Rift Valley Fever Virus: Diagnosis and Vaccines

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**RVF Virology**

- **NP**
  - Most abundant component of virion
  - Complexes vRNA in virion, cRNA in infection
  - Needed for virus replication + packaging
  - recN ELISA good diagnostic tool

- **NSs**
  - Not essential for virus replication
  - Blocks production of interferon in vitro
  - Essential for virulence

- **NSm**
  - Not essential for virus replication
  - Shown to suppress virus-induced apoptosis in vitro
  - Other functions unknown

- **G1, G2**
  - Envelope surface glycoproteins
  - Integral membrane proteins
  - Involved in virus attachment and tissue tropism
  - Targets of neutralizing antibodies

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 RVF virions (Neg stain)  

**Virions in hepatocytes**  
By Geisbert TW, USAMRIID, MD.

- **NP**
  - (245aa) [26 kDa]
- **NSs**
  - (264aa) [17 kDa]
- **NSm**
  - (135 aa) [14 kDa]
- **G1**
  - (534aa) [58 kDa]
- **G2**
  - (507aa) [56 kDa]
- **L**
  - 6404 nt

- **vRNA**  
  - S 1690 nt
  - M 3885 nt
  - NSm 271 nt untranslated

- **RdRp**
  - (2092 aa) [237 kDa]
Laboratory Diagnosis – RVF Case Confirmation

**Specimen:** Serum, whole blood, liver tissue, aborted fetus

**Tests performed:** VI, antigen ELISA, PCR, IgM sandwich ELISA, IgG ELISA, VN

**RVF: short duration viraemia**

- **Fever**
- **Virus / Antigen**
- **Neutralizing / IgG ELISA Antibodies**
- **IgM Antibodies**

3-7 days Incubation  |  3-15 days Disease  |  Time
Biosafety in diagnosis

There is no vaccination for humans

– Reduce the risks!

• Special caution when doing PM’s
• Inactivate the sample within the 1st step
• Handling of lab specimen (let serum clot....)
• Reduce pipetting and dilution steps
• Wash plates and equipment with caution
• Wear PPE
Successful diagnosis depends largely on the quality of the specimen and the transport and storage conditions of the specimen before it is processed in the laboratory.
Strategies for diagnosis of RVF

Based on detection of:

- Live virus
- Viral antigens
- Viral nucleic acids
- Acute phase antibodies (IgM) (4 – 42 days)
- Chronic phase antibodies (IgG) (7 - ? days)
Short viremia following RVF infection

- Data shows that $10^{10}$ RNA copies/mL of serum in sheep and $10^{8}$ copies/mL in cattle and humans.
- At day 9 post-infection, calves no longer viremic, and RVF virus can be isolated only from the brain.
RVF Diagnosis

- during outbreak
- post-outbreak
- for routine surveillance*
- for return to trade
- sentinel animals

*For routine surveillance

- is the region endemic or RVF-free?
- has there been vaccination?
RVF Diagnosis (Endemic Region)

During outbreak and post-outbreak
- PCR, antigen and IgM
- Send samples for virus isolation
- IgG NOT useful

For returning to trade after outbreak
- PCR, antigen and IgM
- Send samples for virus isolation
- IgG NOT useful
RVF Diagnosis (Endemic Region)

For routine surveillance
- IgM, IgG for animals born in IEP
- Send samples for virus neutralization

For sentinel animals
- IgM, IgG
- Send samples for virus neutralization
RVF Diagnosis (RVF-free Region)

- IgG sufficient
- May do both IgM and IgG for confirmation
- Send samples for virus neutralization
Antibody profile in infected vs vaccinated

Infected ➔ IgG ➔ IgM ➔ Vaccinated

Fig. 2. Mean ± 1 S.D. IgG (A) and IgM (B) responses in sheep (n = 8) infected with wild type AR 20368 strain of Rift Valley fever virus.

