a significant part in the epidemiology of FMD in Uganda. Establishment of disease control zones is largely dependent on the involved livestock and owner populations, and large scale investment will be required to progressively shift towards establishment of disease free zones.
ANTIGENIC AND GENETIC CHARACTERIZATION OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPE O AND A CIRCULATING IN EAST AFRICA

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

Materials and methods:
Two-dimensional virus neutralisation tests (VNT) were carried out using five different bovine post-vaccinal sera and 50 FMDV serotype A viruses isolated from five East African countries and two neighboring and livestock-trade-related countries, and results represented as antigenic relationship ($r_1$) values; $r_1$ values were also generated for East African serotype O viruses ($n=48$) using three post-vaccinal sera. In addition, full capsid sequence data was generated for all the viruses used in this study.

Results:
Phylogenetic analysis revealed three different genotypes (G-I, IV and VII) of FMDV serotype A are circulating in East Africa. Preliminary vaccine matching results indicate A-ETH-2005 provides the best cross-reactivity with East African serotype A FMD viruses, though no linear correlation was observed between $r_1$ values and no. of capsid amino acid changes. Similar analyses are ongoing for serotype O viruses.

Discussion:
The vaccine matching results indicate A-ETH-2005 as a candidate vaccine against East African serotype A FMD viruses. The serology and capsid sequence data will now be combined to predict vaccine match. This may lead to identification of sequence motifs contributing to the loss of cross-reactivity with the antisera that will be tested in a reverse genetics system to study their impact on the antigenicity of the virus.
EVIDENCE FOR MULTIPLE RECOMBINATION EVENTS WITHIN GENOMES OF FMDVs CURRENTLY CIRCULATING IN WEST EURASIA

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Introduction:
Phylogenetic studies on FMDVs circulating in the West EurAsian region have largely focused on genome regions encoding the structural proteins which determine the serotype. The present study has compared near complete genome sequences of FMDVs representative of the three serotypes (O, A and Asia-1) which are currently circulating in this region.

Materials and Methods:
Near complete genome sequences (ca. 7600nt) were generated for different FMDVs belonging to serotypes O, A and Asia-1, including the O-PanAsia-II⁴ANT-10 strain and members of the A-Iran05 lineage, which are currently predominant and widespread in the West EurAsian region. These sequences were compared and analysed together with other sequences obtained from GenBank.

Results:
Comparison of different regions of the FMDVs genomes revealed evidence for multiple inter-serotypic recombination sites within some FMDVs belonging to the serotype O, A and Asia-1 viruses under study.

Discussion:
Accumulation of genetic heterogeneity within FMDV results from a number of different factors including the error rate of the viral polymerase during viral replication and the selection of variants as a result of various selection pressures. The present study shows that more dramatic changes in virus sequences can occur “in the field” as a result of recombination between different FMDV genomes. These analyses provide information about the ancestry of the serotype O, A and Asia-1 FMDVs which are currently circulating within the West EurAsian region.
DETECTION OF FMDV CARRIER CATTLE IN VACCINATED POPULATION THAT WERE INFECTED WITH SEROTYPE A FOOT-AND-MOUTH DISEASE VIRUS

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Introduction:
Since carriers may be considered a risk for transmitting infection, they should be identified by post-vaccination serosurveillance to substantiate freedom from infection and to regain the FMD-free status for the purpose of international trade. Assays against non-structural proteins (NSP) are used to differentiate between vaccination and infection. Recently we have shown that, NSP tests do not exclusively detect the carrier animals as they also detect the animals that were recovered from FMD. In contrast, we have shown IgA antibody as an indicator of oro-pharyngeal replication of O serotype FMD virus and therefore IgA test detects only the present infection. In this present study we intend to detect the carrier cattle that were infected with A serotype FMD virus.

Materials and methods:
We had previously developed and validated an indirect sandwich IgA ELISA to detect serotype O specific FMDV carrier cattle. In this study we have extended this work for the detection of serotype A specific IgA antibody in the mucosal fluids (saliva/nasal fluids) of carrier animals. Further we have replaced the inactivated antigen with recombinant empty capsid in the IgA assay to increase the efficiency of detection of carrier animals. Wherever possible, a comparison (ROC analysis) was made between the performances of detecting carrier animals by different tests viz; mucosal test, NSP test and antigen test (PCR + virus isolation).

Results:
We have analysed saliva/nasal samples of 6 homologous vaccine potency tests and 4 heterologous cross protection studies pertaining to A serotypes carried out at FLI, CVI and IAH. The IgA ELISA performed well to detect the carrier animals in these experiments and comparable to VI and PCR results.

Discussion:
More number of carrier animals were detected in heterologous challenged animals than the homologous challenge animals. Although these high potent vaccines could provide complete clinical protection in full dose vaccine group upon heterologous challenge, IgA ELISA could detect sub-clinical infection in these animals.
USE OF TRANSECTS FOR RAPID IDENTIFICATION OF FMD SPREAD IN OUTBREAKS AMONG SMALL HOLDER FARMERS IN KENYA; LESSONS FROM REAL TIME TRAINING COURSES

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Introduction:
Foot and mouth disease (FMD) is endemic in Kenya and in apparent constant circulation for serotypes O, SAT1 and SAT2 although A and C have been recorded in the past. Since early 2010, EuFMD training courses based in Nakuru have trained veterinarians from EuFMD member states and host countries in clinical management and epidemiological investigation of outbreaks using real case material. As part of the training, transect studies are used to establish local risk factors for infection during the current outbreak.

Methods:
Over two hours, 3-4 investigation teams start at a central point in a village and go in different directions recording farm details and putative risk factor information. In the latest course, EpiCollect smartphone application was used for data collection. Results were combined with data from a pilot study conducted by the primary author using similar methodology. A logistic regression model accounting for clustering was used in the analysis.

Results:
Of ten outbreaks investigated, the mean number of households visited was 25.8 (range=18-40) owning a mean of 6.1 cows (range=1-50, SE=0.79). The proportion of households with FMD ranged from 4.4-50.0% (mean=23.0, SE=0.05). Households that mixed their animals with susceptible species from other households were at five times the odds of having a case of FMD (OR=5.0, 95%CI 1.6-16.0, P=0.012). There is good evidence that the odds of a household having FMD increases for each additional cow in the herd (OR=1.1, 95%CI 1.0-1.2, P=0.045). Households that owned small ruminants were at 2.8 times the odds of having FMD (95%CI 1.0-8.0, P=0.045).

Conclusion:
Real-time assessment of FMD outbreaks can offer a rapid, simple way of establishing local risk factors, outbreak source and spread, and relevant control measures. Although in this analysis the methodology is inconsistent due to differing trainee-led investigations, the approach is useful for investigating outbreaks in endemic countries.
FMDV SEROTYPES IN BUFFALOS IN QUEEN ELIZABETH NATIONAL PARK, UGANDA (2011)

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Introduction:
Foot-and-mouth disease is endemic in Uganda, and Buffalos (Syncerus caffer) are known reservoirs of FMDV SAT serotypes. We determined the FMDV serotypes circulating in Buffalos in Queen Elizabeth National Park (QENP) in South Western Uganda, with high level of livestock–wildlife interaction.

Materials and Methods:
Serum and oro-pharyngeal probang samples were collected from 12 buffalos in QENP in August, 2011. Serum was screened using Priocheck FMDV NS ELISA. All buffalo samples were screened using Real-Time PCR. Serotypes were determined by solid phase blocking ELISA (SPBE) (sample titers ≥80 were considered positive) and Virus Neutralization Test (VNT).

Results:
58% (7/12) of buffalos were positive for antibodies against FMDV by Priocheck FMDV NS ELISA. Serotyping of these antibodies by SPBE showed antibodies against serotypes SAT 2 (5/12; 42%) and SAT 3 (3/12; 25%). VNT confirmed antibodies against SAT 2 (10/11, 90%) and SAT 3 (1/11, 9%). Real-Time PCR revealed that 92% (11/12) of all investigated buffalos were positive for FMDV genome in OP samples.

Discussion:
This study was undertaken on wildlife without any sign of infection. Priocheck FMDV NS ELISA identified antibodies against FMDV in 58% of the buffalos, and the antibodies serotyped were mainly directed against SAT 2, and possibly against SAT 3 in one animal. This confirms serological findings in buffalos in previous work (Kalema-Zikusoka et al., 2005; Ayebazibwe et al., 2010). Serological evidence from this paper and serological results from Dhikusoka et al. indicate that the FMD viruses circulating in buffalos and cattle in this area may not be same. The on-going virus sequencing studies will be useful in understanding the molecular epidemiology of FMDV in livestock and wildlife and in clarifying the role of wildlife in the epidemiology of FMDV in the area around QENP.
SERO-SURVEILLANCE OF FOOT AND MOUTH DISEASE IN SMALL RUMINANTS OF INDIA

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Introduction:
Foot and mouth disease (FMD) is a highly contagious disease of cloven-hoofed domesticated and wild animals and it continues to be a major threat to livestock trade worldwide. India ranks second in goat population (124.35 million) and third in sheep population (67.198 million) in the world. Despite representing a huge part of the world’s domestic livestock population, the role of small ruminants in the epidemiology of FMD has been largely ignored. This study was aimed at determining the sero-prevalence of antibodies against non-structural protein of FMDV in small ruminants in India.

Materials and methods:
A total of 7401 serum samples (3680 from sheep and 3721 from goats) were collected from various states of India during 2010-2012. An indirect ELISA was performed using r3AB3 DIVA ELISA kit (PDFMD, Mukteswar) having a diagnostic sensitivity and specificity of 95% and 98%, respectively to assess antibodies against 3AB-non-structural protein (NSP) of FMDV.

Results:
A total of 780 of 3680 (21.19%) sheep and 545 of 3721 (14.64%) goats were found to be 3AB3 NSP reactors providing a serological evidence of viral activity in small ruminant population of India. In India, the NSP antibody prevalence in large ruminants during the same period appeared to be nearly 27% (Annual Report, PDFMD, 2011-12), which is comparatively higher than those observed in small ruminants. Concurrent asymptomatic FMD virus infection in small ruminants in outbreak areas involving large ruminants could pose a potential risk of virus dissemination.

Discussion:
Sheep and goats carry FMD virus as subclinical respondents and may disseminate the virus in the environment. Hence, a requirement does exist to bring these species under the umbrella of ongoing national surveillance and control measures including routine vaccination coupled with zoosanitary measures to reduce silent amplification, excretion and transmission of the virus, to achieve comprehensive freedom from FMD.
SUBCLINICAL FOOT-AND-MOUTH DISEASE VIRUS OCCURRENCE IN UNVACCINATED CALVES AT THE WILDLIFE INTERFACE AREAS OF QUEEN ELIZABETH NATIONAL PARK

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Introduction:
Uganda has many protected areas that harbor large numbers of wildlife believed to be maintenance hosts of Foot-and-mouth disease virus (FMDV). In this study we investigate the prevalence of FMDV among unvaccinated calves that constantly interact with wildlife in Queen Elizabeth National Park (QENP) where FMD has not been reported since 2006.

Materials and methods:
248 calves aged 6 to 24 months were randomly sampled in 25 pastoral herds in 12 parishes, from two interface Sub-counties of Katwe Kabatooro and Nyakatonzi in August 2011. The Serum collected was screened for antibodies against FMDV using Priocheck\textsuperscript{®} FMDV-NS ELISA, and antibodies were serotyped using Solid Phase Blocking ELISAs. Serotype-specificity was confirmed using virus neutralization test (VNT). Probang samples from the same animals were tested for FMDV RNA content using real time RT-PCR.

Results:
Sera from 31/248 (12.5\%) calves that were never reported sick nor vaccinated against FMD tested positive to antibodies against NSP of FMDV. 14/23 (60.8\%) of the selected herds had evidence of antibodies against the NSPs. Investigations of serotype-specificity of these antibodies showed titres of 80 and above against serotypes: O (7/18), SAT 1 (4/10), SAT 2 (3/14) and SAT 3 (2/16) meanwhile VNT indicated contact with serotype O. Real time RT-PCR on probang samples from the same seropositive animals identified FMDV RNA in seven animals with Ct-values 18.3, 18.7, 26.5, 34.7, 38.3, 39.2, 41.3 confirming presence of FMDV RNA.

Discussion:
Presence of FMDV RNA in seven young unvaccinated animals without visible or reported clinical symptoms of FMD, supported with positive serological VNT results, is an indicator of exposure to circulating FMD-viruses. This implies that there may be continuous FMDV circulation in livestock and wildlife at the QENP interface in Kasese District. There is need to further investigate the dynamics of FMD at the livestock wildlife interface.
PROTECTIVE IMMUNE RESPONSES ELICITED AFTER CONSECUTIVE INOCULATIONS OF THE SAME ANTIGEN, USING DIFFERENT VACCINE DELIVERY APPROACHES

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Introduction:
New generation vaccines against foot-and-mouth disease virus (FMDV) have been largely explored, though they have not equaled the efficacy of the conventional killed virus vaccine in use. In the present study, we analyzed the immune responses elicited by consecutive immunizations of the same antigen, using different vaccine delivery approaches, in a heterologous prime-boost format.

Materials and Methods:
Herpesvirus and adenovirus vectors encoding FMDV structural proteins and the 3C protease from O1 Campos strain, and inactivated oil adjuvanted O1 Campos virus were used in a heterologous prime-boost schedule. Humoral and cellular immunity and protection after challenge in BALB/c mice was used.

