Controlling Foot-And-Mouth Disease: Challenges and Opportunities

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The NBAF, a new, state-of-the-art biosafety level (BSL) 3 & 4 facility, located in Manhattan, KS, will enable the U.S. to conduct comprehensive research, develop vaccines and anti-virals, and provide enhanced diagnostic capabilities to protect our country from numerous foreign animal, emerging and zoonotic diseases to assist in protecting our food supply and the nations agriculture economy and public health.
Outline

- **FMD overview**
- **Challenges to control**: (partial list)
  - Vaccine production – biosafety
  - Onset of immunity
  - Duration of immunity
  - Genetic/antigenic diversity

Recent reviews:
**Features of FMDV**

- Family Picornaviridae, genus *aphtovirus* (respiratory tract viruses?)

- Small RNA virus, Approximately 8.2 kb

- Seven serotypes: A, O, C, Asia, Sat1, Sat2, Sat3, multiple subtypes

- High mutation rate of $10^{-3}$ mut/site → Fast adaptation – evolution

- Causes persistent infection (carriers)
Foot-and mouth disease: THE MOST contagious disease of animals

FMD is the major animal disease preventing world trade of animals and animal products

Mortality is low but morbidity is high

High mortality associated with some strains and some control methods

Results in persistent infections (carrier state)


S. Korea 2010-14

Japan 2010

Egypt 2012
FMD Outbreaks Reports 2005-2014:
Different serotypes/subtypes on each endemic pool
FMD Vaccines Today

Requires BSL3Ag facilities
Risk of vaccine production with virulent FMDV

On Friday August 3, 2007 FMD was detected in a farm in Southern England located within 6 miles of the Pirbright Laboratory site.
Concerns with FMD Vaccines

• SAFETY: Require adaptation and growth of large volumes of wild type virus in cells ➞ possibility of escape of virus from manufacturing facilities

• EFFICACY:
  • Onset of immunity
  • Narrow antigenic coverage (serotype / subtype specific) and high antigenic variation
  • Short duration of immunity ≤6 months
  • Vaccinated and exposed animals become carriers (ie vaccine protects against clinical disease NOT against infection)
Solutions: SAFETY
Rationally Designed SAFE FMD Vaccine Platform

- Identification of genomic regions determining virulence
- Identification of antigenic epitopes associated to infection
- Engineering FMDV to attenuate and remove antigenic sites
FMDV takes over translation machinery and rapidly replicates to produce large amount of virus

Lpro also translocates to the nucleus and cleaves NFkB \( \rightarrow \) reduces innate response

Self processing papain-like proteinase

Cleavage of eIF-4G

(FMDV Leader protease functions)

Strebel & Beck 1986; Kleina & Grubman, 1992)

(Devaney et al., 1988
Kirchweger et al., 1994)
FMDV Pathogenesis in cattle
Summary of Wild Type FMDV Pathogenesis

0.5-3h 3-12h 12-24h 24-48h 48-96h 96-240h >28 days

Low titer
High titer
LEADERLESS VIRUS IS FULLY ATTENUATED

6-24h  24-48h  72-96h  96-240h  >28 days?

- No fever
- No viremia
- No lesions
- No shedding

No fever
No viremia
No lesions
No shedding

Low titer
High titer

GOOD LIVE VACCINE CANDIDATE?

NO – infection does not induce a strong immune response
Pathogenesis of wt FMDV in Pigs

48 hpi
Pathogenesis of FMDV3B3D in Pigs

48 hpi
Marker FMD-LL3B3D Virus
For Safe Inactivated Vaccine Production

- Safe production: attenuated in cattle and pigs
- Easy production: uses same production system as current FMD vaccines
- Simplified downstream processing: no need for NSP removal
- Non transmissible from cattle and swine
- Negative markers: 2 independent DIVA compatible markers
- Immunogenic: same as current inactivated vaccine
- Cassette construct allows to rapidly insert capsid-coding region from emerging strains
Infection of cells with FMD virus

Filter Virus Harvest to Remove Debris

Transfer to Second Vessel

Concentration & Purification by Chromatography

Liquid Nitrogen

Antigen Bank Room

Select and Thaw Antigen(s)

Blend with Adjuvants & Preservatives

Delivery to Customer In a few days

Final Vaccine

Reconstitute Antigen(s)

Final Vaccine

SAFE
Another SAFE platform: hAd5 Vector for Delivery of Empty FMD VLPs and IFNs

- Contains all protective epitopes present on current inactivated virus vaccine but lacks infectious viral nucleic acid and non-structural protein (NSP)

- Allows to "cleanly" distinguish vaccinated from infected animals using 3D and other NSP diagnostic tests

