Foot-and-Mouth Disease

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Good science, useful science
Foot-and-mouth Disease Virus.

- FMDV is the causative agent of foot-and-mouth disease, an economically important and highly contagious disease of many domestic livestock, such as pigs, sheep, goats and cattle.

- The primary route of infection by FMDV is through the upper respiratory tract.

- FMDV has a strong predisposition for epithelial cells. The initial sites of virus replication are thought to be epithelial cells of the oropharynx and associated lymphoid tissues.

- During the development of disease, virus is widely disseminated throughout the body with secondary sites of replication in many epithelial tissues.

- Epithelial cells of the soft palate and pharynx are most likely the sites of virus replication in persistently infected animals.
The nine genera that comprise the *Picornaviridae*

**Genus: Aphthovirus**
- Foot-and-mouth disease virus
- Equine rhinitis A virus
- ERBV

**Genus: Erbovirus**
- Equine rhinitis B virus
- TMEV

**Genus: Cardiovirus**
- Encephalomyocarditis virus
- EMCV

**Genus: Teschovirus**
- Porcine teschovirus

**Genus: Rhinovirus**
- Human enterovirus A
- Human enterovirus D
- Human rhinovirus A
- Human rhinovirus B

**Genus: Enterovirus**
- Poliovirus
- Human enterovirus C
- Human enterovirus B

**Genus: Hepatovirus**
- HHAV (x5)
- SHAV (x2)

**Genus: Parechovirus**
- HPeV-1
- HPeV-2

**Genus: Kobuvirus**
- Aichi virus
- AiV

**Genus: Eraviridae**
- Equine rhinitis A virus
- ERBV

Neighbor-joining tree based on a comparison of amino acid similarities of P1 (capsid)
The UK FMD outbreak 2001
IMPORTANT EVENTS IN THE SPREAD AND CONTROL OF FMD IN THE UK

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• 23 Feb - Culling on IPs and DCs. National movement restrictions.
• 15 March - Sheep, goats & pigs within 3 km of an IP in Lockerbie, Carlisle and Solway targeted for culling.
• 23 March - Contiguous premises (CPs) were included in the cull.
• 26 March - Epidemic reached its maximum with 54 outbreaks in one day.
• 27 March - 3 km cull started in Penrith valley, Cumbria
• 29 March - 24/48 hour cull policy began, IPs slaughtered within 24 hours, CPs culled within 48 hours.
• 26 April - cattle in farms with high biosecurity exempted from culls
Figure 1: Known movements of FMD-infected animals before 23 February 2001 and location of affected markets, abattoirs and livestock dealers.

Subject to information available on 15/07/01

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Stamping out involves:

- identification of infected animals
- culling of infected herds and flocks
- movement restrictions
- tracing of dangerous contacts
- culling of contiguous premises
- safe disposal of culled animals

Good science, useful science
Foot and mouth crisis

Cattle low mightily, then the thuds begin

Killing fields Cumbria fears disease is now uncontrollable

John Vidal

The three slaughtermen are young, cheerful and clear-eyed. Killing is their business and they have never known trade like this. In the spring evening light they were on a farm near Wigton, Cumbria, shooting more than 100 cows and 500 sheep. Now the Ministry of Agriculture has told farmers to go to Jim Hutchinson's scale End farm near Penrith.

Our mobile death squad ratifies through the lowlands south of Carlisle. We carry, in the back of a 15-year-old white van, cartridges, stun guns, decontamination suits, blue overalls, rubber gloves, sprays, wellington boots, forms and all the paraphernalia of modern slaughter.

The landscape is flat and desolate. Most farms here have been condemned and there are no animals in the field.

The white smoke of incineration pyres drifts into the van. We pass decomposing sheep carcasses piled high in the corner of fields where they have been waiting days to be picked up. The sickly stench of death hangs over some farms; in others the cattle and sheep lie sweetly together, their limbs splayed, ballots swelling, tongues rigid and out.

Scale End was condemned on Sunday, but in the watery sunlight it seems normal. At the end of a long closed road is a tangle of ancient and modern buildings. Seven lambs play in a small paddock behind the farm. A bull stalks the yard. The cattle low quietly in the barns and the sheep are penned in a field beyond. This is spring; there are daffodils on the roadside, birds in the hedges, and the Hutchisons family is waiting expectantly, as if for a relative.

But we bring only death. The three slaughtermen change into their overalls and white suits. They smoke a cigarette and share a joke. They don’t like this, they say. It is messy and too human. Yesterday a farmer cracked up.

We cross the straw border that separates the clean from the uncleans, and dip our feet in disinfectants. There are handshakes and nods. Mr. Hutchison is almost too emotional to speak, but a toothless old cowherd shows them round. “There are plenty sheep and cattle,” he says.