Results:
The magnitude and quality of the protective immune response was dependent on the delivery immunization regimen used. Priming with any of the viral vectors induced a shift of the cytokine balance towards a Th1 type immune response, with a IgG1/IgG2a ratio of 0.5-0.8, regardless of the delivery system used for boosting, while priming with inactivated virus induced a Th2 type response, with a ratio IgG1/IgG2a=1.5. Heterologous prime-boost induced significantly higher specific antibody titers than homologous booster immunization (p< 0.05). Interestingly, similar antibody titers were elicited in mice vaccinated with heterologous combinations including viral vectors, than in mice that received two doses of adjuvanted killed virus. Re-stimulation of mice with inactivated virus after 146 days resulted in a fast increase of antibody titers and memory B cells in all groups. In challenge experiments, after priming of the animals with the adenovirus viral vector, boosting with herpesvirus amplicons or killed virus induced 75% protection, the same percentage obtained with two doses of oil adjuvanted inactivated virus.

Discussion:
Immunization with viral vector-based vaccines combined with protein-based vaccines in diverse delivery formats may represent a promising approach for improving active protection against FMDV.
CO-ADMINISTRATION OF PLASMIDS ENCODING FMDV P12A3C, CD40L OR IL-15 ENHANCED PROTECTIVE IMMUNE RESPONSE AGAINST VIRAL CHALLENGE

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Introduction:
DNA vaccines offer an economical and safer alternative to traditional vaccines employing inactivated virus. Nevertheless one major drawback is the low potency that constrains to employ adjuvant to further enhance DNA vaccine potency. In this study we have evaluated the capacity of inducing a specific and protective immune response using a formulation that includes eukaryotic plasmids encoding the polyprotein pP12A3C form of FMDV O1Campos in combination or not with the molecular adjuvants pCD40L and pIL15 and to evaluate the immune efficacy of these DNA vaccinations in a murine model.

Materials and methods:
Balb/c mice were inoculated i.d, four times with the different DNA vaccines in combination or not with the chemical adjuvant Montanide Essai 903101 (SEPPIC) and a negative control group inoculated with empty plasmids or the adjuvant. Total antibodies against FMDV were evaluated by ELISA and the protective efficacy against viral challenge by infecting mice with 104.5 TCID50 of FMDV strain O1/Campos on day 10 after the last inmunization.

Results:
We found that the group of mice inoculated with pP12A3C and pIL15 and the chemical adyuvants had a 75% of protected animals and with pP12A3C and pCD40L and chemical adyuvants had a 50% protected animals. Surprisingly, no signicative difference in antibody titers was foud among the groups.

Discussion:
Our data suggest that genetic treatment with pIL15 and in lesser extent with pCD40L in the presence of cognate antigen can contribute to immune-enhancement and protection against FMDV challenge.
FOOT-AND-MOUTH DISEASE VACCINES INDUCE PRIMARY IMMUNE RESPONSES IN CALVES WITH PREEXISTING MATERNAL IMMUNITY

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Introduction:
Maternal immunity has been associated with a diminished response of calves to vaccination. The aim of this study was to evaluate the immunogenicity of the current inactivated oil-based Argentinean FMD vaccine in calves with or without maternally-derived antibodies (MdAb).

Materials and methods:
Thirteen calves with high MdAb and twelve without measurable FMDV-antibodies were vaccinated twice (day 0 and 35) with a commercial tetravalent vaccine. Animals were bled at the beginning of the study and at 7, 21, 35 and 55 days post vaccination (dpv). Sera were assessed for specific antibodies against O1 Campos strain by liquid phase blocking ELISA (LpBELISA), avidity ELISA and FMDV-specific IgM ELISA.

Results:
All calves (12/12) without MdAb elicited specific antibodies after the first vaccination, surpassing at 21 dpv a LpBELISA titer of 2.1, that correspond to 75% expectative of protection (EPP-75). Calves with MdAb had LpBELISA titers over 2.9 at day 0, and titers were maintained over protective levels along the study. Avidity maturation profiles were different between both groups. Calves without MdAb reached high IgG-avidity levels at 35 dpv, after one vaccine dose; while two-vaccinations were required to achieve similar avidity levels in calves with MdAb. Both groups induced similar anti FMDV-IgM profile, showing that acquired immunity was equally initiated despite the presence of MdAb at the time of vaccination.

Discussion:
Calves with high levels of MdAb immunized with an inactivated oil-adjuvanted vaccine maintained LpBELISA titers over 2.1 (EPP 75) even when MdAb had decayed. These animals elicited a primary IgM response of the same magnitude and kinetics than those without MdAb. Avidity profiles should be further explored, together with IgG-isotype responses to establish the influence of cell-mediated immunity transferred by colostrum. Vaccination of calves with MdAb allows closing the window of vulnerability created by the natural decay of maternal antibodies.
QUANTIFICATION AND PURIFICATION OF 140S APHTOVIRUS VIRAL PARTICLES BY LIQUID CHROMATOGRAPHY

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Introduction: Whole particle (140S) antigen payload in FMD vaccines is usually determined by ultracentrifugation of the viral suspensions on sucrose gradients (Vazquez et al., 1979). However, this method is time consuming, requires the use of expensive equipment and it is difficult to standardize. Therefore, the main objective of this work was to develop an alternative method based on liquid chromatography (LC), combined with the precise identification of each virus strain present in polyvalent vaccines by means of strain specific monoclonal antibodies (MAbs).

Materials and methods: Viral particles were quantified by LC on Sephacryl S® 400 columns. The methodology was standardized using heat-treated or unheated concentrated samples from inactivated FMDV A24/Cruzeiro suspensions. This virus strain generates a large amount of natural empty capsids (75S) which have been very useful, together with the 12S subunits, as size markers. Determination of antigenic mass by LC and sucrose gradients was compared using intact virus (140S) or heated samples (12S). The fractions were further analyzed using ELISA with strain specific MAbs.

Results: The comparison between the antigenic payload calculated by LC and sucrose gradients gave a good correlation (r>0.9) for A24 strain. Similar results were obtained with the vaccine strains O1 Campos, A/Arg/2001 and C3 Indaial. Quantification of viral particles by LC allowed the detection of antigenic payloads as low as 5µg/ml. However, the use of ELISA with MAbs allowed the detection of lower amounts of 140S particles (5 ng/ml).

Discussion: The results showed that LC is a suitable tool for quantification of FMDV 140 S particles during the vaccine production process as well as in the final product. We are also exploring the use of this method for industrial production of intact FMDV antigen free of undesirable contaminants, such as DNA and non-structural proteins.
DEVELOPMENT OF A PREDICTIVE MODEL FOR VACCINE MATCHING FOR SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUSES FROM serology and capsid sequence data

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Introduction:
Vaccination, an important means to control foot-and-mouth disease (FMD), is constrained by the lack of cross-protection between serotypes and even between some subtypes. Antibody has a crucial role in acquired immunity and the corresponding antigenic determinants of the virus are associated with the capsid surface. The protection afforded by conventional vaccines correlates fairly well with the amount of humoral antibody. Vaccine match is therefore usually done serologically (VNT/ELISA), however are not always accurate to estimate in-vivo cross-protection. The aim of this work is to develop a predictive model for serotype O vaccine matching by identifying regions on the capsid sequence that are good predictors of cross-reactivity. This will allow (i) to speed up the vaccine matching process, and (ii) to develop tools in the future that could more accurately predict cross-protection as that information gets incorporated into the model.

Materials and methods:
Seventy-two serotype O FMD viruses isolated from different geographical regions were selected and sequenced (capsid region), whilst serological antigenic relationships (r₁-values) for the isolates against five well characterized type O vaccine strains were generated by VNT and ELISA. The surface accessibility of the viral capsid residues was also determined using the previously published O BFS structure. Linear mixed effect modelling was used to develop the predictive model.

Results and discussion:
We have developed capsid sequence-based in silico models for both VNT and ELISA that can accurately predict cross-reactivity for serotype O FMDV. The results led to identification of determinants of protection across serotype O virus phenotypes, and these predictions are currently being tested by reverse genetics using a serotype O cDNA clone. If successful, it would suggest that capsid sequence data can be used to rapidly determine the efficacy and spectrum of available vaccines without the need for further serological investigations.
EVALUATION OF FOOT-AND-MOUTH DISEASE VIRUS AEROSOL CHALLENGE METHOD FOR VACCINE POTENCY TEST

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Introduction: Recent publications highlighted the problem of high variability in the current European Pharmacopoeia vaccine potency test. They suggested that the variability can be reduced by increasing the number of animals per vaccine dose group to 25 instead of 5. However, this is highly impractical because of the need for a high biosecurity containment facility to house the animals and the high cost of the cattle. Moreover, animals must be challenged following intradermal-lingual inoculation with virulent virus and so will suffer considerable pain. Therefore, an alternative easy methods of virus challenge is needed which cause less pain and sufferings in animals and help inoculation of virus accurately. Further as naso-pharynx is the major portal of entry in natural FMDV infection, an aerosol challenge method will simulate the infection used to occur in the field.

Materials and methods: Four groups of 3 cattle were challenged with 2ml of A22 FMD virus aerosolised using a nebulizer and face mask. The virus dose was titrated as $10^5$, $10^4$, $10^3$ and $10^2$ TCID$_{50}$ per group. The clinical signs and temperatures were recorded daily. Nasal and mouth swabs, heparinised and clotted blood and probang samples were collected daily. The excretion of the virus is measured from the above clinical samples by real-time RT-PCR. The clinical results were compared with previous results of intradermal-lingual challenge in cattle using $10^5$ TCID$_{50}$ A22 FMDV.

Results: All the $10^5$ and $10^4$ TCID$_{50}$ virus inoculated cattle were clinically infected within 3 to 4 days post challenge. All the $10^3$ TCID$_{50}$ virus inoculated cattle were clinically infected within 6th to 7th day post-challenge. Two animals of the $10^2$ virus group were infected on 5th and 9th day post challenge, respectively whereas one cattle did not show any clinical lesions. The virus excretion in the oral and nasal swabs were evident on 3rd to 4th day in all the cattle inoculated with $10^5$ and $10^4$ TCID$_{50}$ virus whereas the excretion of virus was seen on 5th to 6th day for $10^3$ and $10^2$ TCID$_{50}$ virus groups. The other virological samples are being analysed at the moment.

Discussion: The aerosolised challenged using nebuliser and face mask was easy and painless for the animals. We could show that using nebuliser and face mask, $10^3$ TCID$_{50}$ virus could generate similar level of clinical signs and virus excretion as seen in the cattle inoculated with $10^5$ TCID$_{50}$ virus by intradermal-lingual route.
IMMUNE RESPONSE IN CATTLE AFTER REPEATED IMMUNIZATION WITH FOOT-AND-MOUTH DISEASE VIRUS VACCINE CONTAINING A RECOMBINANT NON STRUCTURAL PROTEIN

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Introduction: It is a requirement that repeated immunization with Foot and Mouth Disease Virus (FMDV) vaccines does not result in seroconversion to non-structural proteins (NSP). All immunized animals should prove to be free of antibodies (Ab) against FMDV NSP, as they reflect replication of the virus in the natural hosts and thus, its circulation in the field. The assessment of NSP content in vaccines is performed by repeated vaccination in cattle therefore, we studied the dose response to NSP in bovines under The World Animal Health Organisation (Office International des Espizooties, OIE) guidance.

Material and methods: 20 Calves aged 18-36 months, from the Patagonia Region (south parallel 42º) were selected. These animals had never been vaccinated. Before the experiment, cattle were serologically tested and shown to be negative for Ab against FMDV SP and NSP. We worked with polyvalent vaccine formulations made by a commercial laboratory that underwent a recombinant 3AB1 protein to obtain the following mass / dose of antigen: group A (2 calves) 100 ng; group B (9 calves) 42.5 ng; group C (7 calves) 10.6 ng: and Negative Control (2 calves) 0ng. Each animal received a dose of 2 mL by intramuscular rute. Inoculations were performed at 0, 30, and 60 days, and serological survey was performed at 0, 30, 60, 90 and 120 dpv.

Results: After the first vaccination the entire group vaccinated with the highest dose seroconverted and (2/9) animals inoculated with 42.5 ng gave doubtful results. At 60dpv, after the second immunization, (8/9) animals were seropositive in this group. As for the dose of 10.6 ng only one bovine was doubtful at 60dpv and newly to 90 dpv (after three immunizations) 4/7 animals were confirmed to be seropositives.

Discussion: This study supported the OIE recommendation to vaccinate three times as control to detect traces of NPS in vaccines.
EMERGENCY FMD VACCINATION IN 2010 OUTBREAK IN JAPAN

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Introduction: Foot-and-Mouth Disease (FMD) occurred in Miyazaki in April 2010. After FMD spread to several pig farms and a large cattle firm, the numbers of outbreaks rapidly increased. About 60,000 of infected animals were waiting to be destroyed. Japan decided to vaccinate all susceptible domestic animals within a 10-km radius of affected farms to decrease the speed of infection.

Materials and methods: O Manisa (6PD50) was vaccinated to 125,556 heads (cattle 45,950, pigs 79,606 and others 118) in one week. The animals with FMD clinical signs after vaccination were subjected to RT-PCR test. Each twenty serum samples were collected on 8 to 10 days after vaccination from fatting cattle, dairy cattle and pigs which had not shown any clinical signs of FMD. Those sera were subjected to LPB-ELISA and NSP-ELISAs.

