GF-TADs
GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

FAO OIE

Standing Group of Experts on Lumpy Skin Disease in Europe

under the GF-TADs umbrella

First meeting (LSD1)
Brussels, Belgium, 4-5 July 2016

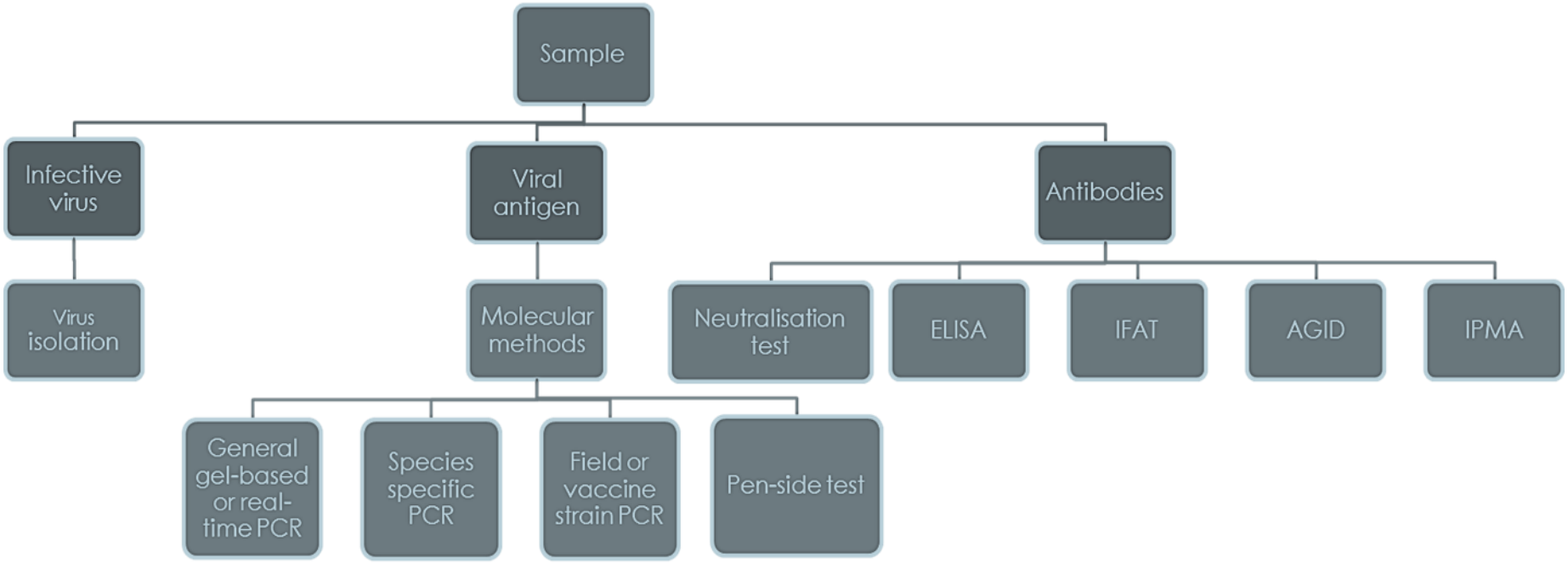
Diagnostic methods for Lumpy skin disease virus

LSD Expert: Dr Eeva Tuppurainen

General characteristics of LSDV affecting laboratory diagnostics

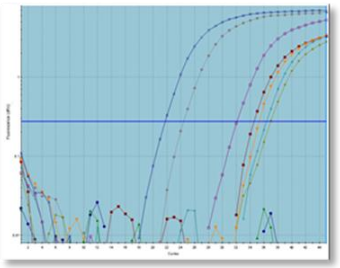
- Capripoxvirus genus comprises lumpy skin disease virus (LSDV), sheeppox virus (SPPV) and goatpox virus (GTPV) which are closely related but phylogenetically distinct
- Large (294±20 nm and 262±2 nm), double-stranded DNA virus
- Stable genome (151 kbp, 156 genes) - Nucleotide changes cannot be used for example tracing farm-to-farm spread of the virus
- Virus can be with or without an envelope - both are infectious but antigenically different
- Sensitive to most disinfectants

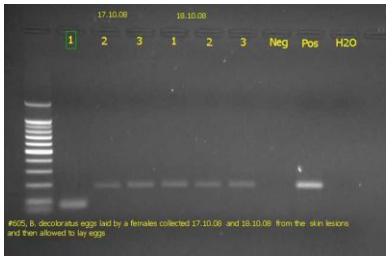
Detection of the antigen/infectious virus and antibodies against LSDV



