



EURL Capripox Workprogramme 2018

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Kris De Clercq

Assist EC and Countries

- Technical input
 - Lab protocols for laboratories
- Trainings on the request of a country: Kazakhstan
- 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

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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)

Capripox Proficiency Testing 2018

PROFICIENCY TESTING 2018

CAPRIPOX VIRUS (CAPX)

Detection of specific antibodies to capripox viruses in serum
and/or

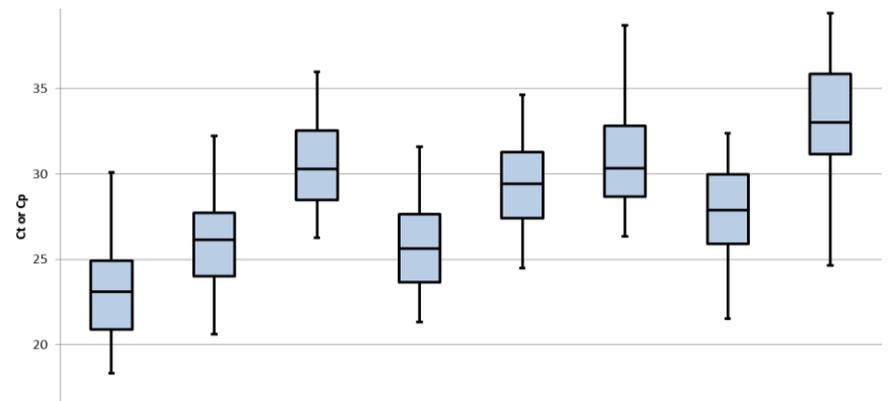
Detection of capripox virus nucleic acid in cell culture supernatant
and tissue homogenate.

**Results presented to NRLs
at EURL annual meeting
Montpellier, 12/10/2018**

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EFSA: Lumpy skin disease: scientific and technical assistance on control and surveillance activities

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:

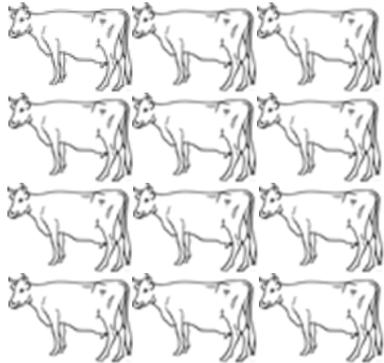
- ELISA: Se = 83%; Sp = 99.7%
- IPMA: Se = 100%; Sp = 100%

Under field conditions:

- ELISA: Se = 59%; Sp = 99.7%;
- IPMA: Se = 53%; Sp = 100%.

Serbia and FYROM studies: ELISA Se 75-80% / Milk ELISA

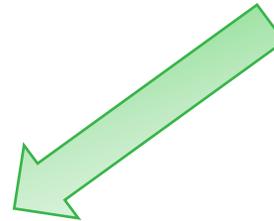
Improved methods for capripox virus diagnosis with focus on molecular DIVA tests to differentiate field virus strains from vaccine strains



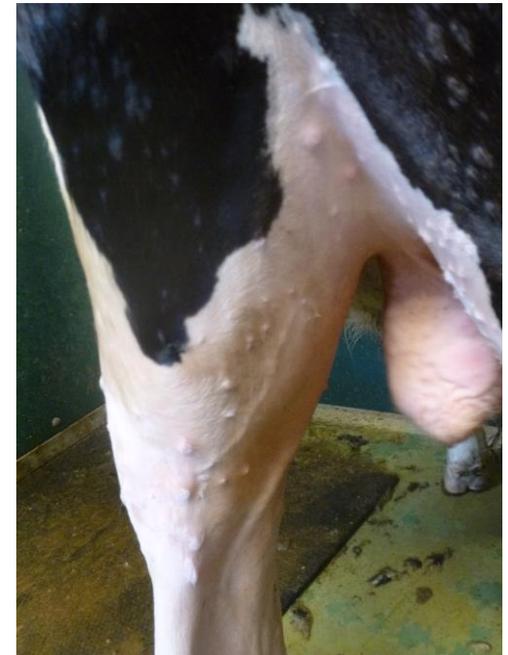
Vaccination with Herbivac
® LS from Deltamune



