Capripox applied research and vaccine comparison at CODA – CERVA, Belgium

Kris De Clercq
Andy Haegeman

EuFMD online meeting Lumpy skin disease

Tuesday 7th April 2015
Diagnostics: real-time PCR

- Development of the Capx real-time PCR panel
  - Consist of 3 Triplex real-time PCRs
    - Targets 3 different genomic regions (D5R, E3L, J6R)
      - (D5R: NTPase, E3L: dsRNA-binding PKR inhibitor and J6R: RNA polymerase sub-unit RPO147; Tulman et al., 2002)
    - Each contains an internal (GAPDH) and external controls
      → Quality

- Validation
  - Diagnostic Specificity (DSp): 160 negatives blood samples
    → No positive and only 1 doubtful result per PCR → 99.38%
  - Diagnostic Sensitivity (DSe):
    - Experimental infection samples (n=591):
      → Individual PCRs: 86% to 97.5% (depending on sample type and PCR)
      → Combining 2 PCRs:
        - Increase in DSe to 96% and 100%
        - DSp remains high 98.75%
Diagnostics: real-time PCR

- Diagnostic Sensitivity (DSe) (continued)
  - Field samples from Morocco (blood, scabs and swabs)
  - Percentage detection: similar to experimental infection samples
    - Individual PCRs: 73% to 94%
    - Combining 2 PCRs: 88% to 100%
    - Combining all three: 91% to 100%

Panel usage? → Fit for purpose

- Endemic regions: 1 PCR is sufficient (High DSp and DSe)
- Evolving to Capx-free status: 2 PCRs in parallel (Higher DSe, still high DSp)
- Capx-free regions/index-case: 3 PCRs in parallel (Highest DSp necessary)
- Samples types with high viral load: 1 PCR is sufficient
Analysis of the Moroccan field isolates (outbreak 2010)

- Isolates from 15 geographical regions + 1 Moroccan vaccine
- Development of 5 new PCRs as potential new phylogenic regions
- 1 published region (RPO30)
- Isolates form 12 out of 15 geographical regions could be analysed

Results

- All isolates are identical (1 SNP in 1 of the 6 PCR regions)
- Vaccine and isolates are very different (No link with vaccination campaign)
- 2 PCRs (1 real-time and 1 classic) developed for distinguishing the field isolates from the vaccine (DIVA)
  - No vaccine found in the field isolates
  - No Wild type found in the vaccine bottle

Transboundary and Emerging Diseases (online early view, 2015)
Investigation of a possible link between vaccination and the 2010 sheep pox epizootic in Morocco.
A. Haegeman, K. Zro, D. Sammin, F. Vandenbussche, MM Ennaji, K. De Clercq
Animal experiments

Set up:

- Refreshment → Titration → Transmission/follow Up
- LSDV (Pirbright), SPPV (Field isolate Morocco), GPV (Pirbright)
- Cattle, Sheep and Goats

### SPPV

<table>
<thead>
<tr>
<th>Animal Id</th>
<th>per ml</th>
<th>Inoculation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1R</td>
<td>10 exp 6</td>
<td>ID</td>
</tr>
<tr>
<td>S2R</td>
<td>10 exp 6</td>
<td>ID</td>
</tr>
<tr>
<td>S1T</td>
<td>10 exp 5</td>
<td>ID</td>
</tr>
<tr>
<td>S2T</td>
<td>10 exp 5</td>
<td>ID</td>
</tr>
<tr>
<td>S1F</td>
<td>10 exp 4</td>
<td>ID</td>
</tr>
<tr>
<td>S2F</td>
<td>10 exp 4,5</td>
<td>ID</td>
</tr>
<tr>
<td>S3F</td>
<td>10 exp 4,5</td>
<td>ID</td>
</tr>
<tr>
<td>S4F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>S5F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>S6F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>S7F</td>
<td>indirect contact</td>
<td></td>
</tr>
</tbody>
</table>