- Can be safely produced in the United States

Mayr et al, 1999, 2001
### Efficacy of Ad5-A24 Vaccine in Swine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Boost</th>
<th>Challenge</th>
<th>Mean Neut Ab (0 dpc)</th>
<th>Viremia (3 dpc)</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – 1</td>
<td>No</td>
<td>42 dpv</td>
<td>&lt;8</td>
<td>Yes</td>
<td>Severe disease</td>
</tr>
<tr>
<td>Commercial Vaccine – 2</td>
<td>No</td>
<td>14 dpv</td>
<td>700</td>
<td>None</td>
<td>No disease</td>
</tr>
<tr>
<td>Commercial Vaccine – 3</td>
<td>No</td>
<td>42 dpv</td>
<td>700</td>
<td>None</td>
<td>No disease</td>
</tr>
<tr>
<td>Ad5-A24 – 4</td>
<td>Yes</td>
<td>14 dpv</td>
<td>400</td>
<td>None</td>
<td>No disease</td>
</tr>
<tr>
<td>Ad5-A24 – 5</td>
<td>No</td>
<td>42 dpv</td>
<td>120</td>
<td>None</td>
<td>No disease</td>
</tr>
<tr>
<td>Ad5-A24 – 6</td>
<td>No</td>
<td>14 dpv</td>
<td>450</td>
<td>None</td>
<td>No disease</td>
</tr>
<tr>
<td>Ad5-A24 – 7</td>
<td>No</td>
<td>7 dpv</td>
<td>36</td>
<td>None</td>
<td>No disease</td>
</tr>
</tbody>
</table>

- **Swine vaccinated IM at 1 site with 5x10⁹ pfu single dose of Ad5-A24 are protected from challenge as early as 7 dpv**

*Moraes et al., 2002*
Efficacy of Ad5-A24 Vaccine in Cattle

A. Viremia

B. Clinical Disease

Cattle vaccinated IM at 1 site with 5x10⁹ pfu of Ad5-A24 are protected from challenge as early as 7 dpv

Pacheco et al., 2005
Solution: Rapid Onset of Immunity
Use of interferons for the rapid control of foreign and zoonotic animal diseases

Teresa de los Santos
Plum Island Animal Disease Center, ARS, USDA
One Health Symposium, Kansas City, August 2017
Interferons

- Cytokines discovered in 1950’s. “Interfered” with influenza virus replication.
- Display antiviral activity against most animal viruses including FMDV.
- Three IFN families: type I, II and III with many subtypes of each
- ARS has led the way on the discovery of livestock IFNs and their use against FMDV
**Ad5- IFN trials in the natural hosts**

**Treatment (3-5 animals/group):**
- PBS or Ad5-Blue
- Ad5-IFN
- Ad5-FMD
- Ad5-IFN + Ad5-FMD
Ad5-poIFNα protects swine against FMD - 3 dpv

Ad5-pIFNα $10^9$ pfu

A. Antiviral Activity

- Units Antiviral Activity vs. DPC
- Graph showing antiviral activity over time

B. Viremia

- pfu/ml vs. DPC
- Graph showing viremia levels over time

C. Lesion Score

- Lesions vs. DPC
- Graph showing lesion scores over time

Ad5-blue $10^9$ pfu

A. Antiviral Activity

- Units Antiviral Activity vs. DPC
- Graph showing antiviral activity over time

B. Viremia

- pfu/ml vs. DPC
- Graph showing viremia levels over time

C. Lesion Score

- Lesions vs. DPC
- Graph showing lesion scores over time

Chinsangaram 2003, Moraes 2003, Dias 2011, Grubman 2012
Combination of Ad5-poIFNα and Ad5-FMD protects swine challenged at 3dpv

<table>
<thead>
<tr>
<th>Group</th>
<th>IFN (U/ml)</th>
<th>Viremia/dpc</th>
<th>Clinical score/dpc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9987</td>
<td>52</td>
<td>2.0x10^5/1</td>
<td>14/2</td>
</tr>
<tr>
<td>9988</td>
<td>1,867</td>
<td>1.0x10^3/1</td>
<td>13/3</td>
</tr>
<tr>
<td>9989</td>
<td>0</td>
<td>6.1x10^4/1</td>
<td>14/2</td>
</tr>
<tr>
<td><strong>Ad5-A24</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9981</td>
<td>0</td>
<td>0/0</td>
<td>2/3</td>
</tr>
<tr>
<td>9982</td>
<td>395</td>
<td>0/0</td>
<td>6/3</td>
</tr>
<tr>
<td>9983</td>
<td>0</td>
<td>0/3</td>
<td>9/3</td>
</tr>
<tr>
<td><strong>Ad5-IFNα</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9975</td>
<td>13,718</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>9976</td>
<td>15,431</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>9977</td>
<td>16,059</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td><strong>Ad5-IFNα + Ad5A24</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9972</td>
<td>17,390</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>9973</td>
<td>18,631</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>9974</td>
<td>17,654</td>
<td>0/0</td>
<td>0/0</td>
</tr>
</tbody>
</table>

Moraes 2003
Ad5-IFNs protect against FMD 1-3 dpv

Combining IFN + vaccine could achieve protection starting as early as 24 h post vaccination and lasting for several months!