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



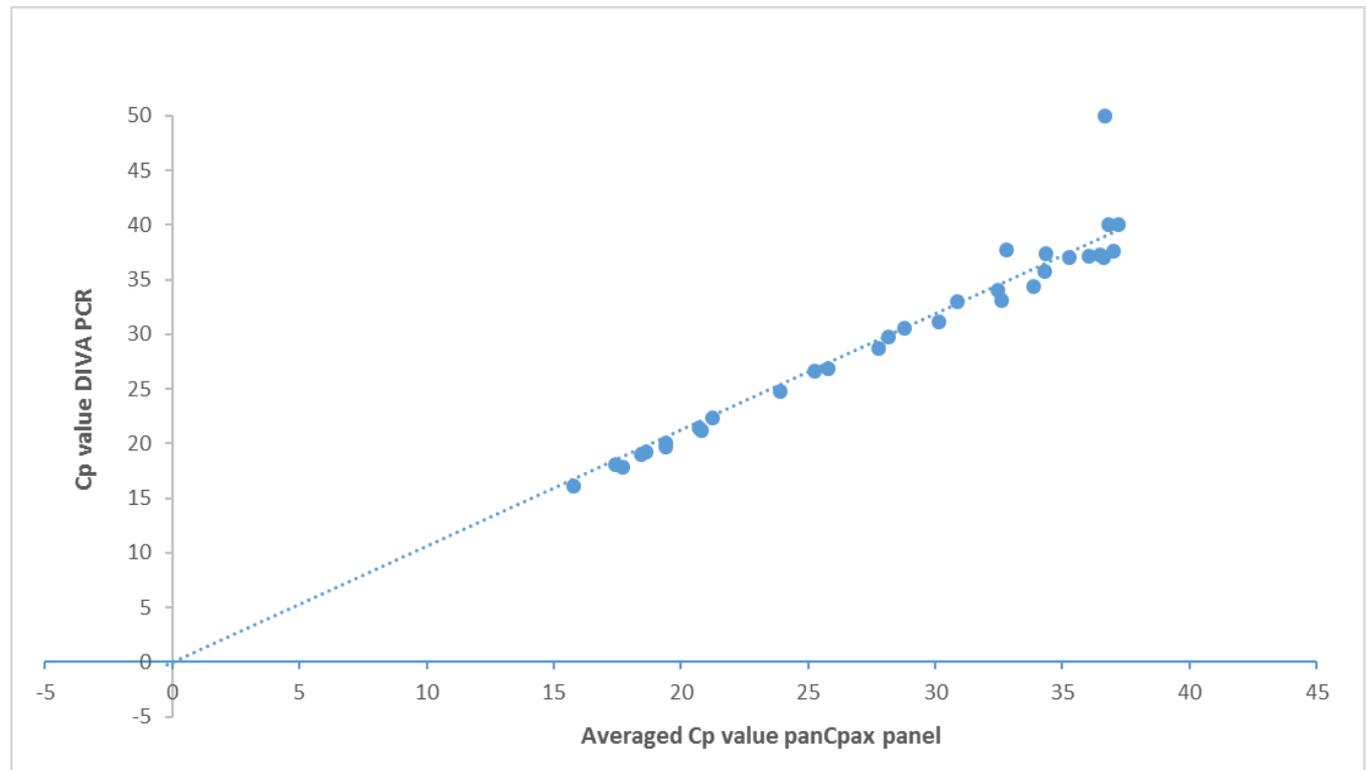
PanPCR positive blood samples, biopsies and organ/tissue samples can be used for the evaluation of the DIVA real-time PCR



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)



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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 ($C_p > 35$) 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 Athropods



Tsviatko Alexandrov .

