FMD Diagnostics

David Paton & Don King
Institute for Animal Health

Research and surveillance to prevent virus diseases of livestock and their transmission to humans
National and International Reference Laboratories

- New & improved diagnostic tests
- Referral diagnosis
- Disease preparedness and emergencies
- International links
- Training and proficiency testing
Talk Outline

- Diagnostic windows
- Suspect case investigation
  - Virus characterisation
    - Surveillance
  - Conclusions
Diagnostic windows

1. Rapid confirmation of clinical signs
2. Active surveillance for infected animals (including pre-clinical cases)
3. Surveillance for FMDV exposed animals

- Clinical lesions
- Antibody response
- FMD virus at mucosal sites
- FMD virus in blood

Representative “in contact” cattle data from Alexandersen et al., 2003 and unpublished data from IAH
Success of reactive strategies, e.g. culling, depends upon how long animals infectious before clinical signs

Proxy measures used based on viraemia/virus excretion

One-to-one contact infection studies suggest shorter interval

Implies early clinical and preferably preclinical diagnosis extremely important and can replace pre-emptive culling

Preclinical screening used in 2007 but currently difficult on large-scale
Clinical diagnosis

![Images of animals with foot, teeth, and map of location]

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<th>August</th>
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FMD tests and samples

1. Detection of FMD virus
2. Detection of FMDV-specific antibody (SP/NSP)

• Samples collected:
  – Tissue (vesicular epithelium)
  – Blood (serum from whole blood)
  – “probang” samples
  – Milk
  – Swabs from mucosal surfaces
  – Environmental samples (air samples etc.)
Assays for FMDV detection/characterisation

<table>
<thead>
<tr>
<th>Assay</th>
<th>Time to report result (hrs)</th>
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<tbody>
<tr>
<td>Virus isolation (BTy or IBRS2)</td>
<td>1-4 days</td>
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<tr>
<td>Ag ELISA</td>
<td>~4 hours</td>
</tr>
<tr>
<td>TaqMan® RT-PCR</td>
<td>~5 hours</td>
</tr>
<tr>
<td>Genome sequencing</td>
<td>~24 hours</td>
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Increasing reliance upon molecular testing

rRT-PCR was widely used to test submitted material, UK 2007

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number submitted</th>
<th>rRT-PCR</th>
<th>Ag-ELISA</th>
<th>VI</th>
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<tbody>
<tr>
<td>Epithelial suspension</td>
<td>50</td>
<td>50</td>
<td>49</td>
<td>50</td>
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<td>Tissue suspension</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Vesicular fluid</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Fluid</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
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<td>Serum</td>
<td>3115</td>
<td>3086</td>
<td>-</td>
<td>585</td>
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<td>EDTA-blood</td>
<td>32</td>
<td>32</td>
<td>-</td>
<td>32</td>
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<tr>
<td>Probang</td>
<td>36</td>
<td>36</td>
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<td>36</td>
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<tr>
<td>Swab</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Faecal suspension</td>
<td>1</td>
<td>1</td>
<td>-</td>
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<tr>
<td><strong>Total samples tested</strong></td>
<td><strong>3246</strong></td>
<td><strong>3216</strong></td>
<td><strong>52</strong></td>
<td><strong>709</strong></td>
</tr>
<tr>
<td><strong>% of samples tested</strong></td>
<td><strong>99.1</strong></td>
<td><strong>1.6</strong></td>
<td><strong>21.8</strong></td>
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</table>
Genome detection and sequencing

variable VP1 region sequences used for isolate characterisation

conserved IRES and 3D regions targets for pan-serotype reactive assays
Tracking spread of FMDV

East Asia incursions: 2010-2011 (O/SEA/Mya-98)

East Asia incursions: 2010-2011 (O/SEA/Mya-98)

Paton et al, Vet Rec 2010
2007 outbreak: real-time forensic tracing

Linking sequence data to infection windows: based on infection dates and lesion aging

**KEY**

- Most likely date of infection (5-7 days prior to clinical signs)
- Range of possible incubation period (14 days)
- Clinical disease evident on the farm

**Legend**

- FMD confirmed
- Preclinical (lab only)
- No evidence of infection
UK 2007 Outbreaks
Next generation sequencing to understand FMDV evolution

Transmission direction

MiSeq (Illumina)

Lost

Drifting

Fixed
Vaccine efficacy

- Potency evaluation
  - With challenge
  - Using correlates of protection
- Vaccine strain selection
  - Serological methods
  - Sequence-based predictions
- Field studies
  - Population immunity
  - Vaccine effectiveness studies: subclinical infection

Serologically predicted probabilities of homologous protection against in vivo outcome of vaccine trials based on VNT alone (A), and combining VNT and LPBE (B), showing that combining assays provides more confidence in protection failures than individual assays, without affecting ability to predict protection successes.
Why do we need field tests for FMD?