Fig. 3. Mean ± 1 S.D. IgG (A) and IgM (B) responses in sheep (n = 10) vaccinated with live-attenuated Smithburn strain of Rift Valley fever virus.

RVF Seroprevalence

Domestic animals
- Cattle, sheep, goat sandwich/indirect EIA, VN
- Camel inhibition EIA, VN

Sentinel animals
- Sheep sandwich/indirect EIA, VN

Wildlife
- Buffalos + others inhibition EIA, VN

Vaccination
NONE
Available test

Commercially:
- BDSL.....
  - inhibition ELISA for detecting IgG (in all species).
  - capture ELISA for IgM (in specified species, bov. capr. ovi)
  - indirect ELISA for IgG (anti-species conjugate)
  - Sandwich ELISA for IgG (in specified species, bov. capr. ovi)

Through research links
- CDC/USA (S. Nichol) - not available commercially
- USDA/USA (W. Wilson) – still undergoing validation
- NICD/RSA (J. Paweska) – most available through BDSL
## RVF Vaccines: Situations and control approaches

<table>
<thead>
<tr>
<th>RVF Situation</th>
<th>Examples of countries</th>
<th>Current Control Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic with regular outbreaks</td>
<td>Kenya, Tanzania, Egypt, Senegal, Mali</td>
<td>Vaccination at sign of outbreak</td>
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<tr>
<td></td>
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<td>Egypt: continuous vaccination</td>
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<tr>
<td></td>
<td></td>
<td>No vaccination</td>
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<tr>
<td>Endemic with sporadic/re-occuring outbreaks</td>
<td>South Africa, Saudi Arabia</td>
<td>Continuous/yearly vaccination</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Free high risk</td>
<td>Middle East, North Africa</td>
<td>(Active) surveillance</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Free low risk</td>
<td>Europe, Americas</td>
<td>Surveillance, talks of vaccine banks</td>
</tr>
</tbody>
</table>

Limited continuous vaccination of livestock in Africa:
- Cost of yearly vaccination
- Safety concerns: difficulties to determine physiological stages of pregnant animals
- Irregularity of outbreaks (years without signs of outbreak)
- Policy aspects: vaccination not always covered by government

Courtesy: Baptiste Dungu, GALVmed
Ideal RVF vaccine (Product profile)…

- **Generic characteristics**
  - **Safety**
    - Safe to produce
    - Safe to all physiological stages of animals
    - No residual virulence
    - No risk of introduction into the environment (shedding, persistence in animals etc.)
    - No risk of spread to human or other species
  - **Efficacy**
    - Protection of all susceptible species
    - Quick onset of protective immunity, including in young animals
    - Long lasting immunity
    - STOP TRANSMISSION: prevent amplification of RVFV in ruminants
  - **Vaccination**
    - Cost effective for producers and users
    - Single vaccination
    - Ease of application
    - Suitable for stockpiling (vaccine or antigen bank) and quick availability

- **Endemic regions**
  - Continuous vaccination: yearly vaccination of susceptible livestock
    - Need to know how many vaccinations may be required to build a life long immunity
  - **Efficacy**
    - Solid protective immunity after 1 vaccination

- **Free regions**
  - Quick onset of protective immunity
  - Protective in young animals and possibly newborn naïve animals
  - Sterilizing immunity
  - DIVA

Courtesy: Baptiste Dungu, GALVmed
Vaccination strategies to be considered

- **Endemic regions**
  - Yearly vaccination
  - Intermittent multiyear vaccination
  - Multivalent or combination vaccine, consisting of RVF antigen & antigen of a vaccine likely to be used regularly
    - RVF+LSD; RVF+ s/g pox; RVF + CBPP
  - Thermostability
  - Use of sentinel animals: need for good diagnostics capability & effective
  - Role of veterinary services

- **Possible suitable candidates:**
  - Multivalents including a safe deleted RVFV vaccine