Results: Total of 48,796 vaccinated animals were found to be infected by clinical signs of FMD or to be suspected by keeping with FMD positive animals. Eight to 10 days after vaccination the fatting cattle, dairy cattle and pigs which possessed more than 1:90 in LPB-ELISA were 50%, 95% and 60% respectively. The specificities of three NSP-ELISAs in the animals were 85-100%. The sensitivity of the kits with field samples (LPB-ELISA more than 1:181) was 38.5-53.8% in cattle and that of one kit was 11.8% in pigs.

Discussion: Even the good matching vaccine (r1 0.7) was used, about 40% of vaccinated animals were found to be infected or suspected. Antibody response to O Manisa in dairy cattle was quicker and higher than those in fatting cattle and pigs. All tested NSP-ELISAs demonstrated high specificity. The sensitivities of the NSP-ELISAs remained around 50% with infected cattle sample. That was very poor with infected pig samples.
EVALUATING THE COVERAGE AND EFFECTIVENESS OF THE SYSTEMATIC FOOT-AND-MOUTH DISEASE VACCINATION AT HERD LEVEL IN ARGENTINA

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Introduction:
Evaluating the coverage and effectiveness of the immunity conferred by the foot-and-mouth (FMD) systematic vaccination campaign is a key component of the FMD prevention program in FMD-free countries in which vaccination is practiced. Since the implementation of the FMD mass vaccination campaigns in 2001, the National Service for Agrifood Health and Quality (SENASA) has evaluated the coverage and effectiveness of the FMD vaccination campaigns at a herd level and on an annual basis. Here, we offer a brief summary of the methods used to conduct such evaluation and some of the results obtained over the last years.

Methods:
Serological studies are conducted on an annual basis in Argentina, with the objective of supporting the FMD-free status with vaccination recognized by OIE. Goals of the serological studies are 1) to demonstrate absence of FMD virus (FMDV) circulation, and 2) quantify, at herd level, the effectiveness and coverage of the FMD mass vaccination campaign. Some of the samples are collected through the region of the country in which FMD mass vaccination is practiced, which is divided in zones. Other samples are collected using a targeted design, to evaluate the extent of vaccine immunity reached in some specific populations. Examples of targeted studies are those conducted in calves shipped from breeding areas into feedlots in fattening regions and the study conducted in animals moved from herds within the high surveillance zone at the northern borders of the country. The antibody title was used to categorize samples as “protected” or “unprotected” based on the cut-off value of the serological technique (ELISA Fl for detection of structural proteins). Consequently, shipments and herds were categorized as unprotected if it was estimated at a 95% confidence interval that less than 80% of the animals in the group were protected; spatial clustering of unprotected herds were identified using the spatial scan statistic.

Results:
The results are presented at population level (proportion of protected cattle per category) and at herd level, with a spatial distribution. In targeted studies, the results are analyzed by group of animals.

Discussion:
Assessment of the coverage and effectiveness of the FMD vaccination campaign is important because it allows for the identification of regions and types of herds in which clusters of unprotected animals and herds are likely to occur. The FMD vaccination campaign in Argentina showed to be successful in terms of its coverage and effectiveness.
AVIDITY AND SUBTYPING OF SPECIFIC ANTIBODIES APPLIED TO THE INDIRECT ASSESSMENT OF HETEROLOGOUS PROTECTION AGAINST FOOT AND MOUTH DISEASE VIRUS IN CATTLE

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Introduction:
Serological assessment of the heterologous response among foot-and-mouth disease virus (FMDV) strains is mainly performed by virus neutralization test (VNT), liquid phase blocking ELISA and complement fixation assay. However, these methods that have been validated for homologous protection are not always accurate to estimate cross-protection supporting the development of new techniques to measure different parameters of cross-reactive antibodies. In this study two high-throughput ELISA techniques to assess antibody avidity and isotype were developed and used to further characterize heterologous antibody responses in cattle.

Materials and methods:
Avidity ELISA was set up using purified 140S particles as target antigen, a single dilution (1:50) of sera and applying an urea washing step to remove low avidity antibodies. Avidity was calculated as an index respect to un-washed serum. IgG subtype ELISA has been developed before. Both assays were applied to a set of previously characterized sera from animals immunized with an inactivated A24 Cruzeiro/Brazil/55 (A24 Cruzeiro) strain monovalent FMDV vaccine and challenged with the heterologous A/Argentina/2001 (A/Arg/01) strain.

Results:
Single dilution avidity ELISA assessment showed that animals that were protected against A/Arg/01 challenge had higher avidity antibodies to this heterologous strain than non-protected cattle, in spite of the VNT titers against this heterologous virus. Animals with low or even undetectable anti-A/Arg/01 serum-neutralizing titers that passed the heterologous challenge presented higher IgG1/IgG2 ratio than non-protected animals. In this study, the three assessments (VNT and both ELISAs) discriminated between protected and not protected animals against a heterologous challenge.

Discussion:
Avidity and subtype ELISA may be applied to complement current indirect serological assessments for vaccine-matching. The measurement of these qualitative parameters can provide additional information to understand the mechanisms underlying FMD heterologous responses and the induction of cross-protection in cattle.
IRANIAN NSP-SEROSURVEY TO ESTIMATE FMD INFECTION LEVEL WHERE IMPURE FMD VACCINE IS USED

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Introduction:
More than 5000 suspected Foot and Mouth Disease (FMD) outbreaks were reported in Iran in 2010 despite mass vaccination of ruminants. The Iranian Veterinary Organisation has partnered with EUFMD/FAO in a 3 year project to strengthen FMD surveillance nationwide and to progress from Stage 1 to 3 on the FMD-PCP (Progressive Control Pathway) in its most western province, West Azerbaijan (WAZB). The objective of this study was to determine the baseline level of FMD infection in WAZB through a sero-survey combined with a questionnaire about putative risk factors for FMD infection.

Materials and methods:
In a 2-stage sampling design, 30 calves (6 - 24 months) in each of 300 randomly-selected epidemiological units (epi-units), stratified proportionally by husbandry system were sampled. Samples were tested for FMD non-structural proteins (NSP) antibodies (Ab) (Prionics). As the FMD vaccine used in Iran is impure, NSP-Ab induction may occur by vaccination. This was accounted for by increasing the cut-off value (75% instead of 50% inhibition) and a sensitivity analysis to evaluate effects of this increased cut-off value. The data were analysed using a random-effects logistic model, accounting for clustering of animals within households and clustering of households within epi-units.

Results:
Over 80% of epi-units had 5 or more calves positive for NSP-Ab, whereas clinical signs of FMD were observed in only 18 % of epi-units in the previous 12 months. Thirty per cent of calves too young to be vaccinated during the last campaign tested positive, compared to 42% old enough to be vaccinated at least once. Calves in dairy and beef farms were more likely to test positive compared with villages as were calves from trading owners compared with non-trading owners. The same risk factors were indicated using 50% inhibition cut-off.

Discussion:
Results have clearly indicated the endemic nature of FMD infection in livestock in West Azerbaijan province. As livestock management in this province is similar to a number of neighbouring provinces, results are considered relevant for the North-Western region of Iran. This information is being used to further determine a targeted FMD control strategy (including vaccination, movement regulation and biosecurity measures).
FOOT AND MOUTH DISEASE IN THE BORENA PLATEAU: VACCINATION BENEFIT - COST ANALYSIS, ETHIOPIA

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Abstract:
Foot-and-mouth disease (FMD) is an acute, highly communicable and economically important disease of livestock and wild animals. This study was conducted to analyze FMD’s vaccination benefit-cost ratio in Borena zone, Oromia regional state of Ethiopia. The study used economic modeling using data generated from participatory appraisal methods and secondary data sources. In this study area vaccination was proposed after realizing that majority of the FMD cases remain untreated and outbreaks remain uncontrolled. The benefit from FMD vaccination was found to be 9.1 times the expense of the vaccination indicating that the control of FMD in Borena zone by vaccination could be justified on economic grounds. The feasibility of FMD vaccination as control strategy needs to be examined in the light of FMD’s significant impacts on livelihoods, its positive benefit-cost ratio for vaccination, absence of an environment suitable for FMD control through movement control, quarantine, culling and other options. The benefit-cost ratio of vaccination was less sensitive to vaccination costs change. Although it is not expected for a vaccination program to lead to a disease free status in herds in the region soon, decreased FMD incidences will lead to increased milk production and calf survival. This would imply less stress on people’s lives, secure food sources and social harmony and also might increase national and international trade opportunities.

Key words: Benefit- cost, economic modeling, participatory epidemiology
DIFFERENCES IN DETECTION OF FMD VIRUS RNA IN ORAL SWABS AND PROBANG SAMPLES DURING EXPERIMENTAL INFECTION OF CATTLE AND PIGS

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Introduction:
Reliable systems for rapid and efficient laboratory evaluation of suspected cases of FMD is of crucial importance for veterinary preparedness in FMD-free nations. In this study, the comparative utility for detection of FMDV RNA was evaluated in oral swabs and probang samples derived from cattle and pigs experimentally infected with serotype O FMDV.

Materials and Methods:
FMDV RNA was measured using quantitative reverse transcription-PCR (qRT-PCR) assays in oral swab and probang samples collected from cattle and pigs during experimental infections with FMDV O UKG/34.

Results:
During acute infection, FMDV RNA was measurable in oral swabs as well as in probang samples from both species. FMDV RNA could be detected in oral swabs and probang samples from a time point corresponding to the onset of viremia in directly inoculated animals, whereas animals which were infected through contact exposure had low levels of FMDV RNA in oral swabs before viral RNA could be measured in serum. Analysis of samples collected from cattle persistently infected with FMDV showed that it was not possible to detect FMDV RNA in oral swabs harvested beyond 10 days post infection (dpi), despite the presence of FMDV RNA in probang samples that had been collected as late as 35 dpi. An interesting feature of the persistent infection in the cattle was the apparent decline in the level of FMDV RNA in probang samples after the acute phase of infection, which was followed by a marked rise again (in all the carrier animals) by 28dpi.

Discussion:
Results from this study indicate that qRT-PCR analysis of oral swabs is a useful approach in order to achieve a time efficient and reliable initial diagnosis of acute FMD in cattle and pigs, whereas probang sampling is essential for the detection of cattle that are persistently infected “carriers” of FMDV.
EVALUATION OF NEW COMMERCIAL KITS FOR ANTI-NSP FMDV ANTIBODIES

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Introduction:
The assays for the detection of anti-NSP antibodies are highly strategic either as FMD-DIVA tests or for general surveillance, provided diagnostic performances are known and tests reliable. New commercially available kits or prototypes were comparatively evaluated to find out their performances and identify potential confirmatory tests.

Materials and methods:
The following commercial kits were evaluated:
- Two pen-side tests based on lateral flow (LF) chromatography, supplied by UBI (India) and BioNote (Korea), both detecting antibody to 3ABC;
- A competitive ELISA kit (BioNote) for detection of anti-3ABC antibody;
- Prototypes of competitive ELISA kits for detection of antibody to 3D, based on two separate anti-3D conjugated monoclonal antibodies (Prionics A-B).

Two international NSP reference serum panels were used: one prepared at the WRL and composed of 36 sera derived from experimental cattle; another prepared at Panaftosa and focused on field samples, composed of 23 sera from infected cattle and 11 from naïve or vaccinated.

Results:
The two reference serum NSP-panels were examined twice with kits under evaluation and in parallel with two reference tests internationally validated (FMDV NS-Priocheck and IZSLER 3ABC-trapping ELISA).

Only one 3D-ELISA (conjugate B) showed performances similar to those of the two reference tests: the detection rate was 91.5% (54/59) compared to 90% (53/59) and 93% of (55/59) of the NS-Priocheck and IZSLER 3ABC tests respectively; interestingly the three assays tend not to miss the same sera. The second 3D-ELISA (conjugate A) resulted less sensitive, with 45/59 infected sera detected, but the two 3D-ELISAs combined compensate each other; however, also two repeatedly vaccinated animals reacted positive in both tests. The BioNote ELISA appeared definitively less sensitive (detection rate 39/59, 66%), although this was more evident with the WRL panel, while both LF devices showed very poor sensitivity (detection rate for UBIO-LFD 54% and only 27% for BioNote-LFD).

Discussion:
Results provided information on the diagnostic performance of new NSP-assays available on the market or coming soon but not officially validated. Only the 3D-ELISA (with conjugate B alone, or both tests with conjugate 3B and 3A run in parallel) has shown sufficient requisites and an extended validation is recommended, having the potential to be incorporated in a screening or confirmatory testing system. In contrast LF devices are definitely unreliable.

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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 animals and is responsible for important economic losses. FMD is caused by 7 immunologically distinct serotypes, which belong to the genus Aphthovirus in the Picornaviridae family. FMD virus (FMDV) shows a high genetic and antigenic variability and induces similar lesions to those induced by other Vesicular diseases. The aim of this work is the development of a multiplex immunoassay for FMD diagnosis by using the Luminex technology. Such a test will thus allow differential diagnosis of FMD but also serological discrimination between FMDV-infected and vaccinated animals in a single reaction and from a single sample.

Materials and methods: Recombinant 6HisTag viral antigens were produced in E. coli, purified and then coupled to luminex magnetic fluorescent microspheres. Luminex immunoassays were performed using bovine sera from uninfected, infected, or vaccinated cattle. Antigen-bead coupling efficiency was monitored in each experiment by anti-his (mAb) reaction.