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type I + Ad5-FMD</th>
<th>Type III+ Ad5-FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td><img src="image" alt="Smiley" /></td>
<td><img src="image" alt="Smiley" /></td>
<td><img src="image" alt="Smiley" /></td>
<td><img src="image" alt="Smiley" /></td>
<td>?</td>
</tr>
<tr>
<td>Cattle</td>
<td>+/-</td>
<td>-</td>
<td><img src="image" alt="Smiley" /></td>
<td>?</td>
<td><img src="image" alt="Smiley" /></td>
</tr>
</tbody>
</table>
Solution: broadening immune response – mosaic vaccines
VACCINES WITH BROAD PROTECTION AGAINST FOOT-AND-MOUTH DISEASE VIRUS SEROTYPES

Elizabeth Rieder PhD.
Foreign Animal Disease Research Unit, USDA-ARS
Plum Island Animal Disease Center (PIADC), New York, USA.

Will Fischer, PhD
Theoretical Biology Group
Los Alamos National Laboratory (LANL)
Background
VACCINES WITH BROAD PROTECTION AGAINST HIGH-THREAT FOOT-AND-MOUTH DISEASE VIRUS SEROTYPES

ENABLING TECHNOLOGY

- The “mosaic” method of vaccine antigen design: natural diversity is computationally incorporated into a polyvalent multiple-sequence cocktail

![Image showing the mosaic method of vaccine antigen design]

PRELIMINARY RESULTS (PHASE I)

- Structurally and antigenically the type A mosaic are distinguishable from A24Cru

![Image showing structural and antigenic differences]

Mosaic Type A (divalent) BEI-inactivated/ISA 202 (Seppic)

- **Sucrose density gradient fractionation of Mosaic 2.1 and 2.2 140S antigen**

![Graph showing sucrose density gradient fractionation]

- **Experimental type A mosaic vaccine: immunization/heterol. Challenge:**

<table>
<thead>
<tr>
<th>TG</th>
<th>IVP</th>
<th>n (# cows)</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>T01</td>
<td>Placebo</td>
<td>3</td>
<td>Field Isolate A24 Cruzeiro (vaccine backbone)</td>
</tr>
<tr>
<td>T02</td>
<td>2-Mosaic cocktail (1x)</td>
<td>4</td>
<td>Field Isolate A Saudi 95</td>
</tr>
<tr>
<td>T03</td>
<td>Placebo</td>
<td>3</td>
<td>Field Isolate A IRAN 05</td>
</tr>
<tr>
<td>T04</td>
<td>2-Mosaic cocktail (2x)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>T05</td>
<td>Placebo</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>T06</td>
<td>2-Mosaic cocktail (2x)</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

21 cattle in three treatment groups Three control groups placebo/vaccine OIE challenge (~4.0 BID50 intradermal lingual)

April 18

Rieder and Fischer
These preliminary results demonstrated that the inactivated FMDV serotype A mosaic bivalent/adjuvanted vaccines (2.1 and 2.2 at 10 μg dose) elicited virus neutralizing antibodies and conferred 100% protection against FMD clinical disease following challenge with three heterologous FMDV serotype A strains.

### Table: Efficacy of bivalent mosaic FMDV serotype A vaccine against heterologous challenge with antigenically distinct serotype A viruses

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Prime (Day)</th>
<th>Boost (Day)</th>
<th>Challenge (Day) /strain</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>22; A24 Cruzeiro</td>
<td>0%</td>
</tr>
<tr>
<td>Mosaic bivalent</td>
<td>3</td>
<td>0</td>
<td>-</td>
<td>22; A24 Cruzeiro</td>
<td>100%</td>
</tr>
<tr>
<td>Placebo</td>
<td>3</td>
<td>0</td>
<td>22</td>
<td>36; A/Saudi Arabia/95</td>
<td>0%</td>
</tr>
<tr>
<td>Mosaic bivalent</td>
<td>4</td>
<td>0</td>
<td>22</td>
<td>36; A/Saudi Arabia/95</td>
<td>100%</td>
</tr>
<tr>
<td>Placebo</td>
<td>3</td>
<td>0</td>
<td>22</td>
<td>36; A/Iran/05</td>
<td>0%</td>
</tr>
<tr>
<td>Mosaic bivalent</td>
<td>4</td>
<td>0</td>
<td>22</td>
<td>36; A/Iran/05</td>
<td>100%</td>
</tr>
</tbody>
</table>

Rieder and Fischer
Summary

- FMD is highly transmissible disease of high economic impact
- Control of FMD outbreaks require effective vaccines, rapid onset of immunity and broad immune response.
- Current FMD vaccine production requires the use of live-virulent virus ➔ safety concern
- ARS scientists have developed vaccine platforms that allow safe production in the USA, with rapid onset of protection and broad antigenic coverage
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