Sohier C.*, Haegeman A.*, Mostin L., De Leeuw I., Van Campe W.,
De Vleeschauer A., De Regge N., De Clercq K.

Method: Set up in vivo experiment 1

Exp 1

4 Donor animals

D1 D2 D3 D4

8 Acceptor animals

<i>Dermacentor reticulatus</i>				<i>Stomoxys calcitrans</i>			
Fed on donor animals				Fed on donor animals			
A1	A2	A3	A4	A5	A6	A7	A8

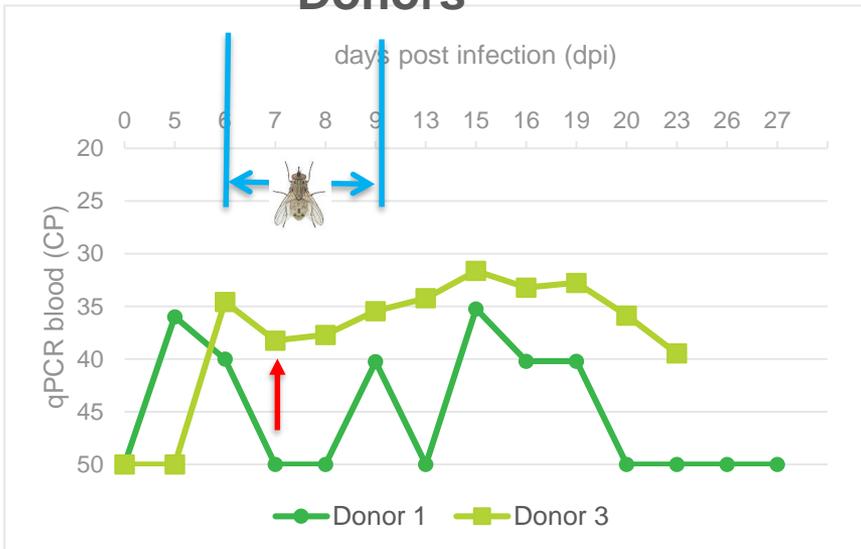
100 ♂ +100 ♀ ticks/
cotton bag on ears Donor
for 5-9 days from 5 dpi
=> On Acceptor for 5-7 days

• flies in cages on viremic donor (10 min/day) from
6-9 dpi => 100-200 flies/acceptor from 6-9 dpi (10
min/day)

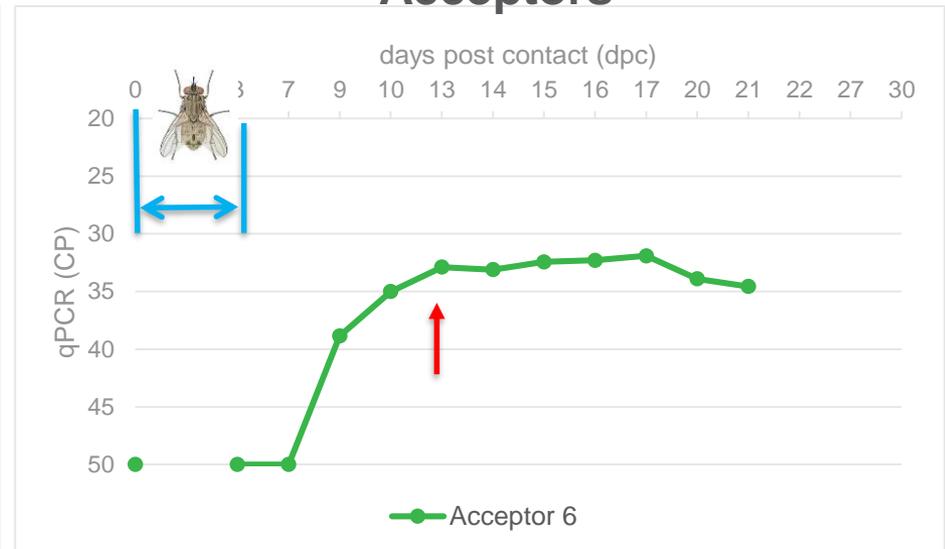


Results: In vivo experiment 1 with *S. calcitrans*

Donors



Acceptors



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

- 1 of 4 acceptors with *S. calcitrans* 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