Results: The first step in developing the luminex test was to perform simplex immunoassays in order to set up and optimize the concentration of each reagent. Using 3D or VP1 Asia coupled beads and bovine sera, Elisa blocking solution formulation, quantity of coupled-bead per reaction, secondary biotinylated antibody dilution and streptavidine-phycoerythrine concentration were thus determined. Encouraging results were obtained and a duplex test (3D/ VP1 Asia 1) was then performed. NSP antibodies (MFI > 1000), were detected with low background (MFI<100). MFI values ranging from 500 to 1000 for sera from Asia1 vaccinated then infected cattle with low background (50<MFI<100) were obtained.

Discussion: The development of this bead-based immunoassay gave so far promising results. Other antigens in production will be included in the test and multiplex tests will be evaluated by using standardized panel of sera for specificity and sensitivity.
READY-TO-USE ELISA KIT FOR FMDV DIAGNOSIS AND SEROTYPING
TAILORED FOR AFRICA

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Introduction:
A simplified ELISA, developed as a stable, ready kit for detection and serotyping of FMD viruses type O, A, C and Asia1, proved satisfying diagnostic performance (EUFMD Open Session 2010) and has been successfully used during the past two years in WestEurasia and Middle East. The kit is based on pre-coated type-specific monoclonal antibodies (MAbs) and a pan-FMDV conjugate as detector MAb. This study describes the development of a similar kit including serotyping of FMDV SAT1 and SAT2, proper for FMD diagnosis in Africa.

Materials and Methods:
The ELISA kit was designed to enable serotyping of FMDV O, A, SAT1 and SAT2. Catching MAbs specific for serotypes O and A, combined with a pan-FMDV detector conjugate (MAb 1F10) were previously selected and validated, while for SAT1 and SAT2 identification several combinations of serotype-specific and pan-FMDV MAbs, alternatively used as catching and detector antibody, were analysed with representative panels of cell-culture grown isolates. Only MAbs that had shown the widest intra-typic reactivity were evaluated.

Results:
For serotyping of SAT1 and SAT2, arrangements that combined best sensitivity and convenience were obtained using a mix of two SAT1 MAbs (HD7+FC12) and another mix of two SAT2 MAbs (2H6+1F5) pre-coated for type-specific capture, combined with a common detector conjugate composed by a pool of three MAbs (HD7-SAT1/2H6-SAT2/1D6 pan-FMDV). The specificity was cross-checked by analyzing heterologous serotypes: 10 isolates of FMDV type O, 10 Asia1, 12 A and 12 C were all negative. Based on MAbs reactivity, the SAT1 and SAT2 tests are expected to detect 87% and 100% of the homologous isolates respectively (Ferris et al, JVM 2011); consistently, in an initial testing 6/6 SAT2 strains including the 2012 Egyptian isolate reacted strongly and specifically, while the combination of SAT1 MAbs identified 13/16 homologous isolates. Of the three untyped isolates, one was detected by the pan-FMDV test based on MAb 1F10, that complements the kit, while one was not even detected by the polyclonal ELISA.

Conclusions:
The kit offers acceptable diagnostic performance and the desired ease for countries undertaking the PCP. It is currently being evaluated at the WRL for validation and in African and neighbouring countries for FMD diagnosis on epithelial lesions. In the present format, the final kit requires use of two different conjugates; however, further improvement should be achieved with incorporation of a pan-SATs MAb recently produced.

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READY-TO-USE KITS FOR DETECTION OF ANTIBODY TO FMDV SEROTYPES O, A, ASIA 1

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Introduction:
The availability of robust and simple kits for FMDV serology may contribute to the standardisation and harmonisation of results, and is crucial for countries involved in the control of FMD. At present, such kits are merely available commercially for NSP and for serotype O SP serology. The objective of this study was the development of a panel of stabilised kits to increase the spectrum of diagnostic tools for FMDV serotype-specific antibody detection.

Materials and Methods:
In-house Solid-Phase Competitive ELISAs (SPCE) for the measurement of antibodies against Structural proteins (SP) of FMDV serotypes O, A, Asia 1 were transformed in stable and simplified kits. The reaction requires only two incubation steps at room temperature: delivery of test sera into plates, supplied pre-sensitized with FMD viruses trapped by monoclonal antibodies (MAbs), followed by addition of the homologous conjugated MAb. 2001, 1620, 2384 naive field sera (including bovine, ovine, porcine) were analysed with the SP-O, SP-A, SP-Asia1 kits respectively; results of experimental sera from vaccine potency tests (178) and from vaccinated and or infected animals (410) were compared with those of VNT or LPBE obtained against homologous strains.

Results:
Specificity values, obtained combining results of an internal (IZSLER) and external (IAH-WRL) validation were: 99.8% for SP-O kit (4 false-positive in 2001 sera), 100% for SP-A kit (1620 sera tested), 99.7% for SP-Asia1 kit (8 false-positive in 2384 sera). Using sera from full dose mono-vaccinated cattle, detection rates were 84.4% for type O (91.7% with VNT/LPBE, 87% concordant results in 109 samples), 89% for type A (96% with homologous VNT/LPBE, 86% concordant results in 49 samples), 100% for type Asia1 (20 sera). An external validation performed at IAH-WRL with VNT positive sera from vaccinated and/or infected animals showed sensitivity for type O, A, Asia1 kits of 90% (261 sera), 83% (196 sera), 100% (53 sera) respectively. The lower kit sensitivity for serotypes O and particularly A is also justified by the presence, within each panels, of sera raised against antigenically different strains, that were examined against the homologous antigens by the reference tests and heterologous by ELISA kits.

Conclusions:
A set of three ready-to-use kits, stable and user-friendly, for measurement of antibodies to FMDV serotypes O, A, Asia1 was developed; internal and external validation proved adequate performances irrespective of the strains that elicited antibodies.

Acknowledgments:
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DEVELOPMENT AND VALIDATION OF CONFIRMATORY NSP ANTIBODY TESTS TO DETECT INFECTION IN VACCINATED ANIMALS

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Introduction:
Purified FMD vaccines mainly stimulate antibodies against the structural proteins of the FMDV. In contrast, infection with live FMDV elicits antibodies to both structural and non-structural proteins (NSP). Therefore, NSP antibody tests can be used to differentiate infection in vaccinated animals (DIVA). In cattle, the best NSP tests (targeting the NSP 3ABC) can detect up to 90% of vaccinated animals that become carriers after exposure to infection, with a specificity of 98-99%. Due to insufficient sensitivity and specificity, detection of a low level of infection is difficult at the population level with a high degree of confidence. The low level non-specificity problem can be overcome by retesting samples scored positive using a second confirmatory test which should have at least comparable sensitivity to the first test. Therefore, the main aim of this study is to develop and evaluate NSP antibody tests as a confirmatory test to the best validated 3ABC screening test.

Materials and methods:
We have developed 2B, 3D, 3CD, 2C and 3ABC in-house indirect ELISAs that detect antibodies against respective NSP. These in-house ELISAs and 3D Prionics ELISA have been evaluated for a large number of samples from naïve, unvaccinated infected and vaccinated sub-clinically infected animals. The sensitivity, specificity and concordance of these assays are compared with the best validated 3ABC NSP antibody test. In addition, we have recently developed a 3B peptide test which needs further optimization and validation.

Results:
The results showed that the 2B and 3D tests have a similar level of sensitivity and specificity and therefore could be used either as a screening or confirmatory test. From the preliminary assessment it is evident that recently developed 3B peptide test detects infection with a higher sensitivity than 2B, 3D and 3ABC tests.

Discussion:
We have shown that 2B peptide and 3D recombinant protein tests (both in-house and commercial ones) could be taken forward as confirmatory test to 3ABC NSP test to detect infection in vaccinated and unvaccinated infected populations. 3B peptide test has potential to detect more infected animals in vaccinated population which needs to be further investigated.
BAYESIAN VALIDATION OF A COMMERCIAL PENSIDE TEST FOR DETECTION OF ANTIBODIES AGAINST FOOT AND MOUTH DISEASE VIRUS NON STRUCTURAL PROTEINS

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\textbf{Introduction:} 
Clinical and serological surveillance are part of the control activities carried out in many countries. Clinical surveillance is routinely done during farm visits or control movements. However, when mass vaccination is used, vaccinated animals might not express clear clinical signs and the sensitivity of clinical inspections will be poor. The objective of this study was to validate a commercial chromatography test (penside test) for detection of antibodies against FMD virus nonstructural proteins (NSP), so that this test can be used routinely in parallel to clinical inspections.

\textbf{Material and Methods:} 
A total of 1897 serum samples were randomly selected from samples that were submitted to the reference laboratory in Bolivia, as part of the active surveillance programme. These samples were tested in parallel using the penside test and the PANAFTOSA’s 3ABC-ELISA test. Tests’ results were analysed using a latent class model implemented in Winbugs.

\textbf{Results:} 
The percentage of agreement between the penside test and the 3ABC-ELISA was 92.7\%, with a kappa statistic equal to 0.42. The estimated sensitivities (Se) [95\% credible intervals (CI)] were 0.72 [0.45, 0.91] for the penside test and 0.86 [0.72, 0.95] for the ELISA test. The estimated specificities (Sp) [95CI] were 0.97 [0.96, 0.99] and 0.91 [0.90, 0.94] respectively.

\textbf{Conclusion:} 
The Se of the penside test is moderate and it could be used to improve the Se of routine clinical inspections during farm visits. However, an economic analysis would give a better indication of the benefit of implementing this test. Given its moderate Se, a high number of samples per visit would be required.
EVALUATION OF THE SPECIFICITY OF TWO COMMERCIAL ELISA KITS FOR DETECTION OF ANTIBODIES AGAINST FOOT AND MOUTH DISEASE VIRUS IN WILDLIFE SPECIES

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Introduction:
FMD is a highly contagious and economically devastating viral trans-boundary disease of domestic and wild cloven-hoofed animals. This study was conducted to evaluate the specificity of two commercial ELISA kits from Priocheck, for detecting antibodies against FMDV 3ABC non structural proteins and Type O serotype from wildlife species.

Material and Methods:
Serum samples (n=1429) were collected from wild boards, zoe deer, red deer and mouflons from eleven French departments between 2009 and 2012. Sera were tested in PrioCHECK FMDV NS and Type O ELISA kits for the detection of serum antibodies against FMDV 3ABC non-structural proteins and Type O serotype respectively. All positive samples were centrifuged and re-tested by the same tests... Samples that remained positive were further analyzed by 3D ELISA or Virus neutralisation test (VNT).

Results:
Among the 1429 samples analysed, 41 samples were found positives by 3ABC ELISA but all were tested negative by the 3D ELISA. Among the 5 samples found positives by Type O ELISA; 3 of them have lead to cellular toxicity in the VNT and the last 2 were tested negatives by VNT.

Discussion&Conclusion:
NSP and Type O ELISA from PrioCHECK showed 2.9% and 0.3% non specificity rates on wildlife sera used in this study,. This could be due to quality of sera (bacterial contamination and haemolysis state). These results are similar to that observed during a serological survey conducted on French livestock in 2011 (0 to 2% of non specificity for 3ABC ELISA and 0 to 2.1% for Type O ELISA). To complete this study, it should be interesting to evaluate the sensitivity of the two ELISA kits on wildlife sera, to know if they can be used to accurately investigate FMDV infection in wildlife.
CHARACTERIZATION OF RECOMBINANT STRUCTURAL PROTEINS AND MONOCLONAL ANTIBODIES FOR ENZYME-ASSAY OF FMD SAT1 AND SAT2

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Introduction:
Current assays for the detection of antibodies to FMDV type SAT 1 and SAT 2 should manipulate live FMDV that requires containment facility and pose a potential risk of leaking out of laboratory in the process. To replace the inactivated FMDV antigen and polyclonal antibodies, recombinant structural proteins were expressed and monoclonal antibodies (mAbs) against FMDV type SAT 1 and SAT 2 were produced. ELISA combining the recombinant proteins and MAb were evaluated.

Materials and Methods:
The recombinant proteins were expressed by infecting insect cells with the recombinant baculoviruses. Monoclonal antibodies were produced in BALB/c mice immunized with VP1 peptides of FMDV type SAT 1 and SAT 2. To investigate time course detection of early antibodies, goats (n=4) were immunized intramuscularly with BEI-inactivated FMDV SAT 1 and SAT 2. The sera were collected at 4, 7, 10, 14, 21, 28 days post-vaccination (dpv).

Results:
The expression of structural proteins was identified by immunofluorescence assay. The P1 precursor was cleaved into subunit proteins by 3C protease, showing VP1 band by Western blotting. Electron microscopy revealed pentamer-like particles. The antigenic properties of recombinant structural proteins were compatible with liquid phase blocking ELISA. A total of four and six Neutralization MAb against SAT 1 and SAT 2 were produced and characterized. The test detected antibodies from days 4 or 7 dpv. These results were equivalent to LPB ELISA.

Conclusions:
We established ELISA using the recombinant proteins and MAb against FMDV type SAT 1 and SAT 2.
AN INDIRECT ELISA FOR DIFFERENTIATION OF FMDV INFECTED FROM VACCINATED ANIMALS USING RECOMBINANT NON-STRUCTURAL PROTEIN 2C

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Introduction:
Among all the non-structural proteins (NSPs) of foot-and-mouth disease virus (FMDV), antibodies against 2C protein is one of the most reliable indicator for differentiation of infected from vaccinated animals as this protein is membrane associated and remain absent in the purified vaccine. However, expression of the 2C protein is difficult in prokaryotic system as it has a predicted N-terminal membrane binding amphipathic helix (residues 17-34). Hence, 34 amino acids from the N-terminus were removed to facilitate the expression in a soluble form. Truncated 2C (2C\(_t\)) protein was used for the development of an indirect ELISA (I-ELISA) for bovine, sheep and goats species.

