Current Vaccine Research For An Evolving Food-and-Mouth Disease Virus

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Plum Island Animal Disease Center, New York, USA.
"We have a large selection of safe and effective vaccines to control the next FMD outbreak in mice and guinea pigs"

**TALK OUTLINE:**
- Challenges of FMD vaccines
- Current inactivated Ag vaccines
- Novel inactivated vaccine
- Peptide and DNA vaccines
- Viral vectored vaccines
- LAV vaccines

**Challenges and gaps**

Recent reviews:

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).

- UK, 2001
- Korea
- Japan
- Egypt 2012
Features of FMDV

- Family Picornaviridae, genus *aphtovirus*
- *Positive sense RNA*
  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
FMD Outbreaks Reports 2005-2011: Endemic Pools

Pool 1: East Asia
- A, O, Asia 1

Pool 2: South Asia
- A, O, Asia 1

Pool 3: Eur Asia
- A, O, Asia 1

Pool 4: East Africa
- A, O, Sat 1-3

Pool 5: West Africa
- A, O, Sat 1-2

Pool 6: South Africa
- Sat 1,2,3

Pool 7: South America
- A, O
Broad vaccine recommendations

<table>
<thead>
<tr>
<th>Pool 1</th>
<th>Pool 2</th>
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<th>Pool 5</th>
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Problem: recommended strains do not always work!
Vaccine matching for specific strains is needed
Addressing antigenic diversity in vaccine banks

Current vaccines
Concerns with FMD Vaccines

• Require adaptation and growth of large volumes of wild type virus in cells
• Escape of virus from manufacturing facilities
• Require banking of multiple antigen concentrates
• Some antigens lack stability (low potency/short shelf life)
• Short duration of immunity ≤6 months
• Vaccinated and exposed animals become carriers
Characteristics of an “Ideal” FMD Vaccine

- Effective, rapid and long-lasting protection with one inoculation
- Prevents viral transmission
- Allow differentiation of infected from vaccinated animals (DIVA)
- Safe: produced without the need for virulent FMDV
- No need for adaptation of field strains to cell culture
- Prevent development of carrier state
- Broad antigenic coverage
- Stable antigen – long shelf life
- Long duration of immunity
Novel vaccines overview

- cDNA-derived inactivated FMD vaccines
- DNA vaccines
- Peptide vaccines
- Subunit vaccines - VLP
- Viral Vectored-vaccines
  - herpesvirus (pseudorabies),
  - poxvirus
  - rabbit haemorrhagic disease virus (RHDV)
  - human defective adenovirus 5 (hAd5) vectors
- FMDV-modified-live vaccines
Genetically Engineered FMDV For Safe Inactivated Vaccine Production

- Safe production: attenuated in cattle and pigs
- Non transmissible to cattle and swine
- Negative markers: DIVA compatible
- Cassette construct allows to rapidly insert capsid-coding region from emerging strains
- Currently under development

Double marker cDNA-derived Killed FMDV Vaccine Platform

Vaccine seed antigens
Easy swap of capsid sequences

Attenuating factor
Deletion of Leader protein (543 bp)
Safety Data FMD-LL3B3D
versus $A_{24}^{WT}$ and $3B_m3D_m$ viruses in cattle

- Cows were inoculated by IDL ($10^6$ pfu live virus per cow)
- Clinical signs and temperatures were recorded for 10 days
- Samples collected for 10 days
  - Sera
  - Nasal swabs
  - Oral swabs
  - Room air samples
  - Vesicular fluid/tissue collected from lesions (if present)
## Safety Data Cattle

<table>
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<tr>
<th>Bovine #</th>
<th>Virus</th>
<th>Viremia, Maximum Titer</th>
<th>Virus in Saliva, Maximum Titer</th>
<th>Fever</th>
<th>Maximum Clinical Score/Maximum achievable</th>
<th>Neutralization Titer maximum (Starting DPI)</th>
<th>Shedding in air. Maximum Titer</th>
<th>(DPI)</th>
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<td>8.90 (3)</td>
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<td>5/5 (7)</td>
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FMD-LL3B3D is fully attenuated in cattle!
Safety Data Pigs
Determined with FMD-LL3D

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<th>4 dpi</th>
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<tbody>
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<tr>
<td>Virus in oral swab</td>
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<td>Negative</td>
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<tr>
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<td>Negative</td>
<td>Negative</td>
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</table>

All room air samples negative
FMD-LL3D completely attenuated and non-transmissible in pigs!
Efficacy Data Cattle

BEI-inactivated Vaccine formulated with montadine ISA 260 adjuvant (Sepic-WOW) 21 dpv challenge with FMDV-A24