Exp 2



5 Donor animals				
D5	D6	D7	D8	D9



6 Acceptor animals

<i>Haematopota sp.</i>		<i>Stomoxys calcitrans</i>		
Fed on donor animals		Fed on donor animals		
A13	A16	A14	A15	A17
			A18	

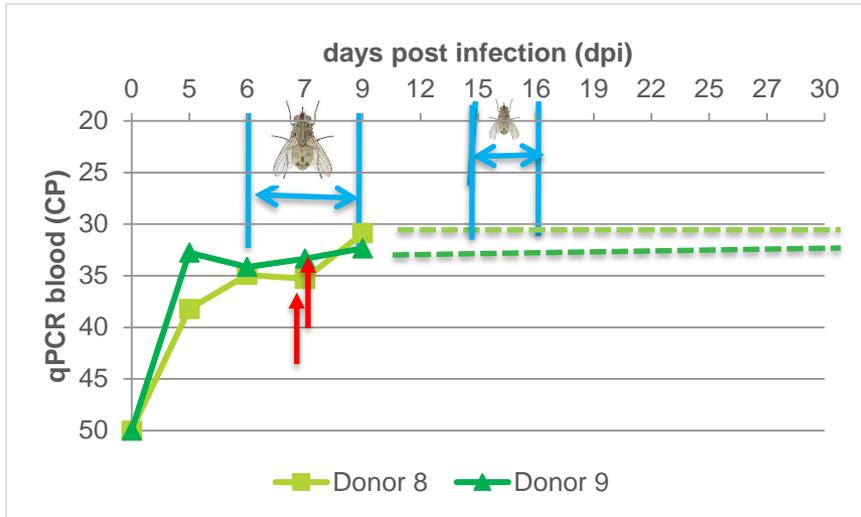
- Horse flies on viremic donor & acceptor from 7-9 dpi (10 min/day)
- 40 *Haematopota sp.*/on each acceptor