The slaughtermen reckon several hundred cows and 600 sheep. It will take four hours, they say, and start moving machinery around the barn to prevent the animals trying to bolt. Now the cattle squeal low and the sheep bleat hard. The legs of a cow give way and it droops to the ground. Five heads strain out of the barn.

31st March 2001
The making of an epidemic

How foot and mouth spread

1. Foot and mouth outbreak at Burnside farm, Heddon-on-the-wall, Northumberland
   - Animals at nearby Prestwick Hall farm, Ponteland, are infected, probably as the virus is carried on the wind

2. 40 sheep from Prestwick Hall are among 3,500 animals sold at Hexham.
   - The buyer is Willy Cleaver, a Devon farmer

3. 348 sheep sent from Devon to Germany, via Dover. Yesterday they were destroyed as a precautionary measure
   - The sheep were shipped to Longton market, Carlisle, which acts as a holding centre

4. The sheep are sent from Mr Cleaver's Burden farm, Highampton in Devon
   - Sheeps from Highampton taken to Bromham slaughterhouse in Wiltshire where they develop foot and mouth.

5. The sheep were sold to Hill farm in Llandudno, Herefordshire, and another batch were sold at auction in Northampton.

Main points

- Number of confirmed cases stood at 52, with 20 in the South West, 15 in the South East, and 10 in the North West
- Countries of origin include France, Belgium, and the Netherlands
- Officials are now planning for public health measures

27th February 2001
What was the causative virus?

Within 24 hours nucleotide sequencing had shown it to be serotype O Pan Asia strain

*Good science, useful science*
The seven FMDV serotypes

Neighbor-joining tree based on partial VP1-coding sequences
Genetic relationships between the UK outbreak virus and other FMDV type O strains

Unrooted Neighbor-joining tree based on the complete VP1 nucleotide sequences
Occurrence of the PanAsia strain of FMDV-O

- PanAsia strain
- FMD type O present (genotype different to PanAsia)
- FMD type O present (genotype not known)
- No FMD or disease status unknown
Neighbor-joining tree depicting the genetic relationships between various FMD type O viruses

N.J. Knowles and P.R. Davies, 15 September 2001
MECHANISMS OF SPREAD OF FMD

• CONTROLLABLE SPREAD
  – movement of infected animals
  – movement of animal products e.g. meat, milk, offal
  – farm equipment, milking machines, vehicles, people etc

• UNCONTROLLABLE SPREAD
  – spread by the carriage of virus on the wind
  – role of wildlife and birds?
AIRBORNE SPREAD FROM HEDDON, NORTHUMBERLAND

Key:
0.01  
0.1  
1.0  
5.0  

Scale 10 km
**Effect of species and number of animals excreting virus on the PREDICTED risk for spread of FMDV O UKG 2001 downwind.**

<table>
<thead>
<tr>
<th>Species excreting virus</th>
<th>Distance downwind at which species may be at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
</tr>
<tr>
<td><strong>1000 infected animals</strong></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td>6-20 km</td>
</tr>
<tr>
<td>Cattle</td>
<td>0.7 km</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.7 km</td>
</tr>
<tr>
<td><strong>100 infected animals</strong></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td>2-6 km</td>
</tr>
<tr>
<td>Cattle</td>
<td>0.2 km</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.2 km</td>
</tr>
<tr>
<td><strong>10 infected animals</strong></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td>&lt;2 km</td>
</tr>
<tr>
<td>Cattle</td>
<td>&lt;0.1 km</td>
</tr>
<tr>
<td>Sheep</td>
<td>&lt;0.1 km</td>
</tr>
<tr>
<td><strong>1 infected animal</strong></td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>&lt;0.5 km</td>
</tr>
<tr>
<td>Steer</td>
<td>&lt;0.1 km</td>
</tr>
<tr>
<td>Sheep</td>
<td>&lt;0.1 km</td>
</tr>
</tbody>
</table>

PREDICTED distances at which virus concentration in a plume may be sufficient to infect.

Estimated using the airborne excretion values for UKG 2001.

Much further airborne spread may be possible for certain other strains, for example C Noville.

It should be noted that these estimates assume ideal topographical and meteorological conditions for airborne spread and that considerable variability may occur.
### Most likely method of spread for cases in each geographic group and overall