Materials and methods
The histidine-tagged 2C\(_t\) protein was expressed in prokaryotic host system and purified using Nickel-nitrilotriacetic acid metal affinity chromatography. An I-ELISA was standardized and validated by testing bovine sera from naïve (n=186), vaccinated (n=286) and FMD infected animals (n= 227). Sera from bovine (n=6750), ovine (n=1090) and caprine (n=611) collected at random from different parts of India were also tested.

Results
The cut-off value for 2C\(_t\) I-ELISA was established to 40 percent positivity based on the frequency distribution at which the diagnostic sensitivity for infected samples {21-365 days post infection (dpi)} was estimated to be 84.1% which decreased to 69.6% when infected samples between 21-1000 dpi were considered. The diagnostic specificity values varied between 97.8% and 88.4% on naïve and vaccinated sera, respectively. The antibodies towards 2C could be consistently detected in this ELISA from 12dpi to 720dpi in experimental animals.

Discussion
With the entry of India into third phase of progressive control pathway, there is increase in multiple vaccinated animals and decrease in incidence of clinical disease. Hence, this developed 2C\(_t\) NSP ELISA with adequate performance characteristics can be used alongside the currently used 3AB3 and 3ABC NSP ELISAs (PDFMD, Mukteswar) to increase the confidence in surveillance results.
DEVELOPMENT OF A SINGLE TEST FOR DETECTION AND TYPING OF FOOT AND MOUTH DISEASE VIRUS AND OTHER VESICULAR DISEASE VIRUSES

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Introduction:

Foot and mouth disease virus (FMDV) belongs to the Aphtovirus genus in Picornaviridae family. This virus causes a highly contagious disease of domesticated and wild cloven-hoofed animals and is responsible for important economic losses. FMDV are commonly clustered in seven serotypes and induced similar lesions to those induced by Vesicular Stomatitis (VS) and Swine Vesicular Disease Viruses (SVDV). The aim of this work is to develop a single test for both detection and exclusion of vesicular diseases and also for molecular typing of FMDV strains.

Materials and methods:

The test is based on the Luminex and Xtag beads technology. Primers sets were specifically designed for Luminex to allow amplification of several target genes in a multiplex test as follow:

I. FMDV 3D gene: detection of FMDV strains;
II. FMDV VP1 gene: molecular typing of each isolate of FMDV;
III. SVDV VP1 gene: detection of SVDV strains;
β-actin g

Results:

A 6-plex Luminex test was developed that allowed both detection and typing of FMDV in the same reaction. The 3D FMDV primer set detected specifically all the reference as well as field strains tested. Primers specific for A, O and Asia1 serotypes gave a specific signal for each ene: positive internal control.

Serotype on reference and fields samples. Furthermore, SVDV primers set detected specifically the reference strain tested.
A lack of sensitivity has been noticed for serotype O detection from field samples.

Discussion:

This test gave good preliminary results: it allowed differentiation between SVDV and FMDV as well as molecular typing of serotypes O, A and Asia1.
However, the test should be improved in order to increase its sensitivity for serotype O detection from field samples. We are currently developing primer sets to allow (i) molecular typing of SAT strains and (ii) detection of VS viruses.
READY-TO-USE MULTIPLEX PCR KIT FOR RAPID DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS SEROTYPES IN INDIA

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Introduction:
A multiplex PCR assay is a sensitive and a rapid method for FMD diagnosis and serotyping. However, due to some factors including cost, lack of infrastructure and the complexity of the reaction mixture, the method could not be utilized to its full potential in India and hence the samples from FMD suspected animals are referred to the central laboratory for FMD diagnosis by mPCR. During the procedure, sometimes, quality of samples gets deteriorated due to break in cold chain leading to the false negative diagnosis by mPCR. A thermo-stable lyophilized ready to use mPCR kit was optimized and validated for rapid diagnosis of FMD at the level of field laboratories in India.

Materials and methods:
Glycerol, PEG-8000 and trehalose were tested individually in different concentrations for stabilization of the RT-PCR mixture during and after lyophilisation. The lyophilized mixtures were tested for thermo-stability by storing at different temperature. The stored mixtures were tested periodically up to 1 year. Inter laboratory validation was done by shipping the kit to the regional FMD diagnostic laboratories without cold chain for testing.

Results:
The lyophilized RT-PCR mixtures could be successfully stabilized with trehalose and PEG-8000, but glycerol stabilized reagents did not withstand higher temperature. The trehalose stabilized reagents worked for 4 days at 37°C and 7 days at room temperature. The kit was found robust enough to be transported without any cold chain and used in the regional level FMD laboratories across India.

Discussion:
The ready-to-use mPCR kit has low reaction mixture complexity, little chance of cross contamination, and is provided with internal lyophilized positive and negative mPCR RNAs controls eliminating the requirement of live FMD virus in the regional diagnostic laboratories. In addition to high sensitivity, the current molecular diagnostic reagents are much quicker in generating results at the local or regional level which would help the surveillance programme.
NEW DIAGNOSTIC TOOLS FOR FMD TO DETECT ANTIBODIES AGAINST STRUCTURAL PROTEINS OF A AND ASIA SEROTYPES AND 3D NON-STRUCTURAL PROTEIN.

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Introduction:
Several diagnostic ELISA tests are available to screen sera for the presence of structural and non-structural antibodies against FMDV. Commonly used tests are directed against the non-structural protein 3ABC and some type specific tests, for example, type O structural antibody test. Here we present the results of evaluation of three new diagnostics kits to detect antibodies against structural proteins of FMDV A and Asia serotypes and 3D non-structural proteins.

Materials and methods:
Different sera from natural or experimentally infected or vaccinated sheep, goat, cattle and pigs have been used to validate these tests.

Results:
All the three tests are able to detect appropriate antibodies against FMDV in the sera originated from FMD infected/vaccinated pigs, cattle, goat and sheep. Generally, the specificity is more than 99% whereas the sensitivity is more than 90%, although some cross reaction between the serotypes are observed. 3D NSP test results are comparable to the existing validated Prionics 3ABC NS test.

Discussion:
Two new type specific ELISA tests have been developed and evaluated which will complement the currently available type specific test for the detection FMD. Although a good NS antibody test could detect antibodies against all the seven FMDV serotypes, a clear need of type specific tests are observed from previous outbreaks. The currently developed SP tests will be valuable tools to suit these needs, although some cross-reactivity is observed between the serotypes. The newly developed 3D test is the first commercially available NS confirmatory ELISA that can reliably confirm positive results originated from the 3ABC screening test.
**LUMINEX TECHNOLOGY FOR THE SIMULTANEOUS DETECTION AND SEROTYPING OF FMD VIRUSES**

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**Introduction:**  
Luminex technology allows for multiplexing of different analyses simultaneously in a single reaction using differently dyed (fluorescent) microspheres as adsorbing surface. The aim of this study was to explore the potential of this multiplexing testing system for the differential diagnosis and serotyping of FMD viruses.

**Materials and methods:**  
The multiplex assay was implemented using six different sets of beads coupled with catching monoclonal antibodies (MAbs) previously validated for an FMDV antigen-detection ELISA kit: one beads-set for serotype O, two for A, one for Asia 1, one for C plus one for pan-FMDV catching were mixed. The biotinilated pan-FMDV MAb 1F10 was used as universal detector; its binding to the fluorescent streptavidin-phycoerytrin protein allows tracking and quantification of the analytes.

Fifty negative tongue epithelium suspensions of different species and 21 FMDV isolates, including 7 FMD strains of type O, 7 A, 1 C and 6 Asia1 were analyzed in the multiplex assay at sequential dilutions.

**Results:**  
Each sample was tested simultaneously against six different MAbs in a single well; the MAbs-based ELISA kit was run in parallel. No false-positive was found in negative epithelial suspensions, while all 21 FMD strains were correctly detected and serotyped. The Luminex multiplex test showed analytical sensitivity on average 5-fold higher than that of ELISA.

**Discussion:**  
This study shows that the multiplex test based on Luminex technology can be a valid, additional tool for FMD diagnosis, with improved sensitivity compared to ELISA. Multiplexed reactions and reduced sample consumption are strategic features for analyses of different FMDV serotypes and other pathogens in a sample. In addition, the potential to increase the number of simultaneous analyses allows in principle screening of samples against many more MAbs-coupled microspheres, improving the reactivity spectrum and providing an accurate antigenic profiling. Next step will be assay validation on field clinical samples and further multiplexing to include detection of SATs serotypes.
MODELLING OF ECONOMICAL CONSEQUENCES OF FMD OUTBREAKS IN TWO DIFFERENT REGIONS IN AUSTRIA

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Introduction:
An economic model for the assessment of possible expenses in case of an outbreak of FMD in two regions in Austria was developed on the basis of a stochastic epidemiological model (Hiesel et al. 2012). Region I is defined with an area of 39.541 km² and a total of 43.973 farms, Region II, is defined with an area of 15.249 km² and consists a total of 15.805 farms.

Material and methods:
The epidemiological model was created in Interspread Plus® version 2.1.14. Generally, an extension through implementation of an economic model is possible (Nielen et al., 1999). The cost positions were chosen on the basis of previous scientific studies (Harvey et al., 2007; Bates et al., 2001; Krämer 2010). Primary expenses of an outbreak, result from culling expenses for each farm, expenses for cleaning and disinfection, compensation per animal, monitoring costs per farm, surveillance and vaccination zone, including voluntary testing of the farms and vaccination costs per animal. Secondary costs include monetary loss for dairy farms caused by failed collection of milk and impossibility to sell the product, and losses for pig and piglet farms caused by failed marketing measures.

Results:
The average costs in region I were calculated between € 8.15 million and € 9.7 million. The average costs in region II were determined at € 6.3 million and € 7.1 million. The main costs in both regions result from the necessary surveillance measures and the respective labour- and laboratory costs.

Discussion:
The economic model has the tendency to underestimate the actual costs of an outbreak of FMD. The resulting financial hardships for tourism, humane kills, possible vaccine damages, etc. were not taken into consideration.
OUTBREAK OF FMD IN EGYPT IN 2012

Rehab Abd elkader Sayed Mohamed

Summary:

During February 2012, multiple incidence of FMD events were reported; SAT2 Strain of FMDV was isolated from collected samples of infected cases in Egypt’s national laboratory (Animal Health Research Institute) "AHRI" and confirmed in Pirbright Lab. / UK.

Emergency plan of GOVS to control outbreak of SAT 2 depended on 4 stages viz.; prevent animal movement, treatment of animals in the house, surveillance and proper vaccination. The overall number of epidemiological units (village) notified since the first recorded incidence on 26th February 2012, stands now at 3361 epidemiological units and 92217 suspected cases and 26161 dead cases which are being notified from all governorates. Most notifications originated from the Delta area. 118, 8, 4 out of 209 missions were confirmed as SAT 2, O and A respectively during our passive surveillance in response to governorates notification.

Since the onset of outbreak, 4 active surveillance has been done with different objectives. Of the 74 samples submitted through active surveillance in April, 7 samples tested positive (serotype O was identified in one sample and serotype SAT2 in 3 samples) and 4 tested negative. Second active surveillance has been done in May to complete the picture of the circulating types and subtypes all over Egypt by serotyping, subtyping and possibly sequencing in seventy randomly selected villages, proportional to the 5 Egyptian regions which had notified incidence of clinical FMD.

Other Sero-surveillance study is on 48 (out of 182) commercial farms that had used the imported polyvalent vaccine. In these herds, antibody titers will be measured to estimate the level of protection against FMD-SAT2, A, O. Vaccination for locally-produced SAT2 monovalent vaccine were started in 9 provinces with no or few FMD notifications. Then vaccination campaigns were extended to other governorates and booster doses in 6 governorates. Between 19 April and 30 July 2012, more than 716515 animals were vaccinated with first dose and 127732 with booster dose. In addition, some commercial farms were vaccinated with, Botswana monovalent SAT2 imported vaccine. Sero-surveillance for locally-produced SAT2 monovalent vaccine was conducted to detect the herd immunity gained by vaccination. The FMD SAT2 situation in Egypt is shifting from emergency-mode to a long-term management perspective.

Notifications of suspected new cases are decreasing and considerable attention is devoted in implementing the vaccination campaign and active surveillance about the circulating FMD virus. Modified procedures have been put in place in response to this SAT2 epidemic and retained to improve FMD surveillance and response in the long-term.
RISK ANALYSIS: ASSESSMENT OF THE RISK OF FMD TRANSMISSION POSED BY CONTINUED PUBLIC ACCESS TO THE COUNTRYSIDE IN SCOTLAND DURING AN FMD OUTBREAK

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Introduction:
During the 2001 UK foot-and-mouth disease (FMD) outbreak, strongly encouraged by the UK Government, local authorities took a precautionary approach to disease control and closed all footpaths to recreational users. This decision had major economic consequences for rural businesses. In response to recommendations for contingency planning for future outbreaks, the Scottish Government engaged EPIC to produce veterinary risk assessments (VRAs) to evaluate risks of FMD spread associated with public access to the countryside during an outbreak.

Materials and Methods:
EPIC designed a standardised qualitative VRA template. Exposure and transmission pathways are supported by tabular summaries of available scientific evidence. The likelihood of different recreational activities causing new outbreaks was assessed for the Protection Zone (PZ), Surveillance Zone (SZ) and Restricted Zone (RZ). These activities included walking, cycling, canoeing, fishing, equestrian activities, deerstalking, shooting birds and staging other sporting events on agricultural land. Possible mitigation strategies were considered.