- **Free regions/ Prevent epidemics**
  - Elimination of possible source of re-infection
  - Use of non-replicating antigen vaccine
  - Early and rapid onset of immunity, even in young animals

- **DIVA**
  - Positive marker: export of animals from endemic countries
  - Negative marker: for detecting infection

- **Possible suitable candidates:**
  - Replication deficient, deleted, marker vaccine

- **Set up regional vaccine bank**
  - FAO/ NGO/ Private company
  - Need storage facility

*Suitable vaccination strategies more critical than improved vaccines*

Modified from: Baptiste Dungu, GALVmed
### RVF traditional vaccines

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>STRAIN</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inactivated</strong> (OBP, VSVRI)</td>
<td>Pathogenic field strain</td>
<td>● Safe in pregnant animals&lt;br&gt;● Can be used in outbreak</td>
<td>● Short term immunity&lt;br&gt;● Multiple vaccinations required&lt;br&gt;● Risk of handling virulent strain during production&lt;br&gt;● Colostral immunity present but poor&lt;br&gt;● Sheep better protected than cattle&lt;br&gt;● 100 x more antigen required than for live attenuated&lt;br&gt;● Longer production lead time</td>
</tr>
<tr>
<td><strong>Live Attenuated</strong> (OBP, KEVEVAPI)</td>
<td>Smithburn</td>
<td>● Highly immunogenic&lt;br&gt;● Single dose&lt;br&gt;● Good immunity (within 21 days)&lt;br&gt;● Effective and easy production&lt;br&gt;● Safer production&lt;br&gt;● Large batches: &gt;4m doses</td>
<td>● Potential residual virulence&lt;br&gt;● Teratogenic for foetus&lt;br&gt;● Potential risk of reversion to virulence&lt;br&gt;● Not advisable for use in outbreaks&lt;br&gt;● Theoretical possibility of transmission by mosquitoes (?)</td>
</tr>
</tbody>
</table>

Courtesy: Baptiste Dungu, GALVmed
## New candidates evaluated in target animals

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<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated</td>
<td>MP12</td>
<td>● Effective and good protective immunity</td>
<td>● Teratogenic for foetus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Easy and safe to produce</td>
<td>● Abortion in early pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Better safety than Smithburn in most species and age groups</td>
<td>● Not available commercially</td>
</tr>
<tr>
<td>Avirulent natural mutant</td>
<td>Clone 13</td>
<td>● Good protective immunity in sheep &amp; cattle</td>
<td>● Only registered to date in South Africa &amp; Namibia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Safe in pregnant animals</td>
<td>● Large scale field data in other regions needed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Safe in outbreak</td>
<td>● No evidence of DIVA to date</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Produced as standard freeze-dried live vaccine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Safe, effective and easy to produce</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Possible DIVA (NSs ELISA?)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Registered 7 used extensively in South Africa</td>
<td></td>
</tr>
<tr>
<td>Recombinant Lumpy skin virus expressing RVF</td>
<td>LSD Neethling strain expressing RVF glycoproteins</td>
<td>● Dual vaccine</td>
<td>● Only proof of concept to date</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Safe in all animals</td>
<td>● Currently grown in primary cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● DIVA</td>
<td>● Possible GMO regulation challenge (?)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Long shelf life (LSD)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>● More thermo-tolerant than others</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Efficacy shown in animal trials</td>
<td></td>
</tr>
</tbody>
</table>

Courtesy: Baptiste Dungu, GALVmed
RVFV clone 13 deletion

RNA segments

Large (L)
Medium (M)
Small (S)

Proteins

Nucleocapsid protein (N)
Viral RNA polymerase (L)
Glycoprotein G₁
Glycoprotein G₂
NSm 14 & 78 KDa

100 nm

Courtesy: Baptiste Dungu, GALVmed
Clone 13 sheep efficacy data

Experiment 3: average temperature per group post-challenge

Experiment 1: Mean temperature reactions of sheep after challenge

Dungu et al., 2010
Clone 13 cattle efficacy data

Mean Body Temperature for each group through the viral challenge phase. DPC: Days post-challenge.