<table>
<thead>
<tr>
<th>Group Name</th>
<th>Airborne</th>
<th>Milk tanker</th>
<th>Infected animals</th>
<th>Local</th>
<th>Other fomite</th>
<th>Person</th>
<th>Swill (suspected)</th>
<th>Vehicle</th>
<th>Under investigation</th>
<th>Total</th>
<th>Percent due to local</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anglesey</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>92%</td>
</tr>
<tr>
<td>County Durham</td>
<td>3</td>
<td>5</td>
<td>82</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>103</td>
<td>80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumbria</td>
<td>2</td>
<td>8</td>
<td>41</td>
<td>927</td>
<td>3</td>
<td>23</td>
<td>10</td>
<td>55</td>
<td>87%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Devon</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>146</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>19</td>
<td>79%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essex and Kent</td>
<td>4</td>
<td>5</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>16</td>
<td>31%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereford</td>
<td>11</td>
<td>118</td>
<td>3</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>70%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Yorkshire</td>
<td>3</td>
<td>2</td>
<td>81</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>27</td>
<td>121</td>
<td>67%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northumberland</td>
<td>4</td>
<td>2</td>
<td>76</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>11</td>
<td>101</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporadic</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>19</td>
<td>11%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staffordshire</td>
<td>8</td>
<td>52</td>
<td></td>
<td>4</td>
<td>17</td>
<td>4</td>
<td></td>
<td>85</td>
<td>62%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wales</td>
<td>3</td>
<td>2</td>
<td>44</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>18</td>
<td>80</td>
<td>53%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yorks &amp; Lancs</td>
<td>4</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lancashire</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>31</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>45</td>
<td>69%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>18</strong></td>
<td><strong>13</strong></td>
<td><strong>92</strong></td>
<td><strong>1587</strong></td>
<td><strong>12</strong></td>
<td><strong>73</strong></td>
<td><strong>1</strong></td>
<td><strong>29</strong></td>
<td><strong>198</strong></td>
<td><strong>2023</strong></td>
<td><strong>78%</strong></td>
</tr>
<tr>
<td>Total percent</td>
<td><strong>0.9%</strong></td>
<td><strong>0.6%</strong></td>
<td><strong>4.5%</strong></td>
<td>78%</td>
<td><strong>0.6%</strong></td>
<td><strong>3.6%</strong></td>
<td><strong>0.0%</strong></td>
<td><strong>1.4%</strong></td>
<td><strong>9.8%</strong></td>
<td><strong>100%</strong></td>
<td></td>
</tr>
</tbody>
</table>

Based on all cases with data to 21st October 2001
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• 26 April - cattle in farms with high biosecurity exempted from culls
Introduction of the 24/48 cull policy
FMD in GB: control options

Model predictions by Dr Neil Ferguson, Dr Christl Donnelly & Prof. Roy Anderson, Imperial College

A: Several days to slaughter

B: Slaughter on infected premises within 24 hours

C: Slaughter on infected and neighbouring farms within 48 hours

Data
Epidemic curve by confirmation date (n = 2030)

Epidemic curve by date of first clinical signs (n=2019)
Animals slaughtered for disease control measures*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>595,884</td>
<td>15</td>
</tr>
<tr>
<td>Sheep</td>
<td>3,297,385</td>
<td>82</td>
</tr>
<tr>
<td>Pigs</td>
<td>144,931</td>
<td>4</td>
</tr>
<tr>
<td>Goats</td>
<td>2,368</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Deer</td>
<td>1,017</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Other</td>
<td>581</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total</td>
<td>4,042,166</td>
<td></td>
</tr>
</tbody>
</table>

Note: Figures are provisional and subject to change.
The need for rapid diagnosis
Current diagnostic tests for FMD:
• Antigen detection by ELISA - ~4 hours
• Infectious virus detection through infection of bovine thyroid cells - ~4 days
BUT
• time is taken to get samples to the Laboratory

Future tests may be based on:
• PCR, both laboratory based and portable
• Penside stip tests
The big debate:
to vaccinate or not

Good science, useful science
FMD VACCINES

- FMD vaccines are inactivated, concentrated, purified preparations of virus mixed with an adjuvant.
- All clove-hoofed species can be immunised.
- One dose of emergency vaccine will protect for 4 to 6 months.
- Two doses of commercial vaccines are required to give protection for 6 to 9 months.
- Do not prevent establishment of carrier state.
FMD VACCINATION IN AN EMERGENCY

• Time is taken to identify protective vaccine strain and formulate vaccine.

• If incubating animals are vaccinated then disease can be expected.

• The period from vaccination to protection is 3 to 4 days with a potent emergency vaccine. (With a commercial vaccine the period is 2 to 3 weeks.)

• Re-excretion of virus and spread to animals in-contact can be expected if vaccinated animals are exposed to virus in the period before they become fully immune.
The problem of persistent infections and the carrier state
Duration of FMDV persistence
(Ruminants only - not pigs)

- **Cattle up to 3.5 years**
  (around 50% of recovered animals are carriers at 4 weeks, but can range from 0-100% depending on the strain of virus and probably the challenge dose. The % decreases over time, but may be up to 40-50% at 6 months, 20% at 8 months and up to 12% at 12 months)

  **Sheep up to 9 months**
  (about 45-50% of recovered animals are carriers at 8 weeks, 25% are at 12 weeks)

  **Goats up to 4 months**

- **African buffalo at least 5 years** - about 55-70% may be carriers under free-living conditions

- **Virus levels decline over time - final elimination of persistence?**
ISH-TSA detection of FMDV RNA in persistently infected bovine soft palate tissues
Duration of FMDV persistence

II

- Vaccinated animals or naturally protected animals exposed to live virus may also become infected in the pharynx and develop a subclinical infection but, in many cases, leading to persistent infection.

