Real-time RT-PCR for the detection of FMDV in Milk

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The Pirbright Institute
Foot-and-mouth disease

- There is a recognized need for rapid diagnostics and surveillance to enable early diagnosis of foot-and-mouth disease in cattle.

- FMDV detection in milk presents unique opportunities for surveillance and early detection both prior to and during an outbreak.

- Screening milk would involve non-invasive sampling, on material that is already collected on a regular basis.

- At present there is a lack of a high-throughput screening tool and bulk tank milk surveillance plan.

Previous studies have shown that:
- Mammary gland = site for FMDV replication.
- FMDV is shed in cows’ milk at high titers ($10^4$ TCID$_{50}$ per ml).
- Studies have observed various first detection points in milk, both preceding the onset of clinical signs and at the same time.

(Burrows, 1968; Reid et al., 2006, Thurmond and Perez, 2006).
Multi-centre collaboration between:
- Institute for Infectious Animal Diseases (IIAD) (previously FAZD)
- Foreign Animal Disease Diagnostic Laboratory (FADDL)
- The Pirbright Institute

- To evaluate the effectiveness of preclinical indicators of FMDV infection.
- To evaluate a high-throughput screening tool using real-time(r)RT-PCR which could be scaled up for use with bulk tank milk.
- To compare real-time PCR protocols developed in the US with the routine diagnostic assay currently used in the UK.
- Both assays based on the pan-serotypic rRT-PCR targeting 3D.
- To obtain data suitable for the development of a national surveillance plan for screening milk for FMDV.
- Need to be suited to high-throughput screening in the event of, or during recovery from an outbreak.
Experiment Protocol

Dpi -1

Dpi 0

Dpi 5
Samples collected

- Whole milk:
  - skimmed milk
  - cell fraction
  - cream

- Blood (serum and whole blood)
- Mouth and nasal swabs
- Probangs
- Temperature
Sample processing

Two parts:

- Real time diagnostic tests (on the day of collection):
  - Virus Isolation  BTY cells (whole and skimmed milk) IB-RS2 cells
  - US rRT-PCR MagMax Express-96 & ABI 7500 Fast Real Time PCR System.

- Quantitative tests:
  - Virus titrations  BTY cells
  - Pirbright rRT-PCR MagNa Pure LC Extraction Robot & Stratagene Mx3005.
FMDV Detection Points

867

- Cows mixed
- Pirbright qRT-PCR-Serum
- US RT-PCR-Serum
- Virus Isolation-Serum
- Foot lesions
- PCR-Milk
- Virus Isolation-Milk
BTY Titration Results

**Whole Milk**

**Skimmed Milk**
Milk samples collected from field cases from the UK 2007 outbreak were used as a positive cohort to evaluate diagnostic sensitivity.

### Positive Cohort

<table>
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<tr>
<th>Sample</th>
<th>TaqMan EZ</th>
<th>Path-ID</th>
<th>Pirbright 3D</th>
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<tr>
<td></td>
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<td>Average Ct</td>
<td><strong>Ct</strong></td>
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</table>

* Indicates sample testing was repeated
Conclusions

• FMDV can be detected in whole milk, skimmed milk and the cell fraction, by all methods tested.
• FMDV was detected in milk before the onset of characteristic clinical signs.
• The greatest window for virus detection was by rRT-PCR in the milk up to 21 days post contact.
• Both rRT-PCRs detected virus for a longer period than seen in virus isolation.
• Data not included in this talk suggest that rRT-PCR of milk from a bulk tank in a large herd could detect a single infected cow in the early stages of infection.
• Milk could be an excellent sample type for the detection of FMDV and could be used for the development of a national FMD surveillance plan in the event of an outbreak.

Further Work

• Test samples from dairies in endemic countries.
Acknowledgements:

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IIAD, FADDL, DEFRA
Samples with high medium and low titers of FMDV were tested in serial dilutions.

Jersey milk revealing positive Ct at 250 fold to 30,000 fold dilutions

Inconclusive Ct at >1,000 fold dilution for the sample with the lowest titer and >700,000 fold dilution for the sample with the highest titer.

Detection from one study sample also yielded positive Ct up to a 100,000 fold dilution.

Milk production was reduced by no more than 50% on a given day.

Taken together this suggests that early in infection, rRT-PCR may reasonably detect a single infected cow from a herd size of 100 to 1,000 milking into a bulk tank.
qPCR Results

Whole Milk

Skimmed Milk
qPCR Results

Serum

- 825 Serum
- 951
- 108

Probangs

- 108 Probangs
- 867
- 951

- 825 probangs
- 867 Serum