General Capripoxvirus gel-based and real-time PCR methods

- Widely used basic methods
- Several conventional and real-time PCR methods have been validated
- Real-time PCR is faster, more sensitive and less prone to contamination
- A gel-based PCR is described in the OIE manual LSD chapter (Ireland and Binepal 1998, Tuppurainen *et al* 2005)
- Bowden *et al* 2008 assay was validated by Stubbs *et al* 2010
- Haegeman *et al* 2013
- Gel-based PCR is reliable sensitive and still a good back-up method in every lab

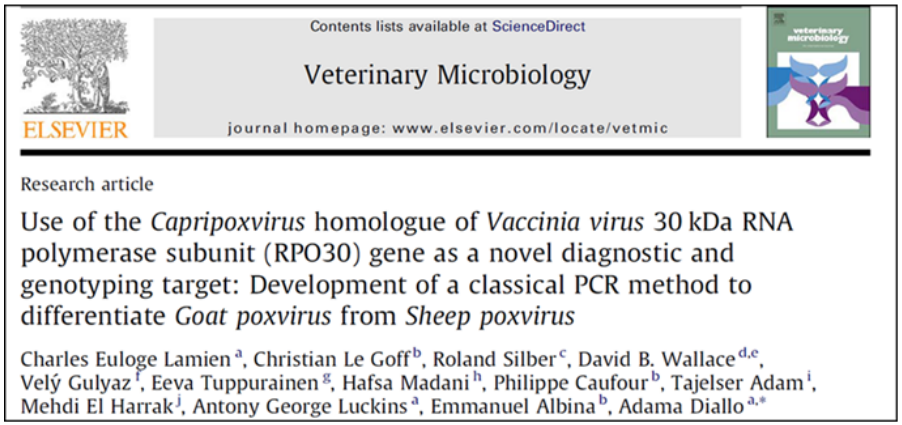




Multiplex PCRs detecting CaPV simultaneously with other viruses

- One-step multiplex real-time qPCR assay
- For simultaneous detection of peste des petits ruminants (PPR) virus, CaPV, Pasteurella multocida and Mycoplasma capricolum subspecies (ssp.) capripneumoniae (Settypalli *et al* 2016)
- Multiplex PCR and TagMan based duplex real-time PCR for detecting ORF and SPPV and GTPV (Venkatesan *et al* 2014 a,b)

Species-specific molecular assays - A gel-based method - differentiates SPPV from LSDV/GTPV – useful when SPPV vaccine used in cattle against LSDV



Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Research article

Use of the *Capripoxvirus* homologue of *Vaccinia virus* 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: Development of a classical PCR method to differentiate *Goat poxvirus* from *Sheep poxvirus*

Charles Euloge Lamien [a], Christian Le Goff [b], Roland Silber [c], David B. Wallace [d,e], Velý Gulyaz [f], Eeva Tuppurainen [g], Hafsa Madani [h], Philippe Caufour [b], Tajelser Adam [i], Mehdi El Harrak [j], Antony George Luckins [a], Emmanuel Albina [b], Adama Diallo [a,*]

Step 1 (1X):	95^{0}C for 4min
Step 2 (40 cycles):	95^{0}C for 30sec, 55^{0}C for 30sec and 72^{0}C for 30sec
Step 3 (1X):	72^{0}C for 7min
Step 4:	Keep at 4^{0}C

	Volume for one tube(ul)	Final concentration
IQ supermix (BioRad)	10	
Primer SpGpRNApol F (5 pmole/ul)	2	500nM
Primer SpGpRNAPol R (5 pmole/ul)	2	500nM
Water	4	
DNA	2	
Total	**20**	

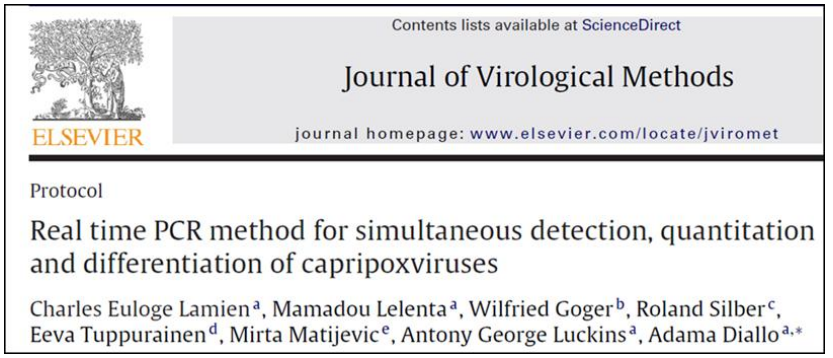
Primer SpGpRNApol F (5 pmole/ul)	5'-TCTATGTCTTGATATGTGGTGGTAG-3'
Primer SpGpRNAPol R (5 pmole/ul)	5'-AGTGATTAGGTGGTGTATTATTTTCC-3'