Results:
For most activities, the likelihood of causing new outbreaks of FMD is considered to be medium (could occur regularly) in the PZ, low (rare but could occur) in the SZ and very low (very rare but could not be excluded) in the RZ, assuming compliance with specified mitigation strategies. However, the likelihood of new outbreaks associated with hunting, shooting, stalking and equestrian activities is considered to be greater. Considerable uncertainty exists around these assessments due to a paucity of data on the likelihood of transmission via fomites.

Discussion:
These VRAs will be used to provide evidence to guide decisions regarding access to the countryside during an outbreak. Due to the substantial uncertainty around these risk estimates, a precautionary management approach is favoured in high risk areas, particularly during the early stages of an outbreak. Outside the PZ/SZ, decision makers must balance very low risks against potential economic impacts of restricting access.
**VET-GEOTOOLS, A RISK MANAGEMENT SYSTEM FOR ANIMAL DISEASE CONTROL**

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**Introduction:**
Foot and mouth disease outbreaks can cause considerable economic and social upheaval. Controlling FMD epidemics requires operational contingency plans, facilitated information management, evidence-based decision-making, and controlled risk communication. All these actions require extended human and financial resources but can be facilitated by information systems that can integrate different data sources in one decision information environment.

**Objectives:**
To develop a space-time information system that collects disease outbreak data and provides the necessary information for contingency planning and decision-making.

**Materials & Methods:**
Vet-geoTools is a space-time information system that combines a ‘Identification & Registration’ database with a GIS server and allows a space-time representation of the accumulated information, which can be disseminated to different users through web-services. For several standard operating procedures that are applied when controlling an outbreak, information was obtained from the database through complex database queries. The information was then visualised using maps and graphs or translated into instructions and checklists for operators in the field.

**Results:**
The different procedures that were developed in Vet-geoTools are grouped into 6 modules: 1) Data access 2) Restriction zone management, 3) Low-risk logistics routing, 4) Contact tracing, 5) Epidemiological analysis, and 6) Reporting and communication. The modules can be used at different phases during the control of an epidemic. A number of instructions are available as web applications and can be consulted by users through access codes. The systems is currently in its test phase using simulated scenarios of FMD outbreaks and other emerging diseases.

**Discussion:**
An animal disease information system as Vet-geoTools provides a considerable support to decision makers and risk managers as the systems allows saving time and reduce resources necessary for contingency planning. In addition, the system provides decision support through online access to integrated data sources, real-time reporting and integrated simulation exercises.
CHASING THE FIRE OR BLOCKING ITS PATH? DETERMINATION OF GEOGRAPHICALLY MEASURABLE STRUCTURES LIKELY TO ACT AS CRITICAL EPIDEMIC NODES BEFORE EPIDEMICS OCCUR

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Introduction:
The economic impact of FMD reintroduction cannot be estimated unless both the time between the epidemic is detected and a control policy is implemented (critical response time) and the proximity of the critical case to a pre-established connecting network are considered. That is so because exotic (true) FMD epidemics disseminate in an exponential fashion. To explore whether both of these factors are, in fact, critical, two rapidly disseminating epidemics were retrospectively evaluated with high-resolution geo-temporal data.

Materials and methods:
A geo-referenced set of constructs meant to estimate two essential concepts of Network Theory (the node and the link) were created and evaluated in two epidemics: one caused by FMD virus (Uruguay, 2001) and, for comparison, one caused by a different virus (avian influenza H5N1, Nigeria, 2006), in which also 100\% of the population was susceptible.

Results:
After determining, empirically, the radius of ‘epidemic nodes’ (the smallest circles that included >50\% of the cases and were centered on the road network), it was observed that only one of the first 3 FMD cases were located within a node. Similarly, only some but not all first H5N1 cases were within epidemic nodes. Numerous network properties were documented in both epidemics, including synchronicity and directionality. Such properties helped to distinguish (rank) epidemic nodes. The epidemic flow moved from ‘high-rank’ to ‘low-rank’ nodes. ‘High-rank’ nodes were engaged first, and they were also identified by some variables potentially measurable before epidemics occur.

Discussion:
Epidemics may be more easily controlled if critical ‘nodes’ are prioritized –ideally, within the first 48 hs. Because such nodes are associated with geographically measurable structures, the evidence suggests that ‘high-rank’ nodes could be identified before epidemics occur and, consequently, resources and training could target such sites so connectivity can be promptly interrupted once an epidemic is detected.
PRODUCTION OF FOOT AND MOUTH DISEASE VIRUS-LIKE PARTICLES IN MAMMALIAN CELLS BY TRANSIENT GENE EXPRESSION

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Introduction:

The vaccine currently used to control Foot and Mouth Disease Virus (FMDV) has some limitations including the requirements of expensive high-containment manufacturing facilities, the risk of an incomplete virus inactivation and the problem of discriminating between vaccinated and infected animals. The expression of the polyprotein precursor P12A and the 3C protease to produce viral-like particles (VLPs) is an alternative to overcome the production problems that preserves the immunogenicity of the current vaccine. Since viral 3C protease cleaves not only the polyprotein precursor P12A but also intracellular proteins, large-scale transient gene expression (TGE) is an attractive alternative approach to stable cell lines. The main objective of this study was to produce recombinant VLPs of FMDV serotype A2001 in mammalian cells by TGE.

Materials and Methods:

Genes required for VLP production were cloned into pTT5 vector (pTT5-P12A3C). Suspension-growing human embryonic kidney 293 cells (293-6E) were transiently transfected with pTT5-P12A3C by using polyethylenimine (PEI). Cells were cultured in serum-free medium. After 48 hours, cells were harvested and analyzed for protein expression by western blot and ELISA. Recombinant protein extracts were loaded onto 15-45% (w/v) sucrose density gradient and fractions were analyzed by ELISA.

Results:

Capsid proteins were successfully expressed in 293-6E cells. P12A polyprotein was correctly cleaved by the 3C protease into individual structural proteins as confirmed by western blot. Recombinant proteins reacted by monoclonal antibodies that recognize conformational epitopes thus suggesting the formation of subviral particles. When these structures were further analyzed by sucrose gradients, the main peak of antigenic material was observed in the fraction corresponding to empty capsids (75s). The yield of FMDV VLPs was 3.1 µg/ml of cell culture.

Discussion:

This is the first report on the expression of recombinant VLPs of FMDV in mammalian cells by TGE. The high levels found are suitable for vaccine production.
USING ROPES TO DETECT FOOT-AND-MOUTH DISEASE VIRUS INFECTION IN PIGS

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Introduction:
In any high density farming practice, it is important to constantly monitor for infectious diseases. Pigs are known to amplify foot and mouth disease (FMD) by excreting large amounts of virus and it is therefore important to detect the virus quickly and accurately. Ropes were used in an experiment where pigs were infected with FMD virus to collect oral fluids from pigs and the fluids tested for FMD virus RNA.

Materials and Methods:
Groups of pigs with varying loads of FMD virus were sampled for oral fluids daily using sections of cotton rope hung at shoulder height. The rope was left between 15 minutes to an hour for the pigs to suck and chew. The oral fluids were squeezed from the rope and stored in viral transportation media at -70°C. The samples were tested using quantitative Real-Time Polymerase Chain Reaction (qRT-PCR).

Results:
There was a strong correlation between FMD infected groups of pigs and qRT-PCR positive rope samples. Two groups of control pigs that were not infected with FMD all gave negative qRT-PCR results. Three groups of pigs infected with FMDV all gave positive results at the time that disease was detected.

Discussion:
Rope sampling was a simple, cost effective and non-invasive surveillance technique that can be used to detect FMDV in groups of pigs.
COMPARISON OF THE PATHOGENICITY OF TWO SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUSES (CHIMERIC AND FIELD STRAIN VIRUSES) IN PIGS

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Introduction: The surface exposed capsid proteins of foot-and-mouth disease virus (FMDV) determine its antigenicity and the ability of the virus to interact with the host cells. The use of viruses with identical capsid sequences allows the role of sequences outside of this region in pathogenicity to be determined. In the present study we compared the pathogenicity of chimeric (O1K/O UKG) and field strain (O-UKG/34/2001) viruses in young pigs.

Material and Methods: Seven-week-old pigs were exposed to virus, either by inoculation or contact. One group of pigs was exposed to the O1K/O-UKG chimera, a derivative of the cell culture adapted O1K B64 strain with the surface exposed capsid proteins (VP1, VP2 and VP3) from O UKG/34/2001 and a second group of pigs was exposed to the field strain O UKG/34/2001. All pigs were examined for clinical signs of FMD and rectal body temperatures were recorded daily. Blood samples were collected on selected days during the experiment and heart tissue was collected post mortem.

Results: All pigs infected with the O1K/O-UKG chimera or the field strain (O-UKG/34/2001) developed fulminant disease and showed a high level of viral RNA in serum. The pigs that survived the acute phase of infection developed a serotype specific antibody response. However, four of the pigs exposed to the O1K/O-UKG chimeric virus died in the acute phase of infection. High levels of viral RNA were found in the hearts of these pigs.

Discussion: In this study we saw acute deaths in young pigs exposed to the O1K/O-UKG chimera. Four out of 8 pigs were found dead in the pen shortly after initial clinical signs were recorded. Whether this finding was related to the differences between the two viruses (outside of the capsid coding region) or coincidental is not yet clarified.
EVIDENCE OF RECOMBINATION IN STRUCTURAL AND NON-STRUCTURAL CODING REGIONS OF SEROTYPE O FMD VIRUS

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Introduction:
Recombination events seem to play an important role in evolution of FMD virus and occur mainly in the gene regions coding for the non-structural proteins but also sometimes in the gene regions coding for the structural proteins.

Materials and methods:
Forty FMD virus serotype O isolates covering different circulating genetic lineages spanning last four decades were selected for the study. The complete genome sequences of were generated for all the 40 isolates except the polyC and polyA tracts. The recombination analysis was carried out using the SimPlot, v. 2.5 software which plots pairwise genetic similarities (similarity plots) between a query sequence that is suspected to be a mosaic recombinant genome and a set of reference sequences in a sliding window of size 200 nt and step size of 20 nt using Kimura (2-parameter) distance model. The pairwise similarity values were plotted at the midpoint of the 200 nt window.

Results:
In several isolates analysed, many clearly visible mosaic patterns were observed in the P1, P2 and P3 regions. The frequency of recombination events observed in the non-structural protein (NSP) coding regions (P2 and P3) were higher than that of the structural protein coding region (P1). One of the isolate was observed to be a triple recombinant; three out of 4 isolates involved in this event were among the current in-use vaccine strain or vaccine strains which were used earlier.

Discussion:
The present study showed high frequency of recombination in NSP region of FMD virus serotype O Indian isolates. All the recombination events in NSP were concentrated in the P3 region and in one, P2 was involved. These observations are congruent with the discussions on recombination hotspots in picornaviruses in general and FMD virus in particular. The accepted hypothesis for picornavirus genetic transmission is that the capsid genes are inherited as a single evolutionary unit, where as the non-structural genes have been involved in extensive intra- and inter-typic recombinations. The less constraint in recombination in these regions may be attributed to the conserved nature of 3C and 3D proteins as these proteins are involved in crucial functions in virus replication.
IDENTIFYING THE POTENTIAL PHYSIOLOGICAL DETERMINANTS OF FMDV-INDUCED BOVINE EPITHELIAL CELL LYSIS

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Introduction:
FMDV causes vesicular lesions in infected animals but only in certain epithelial tissues. The different susceptibility of tissues to FMDV constitutes a significant knowledge gap. Potential physiological determinants of epithelial cell lysis due to infection are explored, starting with tissue structure and thickness and expanding to other factors such as receptor distribution and interferon response.

Materials and methods:
A mathematical model of the bovine epithelium was constructed. This is an one-dimensional, partial differential equation model, which describes the dynamics of FMDV infection in the epithelium starting at the basal membrane and finishing at the tissue surface. Parameters were estimated from the literature. Additional data on the epithelium thickness and structure were collected from tongue and dorsal soft palate epithelia (DSP), as these regions serve as examples of tissues in which lesions do and do not form respectively.

Results:
Numerical investigation show the model predicts extensive cell death and, hence, lesions for both tongue and DSP. Results demonstrate the viral replication parameters to be the most important in the system, while the uptake parameters have little impact.

Discussion:
Epithelial thickness and/or structure alone do not account for the formation of lesions. Different receptor distribution or different viral replication rates in different cell layers cannot be drivers of bifurcation in cell lysis dynamics. To date, viral replication rates have not be shown to differ amongst different tissues and therefore cannot be considered as determinants of lysis. However, extreme differences in receptor distribution between different tissues could be a determinant of lysis and should be investigated further.
REFINEMENT OF A FOOT-AND-MOUTH DISEASE INFECTION MODEL IN GUINEA PIGS FOR THE EVALUATION OF FMDV VACCINES AND ANTIVIRAL DRUGS

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Introduction:
Guinea pigs (GP) are widely used to evaluate (experimental) FMDV vaccines, but a standardized model suited to substitute the current PD\textsubscript{50} FMDV vaccine efficacy tests in cattle remains to be validated. Firstly, we aimed to refine the GP model by investigating several infection/protection parameters.

