EURL Capripox
Work programme 2018

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Assist EC and Countries

o Technical input
  • Lab protocols for laboratories

o Trainings on the request of a country: Kazakhstan

o Missions:
  • EUVET (CVET) Expert mission Sheeppox Greece
  • GFTADs Expert mission LSD Kazakhstan
  • STM (Sustained Technical assistance) mission LSD Ukraine
  • STM mission LSD Belarus
  • OIE Seminar LSD Kazakhstan
  • Workshop Sheeppox for Greece & Bulgaria
Tender for vaccines to include in the EU vaccine bank for LSD

- Independent Vaccine Quality control
  1. Identity of the vaccine strain
  2. Titration of vaccine strain
  3. Freedom from extraneous agents
     - Evidence of absence of bacterial, fungal or mycoplasmal contaminants
     - Evidence of absence of viral contaminants
       e.g. FMD, BTV, EHDV, BVD, BDV, SPPX, GTPX, Lentiviruses (Maedi-visna virus, Bovine leucosis virus)
PROFICIENCY TESTING 2018

CAPRIPOX VIRUS (CAPX)

Detection of specific antibodies to capripox viruses in serum and/or
Detection of capripox virus nucleic acid in cell culture supernatant and tissue homogenate.

Results presented to NRLs at EURL annual meeting
Montpellier, 12/10/2018
Diagnostic tests to be used for active surveillance purposes

**Clinical detection:** Sensitivity detecting clinical signs in the first 3 weeks after infection: 67-75%

**PCR test of blood or skin:** diagnostic sensitivity 90-100% in blood and 95-100% in tissues

**ELISA and IPMA:** antibodies after 1 month
- Experimentally vaccinated or infected animals:
  - ELISA: Se = 83%; Sp = 99.7%
  - IPMA: Se = 100%; Sp = 100%
- Under field conditions:
  - ELISA: Se = 59%; Sp = 99.7%
  - IPMA: Se = 53%; Sp = 100%

**Serbia and FYROM studies:** ELISA Se 75-80% / Milk ELISA
Improved methods for capripox virus diagnosis with focus on molecular DIVA tests to differentiate field virus strains from vaccine strains

Vaccination with Herbivac® LS from Deltamune

A clear Neethling-like response was seen around 8/9 dpv with the appearance of noduli-like structures in 75% of the animals

PanPCR positive blood samples, biopsies and organ/tissue samples can be used for the evaluation of the DIVA real-time PCR
Evaluation of the DIVA real-time PCR

✓ Biopsies and Tissues (n=47)

⇒ good correlation between both real-time PCRs in the Capx Cp range of 15 to 30 (average difference in Cp of 1.4)
Evaluation of the DIVA real-time PCR

✓ Biopsies and Tissues (n=47)

✓ 13 samples (28%) negative with the DIVA real-time PCR
  ➤ inhibition?
  ➤ DNA extracts 1/10 diluted
  ➤ DIVA-PCR: positive results (vaccine-type)

Conclusion: inhibition in pure DNA samples!

✓ All samples were correctly identified and typed by the DIVA real-time PCR as vaccine strain
Evaluation of the DIVA real-time PCR

✓ Blood samples (n=25)

✓ All samples had a low viral load (Cp > 35) with the panCapx panel of Haegeman et al. 2015

✓ Only 40% of the samples were detected with the DIVA real-time PCR of Agianniotaki et al (2017), but all were correctly identified as vaccine type
Conclusions DIVA evaluation

✓ DIVA real-time PCR: suited for detection and typing of vaccine LSDV in samples with a high (vaccine) viral load, such as skin lesions / nodules

✓ Nodules samples or scabs/tissue: inhibition needs to be kept in mind, diluting the DNA samples 1/10 is recommended

✓ Blood or swabs are not recommended for the confirmation of Neethling like response: vaccine viremia or shedding can be low and missed
Experimental evidence of mechanical transmission of lumpy skin disease virus by biting Arthropods