Size of the amplicon

LSDV /GTPV	172bp
SPPV	151bp

Fluorescence Resonance Energy Transfer (FRET) real-time PCR

- Targets the G-Protein Coupled Chemokine Receptor (GPCR)–gene
- By using Fluorescence Melting Curve Analysis (FMCA) the assay detects different melting point temperatures of LSDV, SPPV and GTPV
- Requires a real-time PCR machine which can accommodate the FRET technology
- FRET channel: FAM excitation/Cy5 emission


Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

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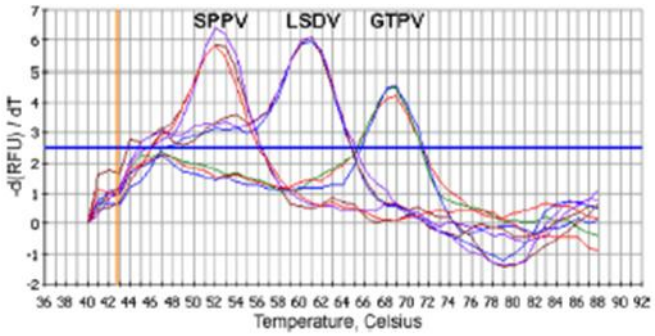
Protocol

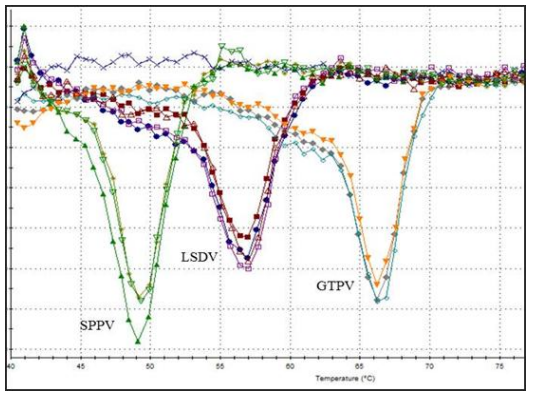
Real time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses

Charles Euloge Lamien[a], Mamadou Lelenta[a], Wilfried Goger[b], Roland Silber[c], Eeva Tuppurainen[d], Mirta Matijevic[e], Antony George Luckins[a], Adama Diallo[a,*]



Inverted species-specific FRET assay

- Allows differentiation using the normal PCR channels (Cy5 -channel)
- Uses the same primers
- Slightly altered labelling of the donor probe
- Publication shortly
- Contact info Dr Charles Lamien, IAEA (C.Lamien@iaea.org)



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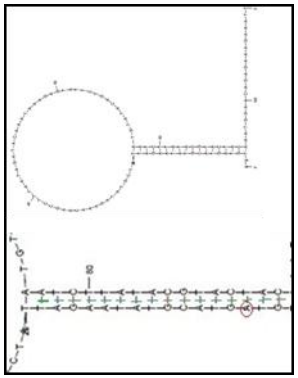
PLOS ONE

Development of a Cost-Effective Method for Capripoxvirus Genotyping Using Snapback Primer and dsDNA Intercalating Dye

Esayas Gelaye[1,5,6], Charles Euloge Lamien[1]*, Roland Silber[2], Eeva S. M. Tuppurainen[3], Reingard Grabherr[4], Adama Diallo[1]



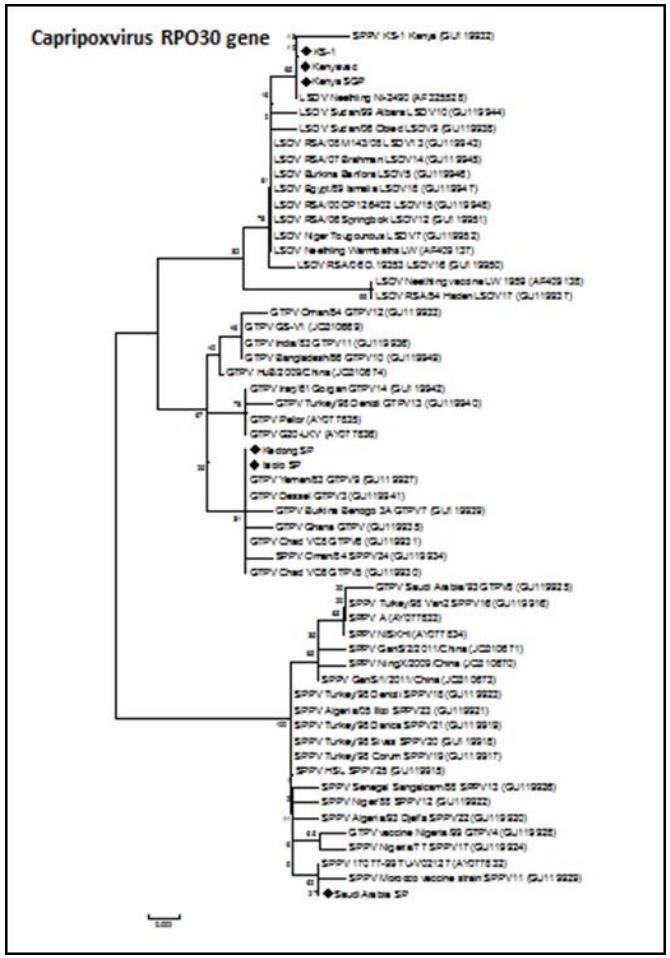
- Targets a 96bp fragment of the RPO30 –gene
- Snapback tail added to the 5’ end of the forward primer: 100% match with GTPV, T:A mismatch with SPPV and T:G with LSDV
- Snapback primer serves as primer and a probe
- Intercalating dye (EvaGreen) between double stranded DNA bonds which is released when the bonds break during melting
- Two melting peaks: one for the stem and one for the amplicon
  High Resolution Melting points and Fluorescent melting curve analysis (FMCA)