Materials and methods:
Dunkin Hartley GPs were vaccinated IM with 125µl, 500µl or 2000µl of a purified DOE O\textsubscript{1} Manisa vaccine. Three independent experiments were performed using a total of 8 animals per vaccine dose and 10 unvaccinated control animals. Three weeks later, all animals were intraplantary challenged with GP-adapted O\textsubscript{1} Manisa and euthanized at 3 or 4 dpi. Clinical signs were recorded daily and serum collected at 2 dpi was examined with real-time RT-PCR.

Results:
Within 2 dpi, 10/10 unvaccinated control animals developed vesicular lesions at the inoculation site and severe reddening with possible vesicles at the other footpads, 8/10 animals developed mouth lesions. All control animals showed depression and weight loss (9.0±6.8%), but no fever. Mean Cp values in serum were 20.5±2.6. Twenty-one vaccinated animals developed moderate reddening with possible vesicles at the inoculation site, and 1/8 animal in the 125µl and 2/8 animals in the 500µl dose group showed reddening at the other footpads. All vaccinated animals were protected against depression, weight loss and mouth lesions. In every dose group, 6/8 animals had viral RNA in their serum, but Cp values were significantly lower than in unvaccinated control animals (39.2±2.8, 36.2±4.7 and 38.3±3.5 for the 125µl 500µl 2000µl dose groups, respectively). Serum collected for serological tests, oral swabs and a series of organs collected for real-time RT-PCR are investigated at present.

Discussion:
The present standardized and reproducible GP infection model will be further validated in order to allow substitution of target species in FMDV vaccine and preliminary antiviral efficacy tests.
The EuFMD
Narrative report

SHORTENED VERSION FOR THE OPEN SESSIONS

Without Annexes or Table of Activities

(Available upon request)
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Summary

This shortened version of our regular 6 month report is provided for the Open Sessions to indicate what we do, where work, and who we work with - and through, in support of international efforts, co-ordinated with FAO and OIE.

The reports are part of our reporting arrangements to the DG-SANCO of the European Commission (EC) in fulfillment of the Contract between FAO and the EC relating to funding for the activities to be conducted by the EuFMD Commission. The current Contract was signed in September 2009 and has a term of 48 months.

The EuFMD Commission, at the 38th General Session in April 2009, adopted a four year Strategic Plan of activities, involving six components, with priorities for in-country actions being to support FMD control in Southeast Europe through greater management of the FMD risk in countries bordering to Turkey, in West Eurasia. These projects are coordinated with those of other Directorates of the EC and other funding agencies, to promote progressive control in the West Eurasian countries along a long term Roadmap.

Following signature of the financing agreement, specific activities of the EuFMD are initiated following response of the EC to proposals from the Secretariat or decisions of the Executive Committee at which the EC are represented.

The EC support is provided through a Trust Fund (TF), MTF/INT/007/EEC, with a total funding of € 8 million for the four year period of the current agreement. Since September 2009, the EC has agreed funding of actions in six of the Strategic Plan components, with by far the largest being for in-country programmes in the Trans-Caucasus and Iran aimed at reducing the risk of new incursions of FMD into Turkey and Eastern Europe. Funding is also provided for training of European veterinarians, for surveillance in the African proximity, for short technical studies, and for surveillance for FMD in Egypt.

In response to FMD outbreaks in Bulgaria in 2011, the TF was used for emergency funding purposes for procurement of FMD vaccines for re-enforcement of immunity in Turkish Thrace, and thereby protection of the three countries with common borders. In 2012 emergency missions have been included to Turkey (Asia-1 vaccination effectiveness) and Egypt and Libya (SAT2 outbreaks). The EC also agreed to supply 500,000 doses of Trivalent A/O/Asia-1 vaccine to the trans-Caucasus to re-enforce the vaccination campaign in Spring 2012.

At the 39th Session in April 2011, the EuFMD Commission recommended three additional components. These are indicated as Components 7-9 below. For two of these, expenditures or activities had not been committed before April 2012, but actions and expenditure has commenced in the current 6 month period (i.e April-September 2012).

In addition, the EC has proposed development of a TRIPARTITE surveillance programme for FMD in Thrace, and the first activity towards this occurred in September and is reported under Component 1 (Risk reduction in South-East Europe).
The nine **Components** reported in detail in the Update Report are:

1. Risk reduction in South-East Europe through support to FMD control in West Eurasia;
2. Activities to reduce FMD risk in the South and East Mediterranean countries;
3. Field based FMD Training Programme;
4. FMD surveillance in the African proximity;
5. Technical studies;
6. Response to FMD Emergencies.
7. Strengthening FMD laboratories in the Balkan Region;
8. Improved Contingency Planning through use of decision support tools;
9. World Reference Laboratory (WRL) contract – FMD surveillance support activities.

The work under each component is scheduled for completion in 2012 or before completion of the term Funding Agreement.

**Annexes: Omitted**

All info is available on our website
http://www.fao.org/ag/eufmd.html

and upon request to Eufmd@fao.org
UPDATE: Main actions undertaken between APRIL AND SEPTEMBER 2012

Component 1

Risk reduction in South-East Europe through support to FMD control in West Eurasia

Projects in this category:

1. Support to the West Eurasia Roadmap (Secretariat, surveillance and annual progress reviews)
2. Trans-Caucasus Countries
3. Iran
4. Thrace – improved surveillance for early detection of FMD

Project #1: Support to the West Eurasia Roadmap (Secretariat, surveillance and annual progress reviews)

Summary of actions in reporting Period:

1. The 2012 West Eurasian FMD Roadmap review was held in Istanbul in March 2012, and attended by representatives of 13 of the 14 Roadmap countries (Syria and Iraq were not present), plus EC, FAO and OIE. Side meetings were held on the risk of SAT2 spread from Egypt/Libya into the mid-east. The PCP Stage was reviewed for each country and a revised Roadmap developed.
2. The Roadmap Report was placed online, with a review of vaccination programmes and recommendations for vaccine use in 2012.
3. A training programme in practical epidemiology for progressive control (PEPc) has been developed by EuFMD and the first course initiated in September 2012, in Istanbul, for 12 trainees from West Eurasia and Egypt. Field work involving FMD outbreak investigation was part of week 1 (Leaders: Melissa McLaws, EuFMD and Theo Knight-Jones, EuFMD).
4. West Eurasia FMD vaccination database: the contract with FLI, Germany has been concluded and the database transferred to EMPRES-I, FAO; four countries (Trans-Caucasus and Turkey) have agreed to send MONTHLY data on FMD outbreaks and vaccination to the database. Training was given in Turkey (T Knight-Jones) in establishing the monthly reporting system into EMPRESi.
5. WELNET: the risk of FMD including SAT2 in Iraq has been recognized and an agreement was reached on shipment across Iraq/Turkey border of samples for typing at the SAP Institute, as air transport seems impossible to arrange.
**Figure 1.** PCP Stage self-assessment in 2012 compared to the previous Roadmap assessment in 2010; the blue boxes indicate the CHANGE to PCP state or in anticipated progress. In almost all cases the revision is downward, a more conservative view on rate of progress, and can be attributed to the more rigorous approach used to assess progress on activities within each Stage and a better understanding of countries of the difficulties to progress in Stages 1 and 2.

**Major Reports online – NEW in Reporting period**


2. Prior Reports:  
   - Roadmap Progress Review Report, for 2010  
Project #2: Strengthening Foot-and-Mouth Disease surveillance and control in the Trans-Caucasian countries to assist progression on the West Eurasia FMD Progressive Control Pathway

Countries: Georgia, Armenia, Azerbaijan
Lead technical officer (LTO) and other principal international experts:
Eoin Ryan (Supervisor since March 2012), Carsten Potzsch (LTO), Tsviatko Alexandrov.
Reporting period: April 2012-September 2012

Summary

The key activities undertaken were:

- Completion of the Spring 2012 vaccination campaigns
- A mission was undertaken to assess the storage, distribution and use of EC-supplied vaccines to the TCC in June (D. Krnjaic & E. Ryan). There was good evidence that the cold chain had been maintained, the vaccine stored and distributed correctly, and used in accordance with the stated plans. The 150,000 dose vaccine reserve for the TCC was inspected and found to be secure and stored correctly.
- Penside tests (lateral flow devices) for FMD were provided to each country.
- A protocol governing the process whereby some or all of the EC-supplied emergency vaccine reserve could be used was written and issued.
- Serological surveys were conducted after the Spring vaccination campaign and the samples analyzed using reagents supplied by EuFMD to the national laboratories.
- A project coordination meeting was held in August to review activities and plan follow-up actions.
- Clusters of NSP-positive animals were identified and follow-up investigations and sampling coordinated and supported, including the provision of technical support and supplies.
- National laboratories participated in WRL proficiency trials.
- Epidemiological training was provided to the TCC under the PEPc program (discussed elsewhere).
- A telephone conference was held with representatives of the United States Department of Agriculture program and the United States Defense Threat Reduction Agency (DTRA) program for animal health support to Georgia, to identify areas of similar activity and ensure no duplication of activities. The information provided about DTRA support in the area of PCR and laboratory capacity development enabled a more efficient approach to be taken, improving outcomes at reduced cost.

Activities planned for October to December 2012:

- October: laboratory training in the use of real time PCR for FMD diagnosis is planned, with follow-up actions to include the provision of diagnostic reagents to the laboratories to facilitate use of PCR to detect viral RNA in samples from NSP positive animal clusters.
- November: a desktop simulation exercise is planned to evaluate the contingency plans for each country, with the results to be used to inform further improvement of plans.
- December: review of project activities, closing workshop.
- December/January: describe the options for further EuFMD/EC support to the TCC, recommending actions to maintain momentum while ensuring efficient use of funds.
Project #3: Combating Foot-and-mouth Disease through enhanced and co-ordinated surveillance activities; Phase III of the FMD surveillance centre initiative

Countries: Iran
Lead technical officer (LTO) and other principal international experts: Melissa McLawns (LTO), Chris Bartels, principal international expert/epidemiologist. Labib Bakkali (France), FMD diagnostic laboratory expert.
Reporting period : April 2012- September 2012
The current project was formally agreed in July 2010 and should run for 3 years. Project activities effectively commenced in October 2010.

Project #4 : Thrace – improved surveillance for early detection of FMD

Countries: Turkey, Bulgaria, Greece
Lead technical officer (LTO) and other principal international experts: Keith Sumption (Supervisor), Angus Cameron (Consultant)
Reporting period : April 2012-September 2012

Summary:
Following a request from DG-SANCO to develop surveillance plans for the early detection of FMD in domestic and wildlife species in Turkish Thrace, and adjoining areas of Greece and Bulgaria, a workshop was held with two participants from each country, in Istanbul (September 18-21st). The workshop was led by Angus Cameron, EuFMD Consultant, and Theo Knight-Jones (EuFMD STP). The workshop aimed to develop a surveillance plan that was risk based and which took into consideration the contribution of all components of the current passive and active surveillance systems; the additional surveillance components will be costed and the proposal provided to EC for potential funding (to begin early 2013-).

Reporting: Draft Report provided within one week of WS. Full one to follow.

Component 2

Activities to reduce FMD risk in the South and East Mediterranean countries

1. One Phase of support to Egypt (150,000 USD) by EuFMD was completed on 29th February 2012, at which time the final workshop became aware of a very high likelihood of a SAT2 incursion, subsequently confirmed by Pirbright.
2. Emergency missions by EuFMD to Egypt were immediately undertaken in March under the Emergency Component (06: Emergencies), and working with the CMC-AH, a co-ordinated response was managed (see 06).
3. Longer term National FMD management was the subject of a National FMD management workshop, Cairo 2-3rd May, organized by FAO and Alexandria University.
4. An FMD management program was developed by FAO for the Bucharest Executive of some 2.7 m USD; subsequently a Surveillance Programme proposal was developed by EuFMD consultants, which after inputs and revisions by FAO-ECTAD and Government of Egypt, was submitted to EC-SANCO in August for support.
5. The EuFMD/EC programme has supported national consultants in Egypt to complete monthly reports, which indicate that SAT2 remains circulating in Egypt, as do other exotic sub-Saharan FMD strains (in September 2012).

6. Co-ordinated activities are unlikely to be easily achieved without a clear agreement from EC on the programme or budget limits.

**Collaboration with:** FAO ECTAD, FAO RNE, EMPRES, CMC-AH

7. Participation in TAIEX workshop in Cyprus, Sept 2012, covering FMD regional threats; EuFMD chaired session on vaccination strategies.

**Coordination with:** TAIEX, OIE, FAO Tunis, REMESA.

### Component 3

**Training Programme – FMD outbreak response**

**(Real-Time Training)**

**Project #1: Real-Time FMD Training Programme**

Lead technical officer (LTO) and other principal international experts:

Keith Sumption (LTO). Nadia Rumich, Communications Officer and Training Course manager, and Eoin Ryan (EuFMD). Naci Bulut, Turkey, and Eunice Chepkwony (Kenya), principal in country focal points for course management.

Countries: All European (EC, EFTA, Western Balkan ) and EuFMD member states.

Reporting period: 6 months to September 2012.

The second (24 month) Phase of Real-Time Training, after the 39th Session, had aim of providing experience of investigating FMD suspect cases in the field, for TWO front-line veterinarians from each member state that are members of the EU and/or EuFMD. (The first Phase had the aim of training THREE persons per country in the European region/EU and EuFMD MS).

The OVERALL number of trainees to September 2012 is 168 (Annex II), with 48 trained in Phase II which is the biennium to April 2013. The target of 2 per MS requires 72 trained in the biennium; or 24 more trainees requiring 3 courses. If the neighbouring countries are included, then another 18 should be added (9 countries in Western Balkans, Eastern European neighbours plus North Africa).