- The % of such animals which become carriers, the virus levels present and the duration of the carrier state have not been shown to be significantly different from non-vaccinated reconvalescent animals (up to 80% carriers have been demonstrated after experimental challenge of vaccinated cattle).
Can vaccines be improved?
The ideal vaccine needs to:

- Have wide specificity
- be safe
- be stable
- be “marked” so that vaccinated animals can be distinguished from infected animals
- prevent persistent infections
- give long term immunity

Good science, useful science
Foot-and-Mouth Disease Virus Capsid Morphogenesis

A

VPg → L^Pro 1A 1B 1C 1D 2B 2C 3A 3C^pol 3D^pol poly(A)

B

P1-2A

3CD

Proteolytic Processing

C

Protomer (5S)

Pentamer (12S)

Virus Particle (140S)

x5

x12

3D^pol

vrRNA

Actin
ASSEMBLY OF FMDV EMPTY CAPSIDS

IN vT7MR1+vTF73 INFECTED CELLS
Foot-and-Mouth Disease Virus Capsid Morphogenesis

A

VPg

L^{106} 1A 1B 1C 1D 2B 2C 3A 3C^{30} 3D^{30} poly(A)

2A

3B_{1-3}

B

P1-2A

Proteolytic Processing

3CD

Virus Particle (140S)

C

Protomer (5S)

x5

Pentamer (12S)

x12

3D^{pol}

vRNA

Actin
Stabilising the *Foot-and-mouth disease virus* capsid

The disassembly of FMDV that occurs in very mild acid is controlled by one amino acid: a highly polarised histidine at position 142 in protein 1C. The virus particle is stabilised by replacing it with a negatively charged amino acid.
Foot-and-Mouth Disease Virus Capsid Morphogenesis

A

B

Virus Particle (140S)

Protomer (5S)
Pentamer (12S)

3D\textsuperscript{pol}
vRNA

Actin

3CD

Proteolytic Processing
Molecular techniques in the identification of FMD virus infected animals

- **FMD virus infection**
- **Enzyme labelled anti-bovine conjugate**
- **Bovine antibody to 3ABC**
- **Anti-3A Mab**
- **Monoclonal antibodies**

- **PGEX**
  - Expression as GST fusion proteins
  - 3A

- **RT PCR Cloning**
  - P1
  - P2
  - P3
Differentiation of infection from vaccination using the 3ABC MAT-ELISA
Can alternatives to emergency vaccination against FMD be considered?

Good science, useful science
Cellular receptors for FMDV

2 types:

**heparan sulphate proteoglycans**

**integrins**

Ligand binding pocket

Extracellular domain.

Transmembrane domain.

Cytoplasmic domain.

‘RGD’ binding integrins

\[ \alpha_v \beta_1 \]

\[ \alpha_v \beta_3 \]

\[ \alpha_v \beta_5 \]

\[ \alpha_v \beta_6 \]

\[ \alpha_5 \beta_1 \]
structural basis of integrin recognition;
the RGD-containing loop (‘G-H loop’) of VP1
FMDV electron density difference maps showing receptor binding loop.

- Reduced - oxidised FMDV
- VP1_{C134S} - wild type

Extra density features due to sequence differences between O_{2}K and O_{2}BS.
Table 1: FMDV specificity for integrin receptors

<table>
<thead>
<tr>
<th>integrin species</th>
<th>binds: in vitro</th>
<th>mediates infection: in culture</th>
<th>in host</th>
</tr>
</thead>
<tbody>
<tr>
<td>αvβ1</td>
<td>nd&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>αvβ3</td>
<td>Y</td>
<td>N/Y&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/Y</td>
</tr>
<tr>
<td>αvβ5</td>
<td>nd</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>αvβ6</td>
<td>nd</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>α5β1</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

<sup>a</sup>nd = not determined  
<sup>b</sup>N/Y = poorly detectable  
<sup>c</sup>preliminary observation
significance of $\alpha v\beta 6$ as receptor for FMDV

- exclusively epithelial
- normally expressed at low or moderate levels
- upregulated in inflammatory conditions/wound healing
- specific
- bidirectional signalling molecule (activates TGF-$\beta$)
- nonessential
Acknowledgements:

My many colleagues at Pirbright including:

Alex Donaldson
Soren Alexanderssen
Andrew King
Nick Knowles
Steven Archibald

Good science, useful science