Method:
Set up in vivo experiment 1

<table>
<thead>
<tr>
<th>Exp 1</th>
<th>4 Donor animals</th>
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<tbody>
<tr>
<td></td>
<td>D1</td>
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<td></td>
<td>D2</td>
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<td>D3</td>
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<td>D4</td>
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</tbody>
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<table>
<thead>
<tr>
<th>8 Acceptor animals</th>
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<tbody>
<tr>
<td><em>Dermacentor reticulatus</em></td>
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<tr>
<td>Fed on donor animals</td>
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<td>A1</td>
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</tbody>
</table>

100 ♀ +100 ♂ ticks/
cotton bag on ears Donor
for 5-9 days from 5 dpi
=> On Acceptor for 5-7 days

• flies in cages on viremic donor (10 min/day) from
6-9 dpi =>100-200 flies/acceptor from 6-9 dpi (10 min/day)
Results: In vivo experiment 1 with *S. calcitrans*

**Donors**
- 2 of 4 donor animals viremic
- Only D3 with noduli on 7 dpi
- noduli PCR confirmed

**Acceptors**
- 1 of 4 acceptors with *S. calictrans* viremic on 9 dpc
- First noduli on 12dpc (PCR confirmed)

First evidence of transmission of LSDV with *S. calcitrans*
Next experiment: => Confirmation with *S. calcitrans*
  => Also possible with the horse fly *Haematopa sp.?*
**Method:**

**Set up in vivo experiment 2**

<table>
<thead>
<tr>
<th>Exp 2</th>
<th>5 Donor animals</th>
<th>6 Acceptor animals</th>
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<tbody>
<tr>
<td></td>
<td>D5</td>
<td>D6</td>
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<td>D7</td>
<td>D8</td>
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<td>D9</td>
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<tr>
<td></td>
<td><strong>Haematopota sp.</strong></td>
<td><strong>Stomoxys calcitrans</strong></td>
</tr>
<tr>
<td>Fed on donor animals</td>
<td>Fed on donor animals</td>
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<td>A13</td>
<td>A16</td>
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<td>A18</td>
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</tbody>
</table>

- Horse flies on viremic donor & acceptor from 7-9 dpi (10 min/day)
- 40 Haematopta sp./on each acceptor
- *S. calcitrans* on viremic donor & acceptor from
  - 6-9 dpi (10 min/day), 100-200 flies/acceptor
  - 15-16 dpi(10 min/day), 100-200 flies/acceptor
Results:
In vivo experiment 2 with *Stomoxys calcitrans*

- 3 of 5 donor animals viremic,
- only D8 an D9 used for *Stomoxys calcitrans*
- Both viremic on 5 dpi
- Noduli: D8 on 8 dpi, D9 on 7 dpi
- Results still in progress (PCR blood)

- 2 of 4 acceptors with *S. calictrans viremic*
- A17 viremic on 15 dpc => viremic from 1st contact
- A15 viremic on 27dpc=> viremic from 1st or 2nd contact
- A 17 noduli on 15 dpc
- A15 noduli on 23 dpc

Re-confirmation of transmission of LSDV with *S. calcitrans*
Results: In vivo experiment 2 with *Haematopota sp.*

- 3 of 5 donor animals viremic,
- only D5 used for *Haematopota sp.*,
- Viremic on 5 dpi, noduli on 7dpi
- Results still in progress (PCR blood)

**Donors**

- **Acceptors**
  - 1 of 2 acceptors with *Haematopota sp.* positive
  - A16 positive on 26 dpc
  - Noduli on 27 dpc

First evidence of transmission of LSDV with *Haematopota sp*

Next experiment:
If *S. calcitrans* can only bite 1 day 10 min to donor & acceptor, will there be still transmission?
Other Studies

- Duration of Immunity and of Protection
- Subclinical infection
- Transmission studies
  - Indirect and Direct transmission
- Sheeppox Vaccine Evaluation
Thanks to EC for support!

EU Reference Laboratory for Capripox viruses

Funded by the European Union