**Reporting in this period**

1. One RT Course was run in the period: NTC10, September 2012.
2. Training Course reports placed online after each course on the common, online network training site: [https://etcrealtimefmdtraining.wikispaces.com/](https://etcrealtimefmdtraining.wikispaces.com/)
Component 4

FMD surveillance in the African proximity

Countries: As per agreement with the Executive and EC, focus is upon countries with close proximity to North Africa and Middle-East in distance and trading connections, with support provided on a Network basis (Eastern and Western Africa).

Reporting period: 6 months to September 2012

Lead technical officer (LTO) and other principal international experts:

Keith Sumption (LTO). Sabenzia Wekesa (Kenya), Joseph Awuni (Ghana), Abdullah Traore (Mali), Network Coordinators for Eastern and West/Central Africa Networks. Joseph Litamoi and Bouabcar Seck for FAO-EARLN and FAO-Resolab networks linkages, respectively. Dr Kees van Maanen (NL), for technical guidance and leadership of the Eastern African Lab network in 2010-12. Jef Hammond, WRL-Pirbright, for linkages to the OIE/FAO Lab network and to WRL support services.

Project basis (background)

As previously reported, the EuFMD support is given to promote FMD reporting via the existing FAO Regional Lab Networks (RESOLAB and EARLN). For both networks, the expected outcomes were:

(a) To provide information on FMD diagnostic and surveillance results in the network areas to the EuFMD, for public dissemination through the EuFMD reports.

(b) To promote the use of reference laboratory services in their regions, and where needed to provide training on FMD typing and technical assistance to member laboratories.

(c) To increase the flow of relevant and informative FMDV samples to the WRL for characterization, sequencing and vaccine matching, to improve the information base on current FMDV circulation in each virus pool/region.

Summary in 6 month period:
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<td>FMD-SAT2 laboratory diagnosis course held in ANSES, Paris (May 2012) with North African and Sahelian zone countries. Surveillance plans developed with each country.</td>
<td>ANSES, FAO Tunis</td>
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<td>FMD diagnostic course held in Accra, Ghana (funded by USAID IDENTIFY project, EuFMD provided lab trainers and planning).</td>
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<td>Nine countries have a new capacity and kits for FMD serotyping, with mainly US funding. Follow up actions identified, to be funded by USAID with technical input from EuFMD.</td>
<td></td>
<td>Monthly FMD report</td>
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<td></td>
<td><strong>Collaboration with:</strong> USAID IDENTIFY, EMPRES, FAO ECTAD, FAO RAF, RESOLAB</td>
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<td>Eastern Africa:</td>
<td>EARLN-FMD workplan could not be adhered to – lack of HQ capacity. Priorities were sample shipment and achieving functional service of vaccine matching in the region, since Kenya supplies SAT2 and other vaccines to East Africa that may be relevant to North Africa/Mid-East.</td>
<td>EMPRES Shipping Service</td>
<td>Monthly FMD reports to EuFMD from EARLN-focal points</td>
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<td></td>
<td>Sample shipment Eritrea, Ethiopia and Sudan to WRL</td>
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<td><strong>Collaboration with:</strong> EMPRES Shipping Service</td>
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<td>FMD Manual developed by network.</td>
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<td></td>
<td><strong>Vaccine matching capacity</strong> – technical advice to establish provided (van Maanen mission).</td>
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</tbody>
</table>
Component 5
Research projects funded by EC through the EuFMD

Reporting period : 6 months to September 2012

Lead technical officer (LTO) and other principal international experts:

Keith Sumption (LTO). Dr Aldo Dekker, (NL), Chairman of the EuFMD Research Group, 2009-11, and Dr Kris de Clercq, Vice-President. Professor David Paton, IAH Pirbright, Chairman of the EuFMD Standing Technical Committee from April 2011. Members of the Research subcommittee of the Executive (Dr Nigel Gibbens, UK, and Alf Füssel, EC). From 4/2011, the Standing Technical Committee (STC) took responsibility for review of proposed CN, and comprised Professors Paton (UK) and Willeberg (DK), Dr C Bruschke (NL) and Dr Matthias Kramer (DE).

Project basis (background)

This component forms part of the overall 4 year Strategic Plan of the EuFMD to ensure that technical gaps to implementing FMD control actions by the member states are identified, and where urgently required, are supported through a Research procurement Process.

Summary in current period:

- Research Co-ordination; supporting the GFRA to produce Annual “State of FMD research “ reviews. (LoA: Onderstepoort Vet Institute, F. Maree). 1st LoA activities completed; second to be negotiated.

- Role of the European wild boar in FMD epidemiology (LoA: SAP Institute, Ankara, N Bulut): activities completed in April 2012.

- Methods for tracking wild boar dispersion and non-invasive surveillance sampling (FAO-EMPRES, S Khomenko and in Bulgaria, T Alexandrov): started 7/2012

- Assessment of FMD Vaccine effectiveness in Turkey/West Eurasia (IAH, T Knight-Jones): started 7/2012

- Development of an FMD Surveillance model for confidence in disease freedom based on multiple types of surveillance activity (Contract: A. Cameron): started 8/2012, for completion 11/2012

In addition, travel to support attendance at research-related meetings was supported, including training meetings, a meeting on FMD in wild boar.

Reporting

For each project, reports were generated and sent to the EuFMD; and each project leader required to report to the next Open Session of the EuFMD research group.
Component 6

FMD emergency responses

Countries: as agreed with EC
Reporting period: Six months to September 2012
Lead technical officer (LTO) and other principal international experts: Keith Sumption (LTO).
Project basis (background): In response to situations arising, and to requests either from the Secretary, EuFMD, or from the EC focal point; the scope of each emergency action is agreed with the EC and communicated by letter of EC to the Chairman, EuFMD Executive Committee.

Summary of actions funded under the emergency response category:

1. Egypt - emergency missions in April-June
2. Training in SAT2 diagnosis, and supply of diagnostic ELISA kits to at risk Mediterranean fringe countries.
4. Surveillance and FMD management regional workshop, Rabat (under UMA/REMESA), funded by EC through EuFMD
5. Asia-1 vaccine effectiveness study, Turkey

Specific outputs to date or other indicators that progress is being made

1. Monthly Reports (Egypt)
2. Monthly Reports on FMD Surveillance (EuFMD/EMPRES), compiled from OIE, FAO, national consultant and media sources
3. Reports from EuFMD missions, from each workshop and training

Specific Reporting

1. Surveillance (Cyprus) Training Report (June 2012)
2. FMD management in Maghreb border zones (Rabat Workshop, July 2012) : report


Component 7

Strengthening FMD laboratories in the Balkan Region

Gap analysis missions to 8 Countries/States planned in period following 83rd Session: to Serbia, Croatia, Bosnia-Herzegovina, Montenegro, Albania, FYROM, Kosovo, Moldova. Missions undertaken by IAH under the Contractual basis of the FMD Diagnostic Services Framework Agreement (FAO/IAH). Missions were delayed in implementation and have not all been completed within the 6 month period.
1. At the 39th General Session, a recommendation was made that member states should consider the use of decision support tools and disease spread models, and the Secretariat was asked to support this. The STC drafted a position paper on how best to proceed, which was endorsed by the Executive Committee at the 83rd meeting. The first Workshop was held as a pre-meeting to the EU CVO Meeting in Denmark, June 2012; this efficient arrangement resulted in high attendance from across the EU with EuFMD supporting the non-EU European countries (EuFMD members) to attend. The STC proposal was discussed; following suggestions from the CVOs, the proposal was revised to specifically train policy makers in acting as “intelligent customers” who could commission, set up and oversee modeling groups in their countries and correctly understand and interrogate the resulting outputs. A half-day workshop for the non-EU countries was also held to identify needs in epidemiology, contingency planning and modeling, with the result that most non-EU countries identified a more pressing need for training in FMD epidemiology than in modeling. Therefore Epi-Training may be needed before modeling could be best used. Two groups of countries thus emerge, those requesting initial Epi-Training and those ready for workshops on modeling in decision support.

2. A first training workshop on the use of modeling and decision support tools will be held in Vienna on the 15th to 19th October, kindly hosted by the Austrian Ministry of Health and the University of Veterinary Medicine, Vienna. The trainees will be senior veterinary policymakers from Serbia, Croatia, Slovakia, Slovenia, Hungary, Czech Republic, Malta and Austria. The trainers will be experts with skills including contingency planning, policy implementation, the use of model outputs to inform disease control, establishing a modeling group, and running simulation models.

It is hoped that, following the successful conclusion of this workshop, the possibility of holding a second workshop or a series of workshops may be supported.

3. Secretary and Chairman of the STC (Professor Paton) participated in RAPIDD policy/modeling for FMD workshop, September (the US funded RAPIDD programme funded travel and participation). This also enables planning of the Vienna workshop, as several US collaborators will assist in this.

Component 8

Improved Contingency planning through use of decision support tools
Component 9

Support to the World Reference laboratory, Pirbright, in 2011-2012

Contract (150,000 per annum US$) developed with IAH covering surveillance activities 2011-12. This has been signed and implemented.
Practical Epidemiology for Progressive Control (PeP-C)
In addition to the Real Time training course, we now have started a Practical Epidemiology for Progressive Control (PeP-C)

The course will emphasize development of the 'epidemiological approach', that is for any activity:

1. Define objectives
2. Design study fit to achieve objectives
3. Data collection
4. Data entry/validation
5. Data analysis (transforming data to information)
6. Write a useful report that clearly presents information

It is held at the Pendik veterinary institute, Istanbul, and the course outline is as follows:

- **Week 1: Outbreak investigation**
  - Including information on prevalence, incidence, diagnostic tests, risk factors
- **Week 2: Value chain, socio-economic impact assessment**
  - Including information about risk, costs and benefits of FMD control, measuring FMD impact
- **Week 3: Surveys: SP, NSP, questionnaires and monitoring vaccination campaign**
  - Including sample size, survey design, data entry, analysis of data
- **Week 4: Control Strategy development**
  - Putting it all together

The course will be based around the Progressive Control Pathway (PCP) and will be very practical with lecture time minimised and students learning whilst working on problems using case-studies. Prior epidemiology training is not required.

Contact point: eufmd-training@fao.org
Progressive Control Pathway (PCP)
The Progressive Control Pathway For FMD control

(PCP-FMD)

The Progressive Control Pathway (PCP) is a set of stages (steps) in the level of control programs that build towards the eventual official recognition of FMD freedom. It is a risk-based approach and the initial actions (Stage 1) are achievable in all countries with limited investment. The PCP promotes investment to step up FMD control in each country and enables regional organizations to compare levels of action in affected countries and thereby to promote and monitor progress at regional scale. The pathway generates information for risk assessment and risk management at every stage, and is supportive for risk-based approaches that promote access to markets for livestock keepers in affected countries. The PCP principles and tools are available to assist assessment and guide new actions/projects and programs.
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Erol Irfan (Turkey)
M.Turcanu (Romania)
Slobodan Sibalic (Serbia)

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Dr Matthias Kramer
Prof David Paton
Dr Preben Willeberg

The Special Committee on Research

Aldo Dekker
Bernd Haas
Emiliana Brocchi
Naci Bulut
Stefan Zientara
Labib Bakkali
Jeff Hammond
Georgi Georgiev
Marisa Arias
Eoin Ryan
Graham Belsham
Kris de Clercq
Michel Bellaiche
The structure of the EuFMD
Join us at the EuFMD with the Short Term Professional program

The EuFMD Commission, based in FAO, has funds to support Short Term Professional (STP) staff to work in Rome (or possibly at other FAO offices including Ankara, Turkey) for the purpose of gaining professional experience in risk management of FMD, and of work in an international environment on FMD control. The duration of such STP attachments are a minimum of 3 months and the EuFMD Commission will support living allowances in Rome to a ceiling of € 2000 per month in 2011. Candidates will be selected on merit, taking into consideration the experience and profile, and the duration (availability).

Trainees are expected to travel and undertake duties as part of the EuFMD Secretariat, participating in meetings and workshops, disease investigations and field missions as required and appropriate to their skill set and aspirations. These missions will be part of the normal EuFMD program and it is expected, but not guaranteed, that trainees will gain experience of FMD control in the field.

Profile of STP trainees

Candidates should be veterinary graduates, have a high standard of spoken and written English, and should normally have recent experience at post-graduate level in disciplines relevant to the EuFMD such as the investigation and control of exotic/contagious animal diseases, epidemiology, pathology/laboratory diagnosis, risk management and contingency planning for disease control. The trainees should normally be working for the State Veterinary Service and be expected to use their experience after return to their full time positions.

Applications can be made directly to the Secretariat. If offered a position we expect the applicant to negotiate their release from their current duties. Applications with supporting letter from their Chief Veterinary Officer may also assist selection. The minimum duration would be 3 months, maximum 11 months.

Applications should include:

- an FAO personal history form or curriculum vitae
- a covering letter indicating, with reasons, which of the above 6 key areas the candidate wishes to focus upon, and the relevance of the position for their current or future profile.
- wherever possible, evidence that if accepted they be released from their normal duties for the duration but would continue to receive their normal entitlements of salary and related entitlements.

A Selection Panel of the Secretary and Chairman of the Executive Committee would agree the Terms of Reference for each position if there is significant interest/competition for places. Interviews (supported by the EuFMD Commission) may be held if necessary.

Contact: Eufmd@fao.org, subject line: STP