Clinical course for the unvaccinated control group

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Peak Fever</th>
<th>Day PC</th>
<th>Duration of fever</th>
<th>Euthanasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1367</td>
<td>41.2</td>
<td>3</td>
<td>8 days (2-9)</td>
<td>9</td>
</tr>
<tr>
<td>1402</td>
<td>41.4</td>
<td>2</td>
<td>1 day (2)</td>
<td>3</td>
</tr>
<tr>
<td>1404</td>
<td>41.0</td>
<td>2</td>
<td>7 days (2–8)</td>
<td>11</td>
</tr>
<tr>
<td>1405</td>
<td>41.3</td>
<td>2</td>
<td>1 day (2)</td>
<td>3</td>
</tr>
<tr>
<td>1406</td>
<td>40.4</td>
<td>2</td>
<td>3 days (2-4)</td>
<td>11</td>
</tr>
</tbody>
</table>

Dungu et al., In Submission
<table>
<thead>
<tr>
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<th>STRAIN</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avirulent (lab generated)</td>
<td>R566: deletion in the M and S segments</td>
<td>● Safer due to deletions in all 3 segments, may never reassort</td>
<td>● Never tested in target animals</td>
</tr>
<tr>
<td>reassortant</td>
<td></td>
<td>● Protection in mice</td>
<td>● More stringent regulatory requirements for registration (?)</td>
</tr>
<tr>
<td>Virus- vectored RVF vaccines</td>
<td>Canarypox-expressing RVF proteins</td>
<td>● DIVA: Positive &amp; Negative marker</td>
<td>● No registered vaccine yet available</td>
</tr>
<tr>
<td></td>
<td>Heterologous virus expressing GP: Newcastle</td>
<td>● Live vaccine</td>
<td>● No large scale field data yet available, although extensive analytical</td>
</tr>
<tr>
<td></td>
<td>disease virus as vector</td>
<td>● Replication deficient</td>
<td>data generated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Multivalent: suitable where annual vaccination is a challenge</td>
<td>● Data to date showing low immunogenicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Potential for improved thermostability</td>
<td></td>
</tr>
<tr>
<td>Virus like particle (VLP)</td>
<td>VLP made of envelop proteins (GP)</td>
<td>● Potentially very safe</td>
<td>● No proof of concept in target animals</td>
</tr>
<tr>
<td></td>
<td>Naslund et al., 2009</td>
<td>● Immunity similar to live vaccine, but no replication</td>
<td>● Large scale production might be a challenge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● DIVA</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>DNA priming + inact. Vaccine Lorenzo et al.,</td>
<td>● DIVA</td>
<td>● Only incomplete protection demonstrated in mice</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>● Potentially long lasting immunity</td>
<td>● Production challenges</td>
</tr>
<tr>
<td></td>
<td>cDNA encoding GP</td>
<td>● Ability to enhance and modulate induced immunity</td>
<td>● Regulatory challenges (use in food animals)</td>
</tr>
<tr>
<td></td>
<td>Lagerqvist et al., 2009</td>
<td></td>
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</tr>
</tbody>
</table>

Candidates not evaluated in target animals

Courtesy: Baptiste Dungu, GALVmed
**Recombinant-multiple deletion virus**

- Reverse genetic generating RVF virus with double deletions in NSs & NSm
  *Bird et al., 2008*
- Less prone to reassortment
- Live vaccine
- DIVA: negative marker
- Easy and safe to produce
- Target animal efficacy & safety data generated

**Advantages**

**Disadvantages**

- Not yet registered

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**RNA segments**

- Large (L)
- Medium (M)
- Small (S)

**Proteins**

- Nucleocapsid protein (N)
- Viral RNA polymerase (L)
- Glycoprotein G₁
- Glycoprotein G₂

Courtesy: Baptiste Dungu, GALVmed