- *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

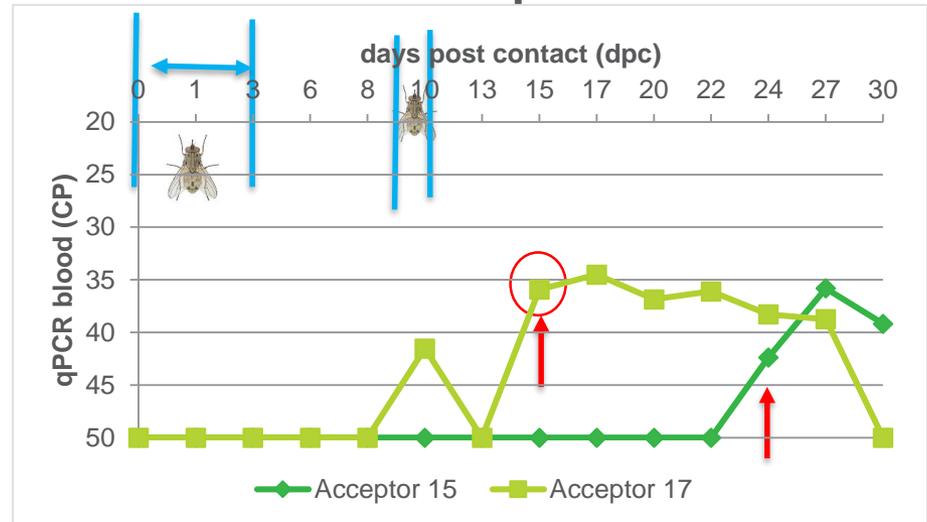


Results: In vivo experiment 2 with *Stomoxys calcitrans*

Donors



Acceptors



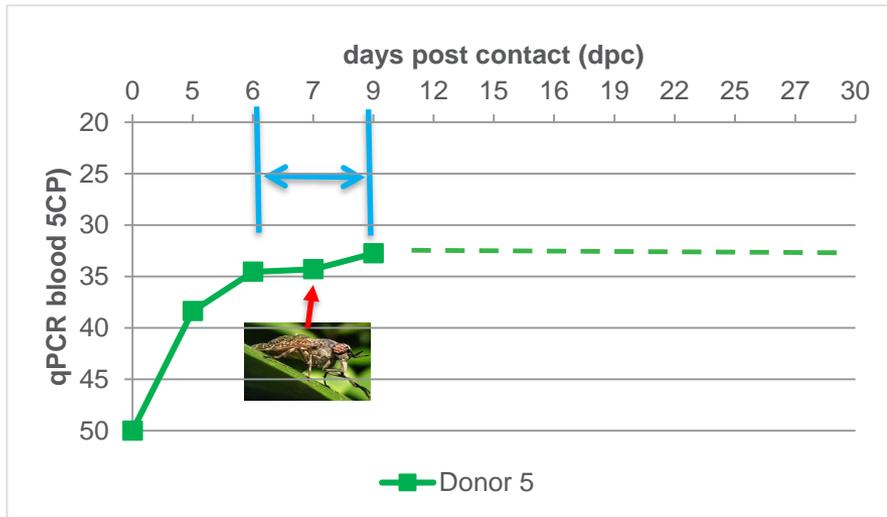
- 3 of 5 donor animals viremic,
- only D8 and D9 used for *Stomoxys calcitrans*
- Both viremic on 5 dpi
- Noduli: D8 on 8 dpi, D9 on 7 dpi
- Results still in progress (PCR blood)

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

Re-confirmation of transmission of LSDV with *S. calcitrans*

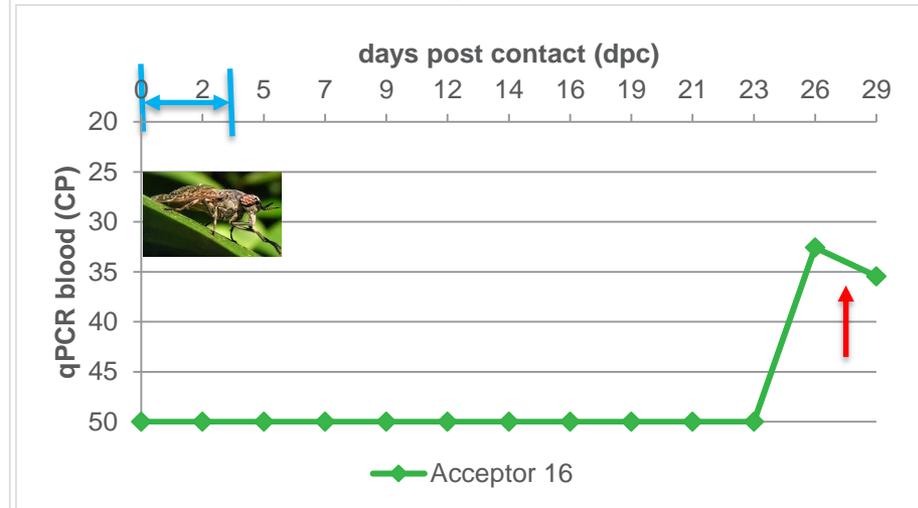
Results: In vivo experiment 2 with *Haematopota sp.*

Donors



- 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)

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!

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