• FMD spreads very rapidly
  – Rapid decision required
  – Average time to receipt of samples >24hrs

• Devolved and POC formats offer potential to significantly decrease assay time
  – Tools to support clinical judgement

• Rapid, simple tests also useful in labs
New assay formats: considerations

- Reliability
  - Simple-to-use
  - Non-specialist
- Performance
  - Limit of detection
  - Ability to correctly identify infected animals with diverse FMDV strains
- Speed
- Scalability
- Cost?
Field testing vs centralised testing

- Field Tests
  - FMDV antigen (positive cases)
  - FMDV na (negative cases)

- Diagnostic Tests
  - Confirmation of 1st case (in a FMD-free country)?
  - Strain characterisation
  - Active surveillance programmes
Pen-side diagnostics

SVANODIP® FMDV-Ag

Mesosystems: non-invasive air samplers

Portable molecular assays: Genie III for LAMP and Enigma diagnostics for PCR

Infra-red thermography
Scatter plot showing hoof temperatures and their dependence on ambient temperature.

Differences between rectal and eye temperature for each animal

Gloster et al, BMC Vet Res 2011
Pre-clinical diagnosis IP2c 2007

• All animals closely checked for clinical signs with negative results
• 19 of 58 animals rRT-PCR positive blood samples
• Indicates near simultaneous infection of multiple animals
• First use of real-time preclinical diagnosis for FMD in field
Active surveillance: use of rRT-PCR for preclinical detection

- Intensively Patrolled Areas (IPA) established within protection zone (PZ) around Outbreak in Egham
- Visited every-other day
- Clinical examination
- Blood samples collected and tested for FMDV by rRT-PCR

- 14 “high risk” cattle herds
- ~ 500 animals
- Reduced un-necessary slaughter
Serological assays

• Targeted towards structural (SP) or non-structural proteins of FMDV
• SP assays – separate assays (VNT or ELISA) required for each serotype
• NSP assays – broadly serotype cross-reactive (ELISA - 3ABC protein and others)
• IgA and IgM serotype specific ELISAs
Note that this figure shows the testing strategy within the Protection Zone where there was evidence of recent active infection. In the Surveillance Zone, where serosurveillance commenced at least 21 days after the last case, it was considered sufficient to use the Cedi-NSP alone (i.e. without VNT) to rule out non-specific positives found in the screening Cedi-O ELISA.
**DIVA tests**

**NSP serology**
- Vaccine: Purified Virus
- Vaccinated
- Abs against Structural proteins
- Vaccinated - infected
- Abs against Structural and NSPs

**Mucosal IgA**
- Parenteral vaccine
- Systemic antibody
- Infection
- Pharyngeal virus replication
- IgA in nasal and oropharyngeal secretions
DIVA Testing with current vaccines

- Relatively specific detection of infection
  - In the past (animals fully recovered)
  - Ongoing
    - in population - virus circulation
    - in individual – virus carrier

- Substantiate freedom from infection
  - Lot of testing required
  - Specificity challenges
DIVA test selection

• NSP serology
  – Not carrier-specific
  – Use in free countries with and without vaccination
  – High quality vaccines – less NSP and less viral replication
  – Use in endemic situations
  – Good commercial kits for 3ABC ELISA
  – Multiplex alternatives to Western Blotting (EITB)

• IgA tests
  – Specific for ongoing virus replication – carriers
  – Need to collect nasal fluids
  – Not commercially available
Outbreak preparedness

• **Lab capacity**
  – central / devolved / local testing
  – **biocontainment**
  – staff
  – biorobotics
  – kits and reagents
  – quality accreditation

• **Coordination between VS HQ, field and lab**
  – sample submission
  – prioritisation
  – **data management**
Conclusions / Diagnostic gaps

• Increasing use of molecular tests
• Preclinical and field diagnosis mostly not in place
• Data management systems rarely optimal
• Molecular tracing improved but further refinements likely
• Improvements in predicting and monitoring vaccine efficacy
• Decision support needed in relation to vaccine selection
• Commercial kits for SP serology – not yet all serotypes
• Multiplex NSP assays under development
• What to do about carriers after vaccinate-to-live
• Identifying serotypes and incidents of new infection in complex epidemiological situations
EuFMD 2012
OPEN SESSION
Appliance of science in the Progressive Control of FMD

Where the world comes to talk FMD science

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