<table>
<thead>
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<th>N=4</th>
<th>N=4</th>
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<td>![Cow]</td>
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<tr>
<td>Commercial Tetravalent FMDV Vaccine</td>
<td>FMD-LL3D FMDV Vaccine</td>
<td>FMD-LL3B3D FMDV Vaccine</td>
<td>Naïve unvaccinated controls</td>
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<tr>
<td>1xBEI- Vx</td>
<td>1x BEI Marker virus</td>
<td>1x BEI Marker virus</td>
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<tr>
<td>No clinical disease (0/4)*</td>
<td>No clinical disease (0/4)*</td>
<td>No clinical disease (0/4)*</td>
<td>100 % clinical disease (4/4)</td>
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Inactivated vaccines prepared with FMD-LL3D and FMD-LL3B3D induced complete protection against challenge!
DNA vaccines


- Construct includes P1-2A-3C-3D + GMCSF – other cytokines
- Electroporation delivery
- Required multiple doses
- 75% protection in cattle
VLPs Protein Vaccines

  - Baculovirus vector in silkworm
  - P1-3C Asia strains, protection at 28 dpv
  - Dose? 30 ug/ml? 6.5 pd50
Peptide vaccines

- Induce narrow immune response that FMDV can easily overcome

- Tests in cattle showed no protection despite strong anti-peptide antibody response

- Some reports suggest peptide vaccines are effective in swine – tested in Asia
  - Dose?
  - Duration of immunity
  - Challenge model?
Viral vectored vaccines – delivering VLP

Viral vectors provide genetic information to express and process all of FMDV structural proteins which are thought to result in the formation of virus-like particles.

Include

- herpesvirus (pseudorabies)
- Poxvirus
- human defective adenovirus 5 (hAd5) vectors
- rabbit haemorrhagic disease virus (RHDV)
A novel FMD vaccine was developed by ARS scientists under the leadership of Dr. Marvin Grubman.

This vaccine utilizes a defective human adenovirus vector to deliver genes coding for FMDV structural proteins.

Human Defective Adenovirus 5 vector
- Lacks necessary proteins for growth
- Delivers and expresses transgenes in target cells
Multi-epitope Immunogen: Empty Viral Capsids (EVC or VLP)

- Contains all protective epitopes present on current inactivated virus vaccine but lacks infectious viral nucleic acid and non-structural protein (NSP)
- Cassette feature: allows to express strain specific capsids without the need to adapt (or even grow) the target virus
- Allows to “cleanly” distinguish vaccinated from infected animals using 3D and other NSP diagnostic tests
- Can be safely produced in the United States

FMDV Empty Capsid Vaccine

- Processed products display epitopes resembling intact capsid.
- “Left-out” proteins can be used for DIVA tests

Dr. M. Grubman
Ad5-FMD Vaccine Prevents Clinical Disease and Viral Transmission

N=6
1° Vx
N=6
1° Vx
N=6
1° Naive
N=4
2° Vx
N=4
2° Naive
N=4
2° Naive

No clinical disease
100% protection (10/10)
No clinical disease
100% protection (10/10)
100% clinical disease (10/10)

DHS- TAD Group
Rationally Designed FMD Vaccines

Foot-and-Mouth Disease Virus

- Identification of genomic regions determining virulence
- Identification of antigenic epitopes associated to infection
- Engineering FMDV **to attenuate** and remove antigenic sites

Protease Cleavage Sites

\[ \text{L}^{\text{pro}} \quad \text{unknown} \quad \text{2A} \quad \text{3C}^{\text{pro}} \]
$L^{pro}$ structural domains

- Putative SAP domain (SAF-A/B, Acinus, and PIAS)
  - Nuclear retention and nuclear localization
  - DNA binding: present in nuclear proteins involved in chromosomal organization
  - Inhibit STATs (signal transducers and activators of transcription) signaling: PIAS (protein inhibitor of activated STAT) contain SAP domains

de Los Santos, et al, J. Virol 2009
Inoculation of Swine with Foot-and-Mouth Disease SAP-Mutant Virus Induces Early Protection against Disease

<table>
<thead>
<tr>
<th>A12-SAP dose (no. of PFU/animal)</th>
<th>Animal no.</th>
<th>Challenge result</th>
<th>Neutralizing antibody PRN$_{70}^c$</th>
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<tr>
<td></td>
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<td>Viremia</td>
<td>Nasal swabs</td>
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$^a$ The dose of the A12-WT challenge virus was $1 \times 10^5$ PFU per animal.
Safe Live Attenuated FMD Vaccines?

- Attenuated vaccines mimic viral life cycle in host
- Induce long lasting immunity (life long in some cases)
- Some success stories: Smallpox, Rinderpest, Polio.
- Concern: reversion to virulence
Solutions For FMD Vaccines

- Understand the barrier of serotype- and subtype-specific vaccine protection (achieving cross-protection and/or increasing the breadth of antigenic coverage)

- Improve the onset and duration of immunity of current and next generation FMD vaccines

- Target FMD vaccines to induce protection at relevant tissues to prevent infection (hence also prevent persistence)
There is a need for vaccines that are inexpensive to produce, easy to deliver and induce long-term immunity. Also there is need for better integrated strategies that fit the specific needs of endemic regions. Only when these critical components are available will the global eradication of FMDV be possible.

Veterinary research can improve the lives of millions of people around the globe!
Thank you !!!