### LSDV

<table>
<thead>
<tr>
<th>Animal Id</th>
<th>Per ml</th>
<th>Inoculation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1R</td>
<td>10 exp 6</td>
<td>IV</td>
</tr>
<tr>
<td>R2R</td>
<td>10 exp 6</td>
<td>IV</td>
</tr>
<tr>
<td>R1T</td>
<td>10 exp 6</td>
<td>ID</td>
</tr>
<tr>
<td>R2T</td>
<td>10 exp 6</td>
<td>ID</td>
</tr>
<tr>
<td>R1F</td>
<td>10 exp 6</td>
<td>IV</td>
</tr>
<tr>
<td>R2F</td>
<td>10 exp 6</td>
<td>IV</td>
</tr>
<tr>
<td>R3F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>R4F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>R5F</td>
<td>IV virus: Passage 2 noduli Refreshment (= 10 exp 6)</td>
<td>IV</td>
</tr>
<tr>
<td>R6F</td>
<td>IV virus: homogenate noduli Refreshment (&lt; 10 exp 1)</td>
<td>IV</td>
</tr>
</tbody>
</table>

### GPV

<table>
<thead>
<tr>
<th>Animal Id</th>
<th>per ml</th>
<th>Inoculation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1G</td>
<td>10 exp 5</td>
<td>ID</td>
</tr>
<tr>
<td>G2G</td>
<td>10 exp 5</td>
<td>ID</td>
</tr>
<tr>
<td>G1T</td>
<td>10 exp 4</td>
<td>ID</td>
</tr>
<tr>
<td>G2T</td>
<td>10 exp 4</td>
<td>ID</td>
</tr>
<tr>
<td>G1F</td>
<td>10 exp 3</td>
<td>ID</td>
</tr>
<tr>
<td>G2F</td>
<td>10 exp 3</td>
<td>ID</td>
</tr>
<tr>
<td>G3F</td>
<td>10 exp 2,5</td>
<td>ID</td>
</tr>
<tr>
<td>G4F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>G5F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>G6F</td>
<td>direct Contact</td>
<td></td>
</tr>
<tr>
<td>G7F</td>
<td>indirect contact</td>
<td></td>
</tr>
<tr>
<td>G8F</td>
<td>indirect contact</td>
<td></td>
</tr>
</tbody>
</table>
Results

- Main findings:
  - Lower morbidity and mortality compared to SPPV and GPV (cfr literature)
  - Variable clinical picture: no signs → sub clinical → clinical
  - Absence of clinical signs although infected (PCR and Ab positive)
  - Autopsy: no generalised typical Capx signs were found
  - No transmission to contact animals

Animal experiments: LSDV
Animal experiments: LSDV

- Clinical picture: Fever
  - Infected:
    peak around 7 Dpi but can be intermittent
Animal experiments: LSDV

Clinical picture:

- First appearance noduli:
  - Infected animals 7 to 10 dpi (2/4)
  - Infected with homogenate (low inf dose): 16 dpi

- Generalisation of noduli:
  - Infected animals 3 to 9 days later

- **No** clinical signs were seen in
  - The contact animals (4/4)
Animal experiments: LSDV

- Serology: Onset seroconversion (SN)
  - Infected: 14 to 18 dpi (4/4)
  - Infected with homogenate: onset 25 dpi
  - Contact: No seroconversion

- Virology: real-time PCR panel
  - Blood:
    - Infected: 2/4 pos, onset 9 dpi
    - Homogenate infected: pos from 14 dpi
  - Organ distribution
    - Reduced number of positive organs (compared to SPPV).
    - Significantly higher Cp’s than SPPV. Most positive were noduli, tongue and apparent healthy skin
    - Virus could be detected in the organs of all the IV infected animals (although only 2/4 pos in blood) and in the homogenate infected animal (animal infected with low dose)
FUTURE PROSPECTS

- Challenge model validation: LSDV infection trial

- LSDV Vaccine comparison (N=6):
  - SPPV–based vaccines (RM-65) Vs LSDV-based (Neethling)
  - GTPV–based vaccines Vs LSDV-based (Neethling)
  - Inactivated vaccines Vs Live attenuated vaccines (LAV)

- Vector competence (Wild type virus and LAV):
  - Ticks
  - Tabanidae

- Transmission: Wild type virus and LAV