Molecular DIVA assays differentiating the vaccine from the field strain

- Needed in case clinical signs are detected in vaccinated herds
- The first assay based on the detection of a 27 nucleotide difference in the extracellular enveloped virion -gene between virulent and attenuated LSDV has been published (Menasherow *et al* 2014)
- High-resolution melting (HRM) assay for LSDV (Menasherow *et al* 2015)
- Gel-based and real-time PCR methods for SPPV (Haegeman *et al* 2015)
- Commercial DIVA PCR kit for LSDV currently available

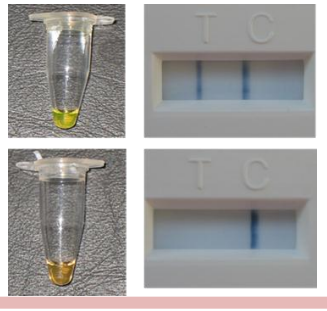
Sequencing tool for phylogenetic studies

- Phylogenetic grouping based on host range genes
- G-protein coupled chemokine receptor
- RNA polymerase subunit (RPO30) (Gelaye *et al* 2015)
- Not expensive by commercial companies
- Data analysing requires highly specific training
- Therefore not suitable for routine screening in many laboratories



LAMP assays for LSDV

- Two basic LAMP assays for the detection of CaPV in general by Murray *et al* 2013 and Das *et al* 2013
- Two species-specific for SPPV and GTPV by Zhao *et al* 2014 and Venkatesan *et al* 2016
- Fast method as isothermal amplification takes less than an hour
- Need to overcome a problem how to do the extraction of the samples in the field settings
- Result are based on cloudiness or colour changes which may complicate interpretation
- Great potential to develop the method into lateral flow device (LFD) pen-side assay



Pen-side tests

- The first PCR method suitable for portable thermocycler has been described by Armson *et al* 2015
- Detects LSDV, SPPV and GTPV
- Validated real-time PCR method by Bowden *et al* (2008) was slightly modified and set up for the machine
- Easy to use in challenging environmental conditions, car cigarette lighter used as a power source
- Freeze dried reagents – no cold-chain required
- Easy sample collection: blood in EDTA, scabs from skin lesions, saliva, eye and nasal discharge
- No separate DNA extraction required

Serology

- Immunity against LSDV is predominantly cell-based but also humoral
- Infected animals develop different kind of antibodies depending on the stage of infection
- Antibodies against LSDV can usually be detected approximately 6 months post outbreak then eventually decreasing below detectable levels
- All serological tests available are for the CaPV group diagnosis (serum/virus neutralization, fluorescent antibody, indirect fluorescent antibody or agar gel immunodiffusion tests)
- None of them is optimal for use as a primary assay nor for testing large numbers of samples

Serology

- Neutralization test (gold standard) is reliable and in slightly modified format it can be used also for serological surveys (by using only two lowest dilutions of the serum)
- LSDV labelled with fluorescence marker can be used in SNT reducing the time required to read SNT
- Indirect ELISAs based on killed whole virus (Babiuk et al 2009) recombinant antigens (Bowden et al 2009) or synthetic peptides (Bhanot et al 2009; Tian et al 2010) have been developed
- AGID (cross-reacts with parapox) and IFAT (background staining, requires careful optimization and validation)

Novel ELISA

- Evaluation of the performance of a promising ELISA (Bowden et al., CSIRO, Australia) has been carried out using a large number of serum samples
- Detects antibodies approximately three months post-infection
- Performs well on herd/flock level
- Sensitivity is clearly better than SNT
- Vaccinated animals and individuals with mild disease show low antibody levels may not be detected

Thank you for your attention!

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