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EU Reference Laboratory
for Capripox viruses

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OIE
Collaborating Centre

LUMPY SKIN DISEASE
DIAGNOSTIC TOOLS

Laetitia Aerts, Andy Haegeman, Ilse De Leeuw
Laurent Mostin, Charlotte Sohier,
Elisabeth Mathijs, Steven Van Borm
Kris De Clercq

9th meeting of the Standing Group of Experts on Lumpy Skin Disease in South-East Europe
under the GF-TADs umbrella (SGE LSD 9)
Athens, Greece, 16-17 October 2019.

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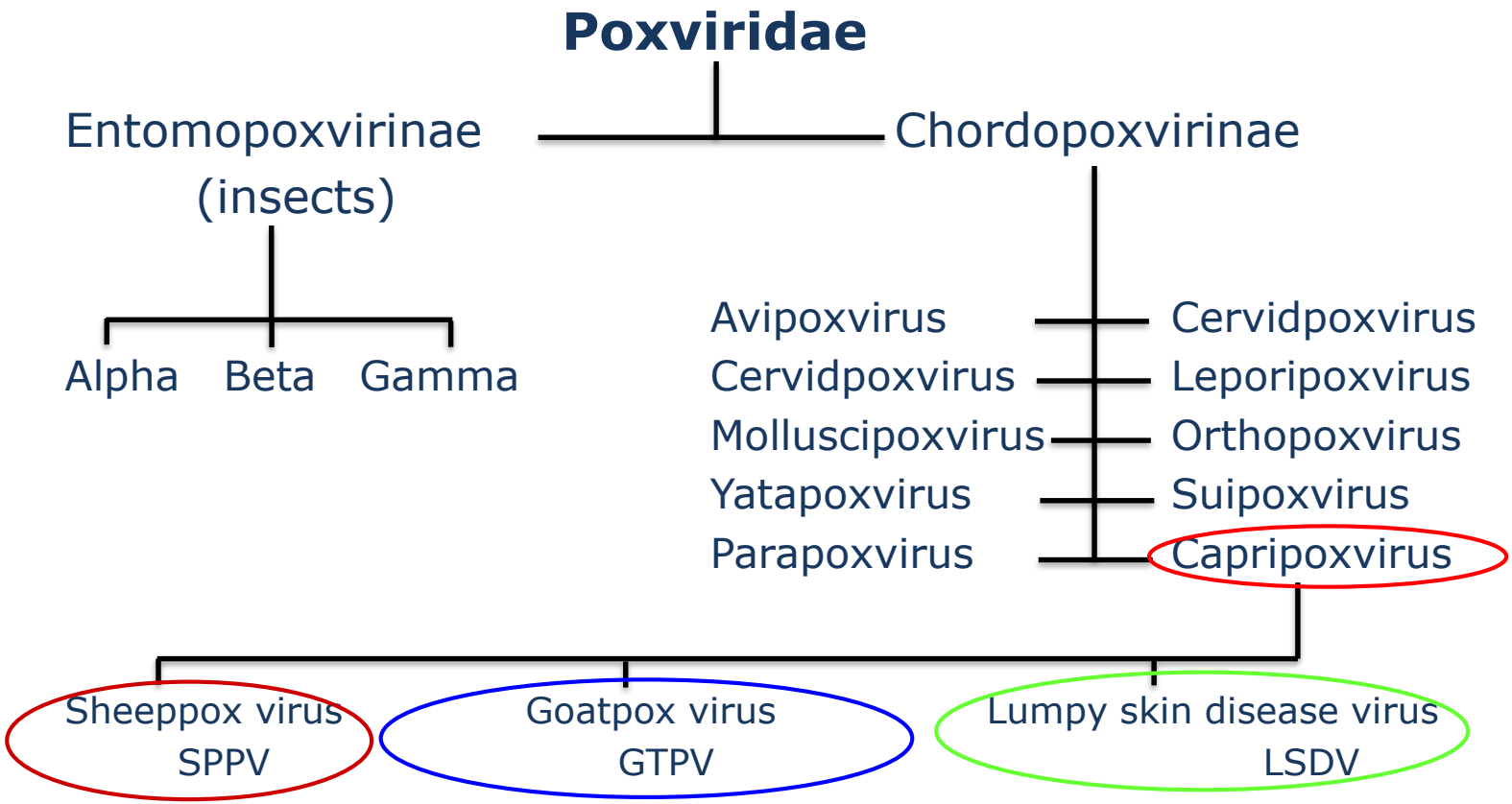
- Capripox
  - Classification



Clinical diagnosis

Passive clinical surveillance

Active clinical surveillance

- Design: population – epidemiological unit – sampling unit
- Sampling method: representative of the populations (probability based)
- Sample size:
  - ~expected prevalence,
  - ~level of confidence desired
  - ~performance of the tests used

General Capripoxvirus real-time PCR methods

- Real-time PCR is faster, more sensitive and less prone to contamination. The assay of Bowden *et al* 2008 was validated by Stubbs *et al* 2012
- Haegeman *et al* 2013 described 3 different Real-time PCRs (for diagnosis and confirmation) each with an internal and external quality control

ELSEVIER

Virology 371 (2008) 380–393

www.elsevier.com/locate/yviro

Capripoxvirus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats

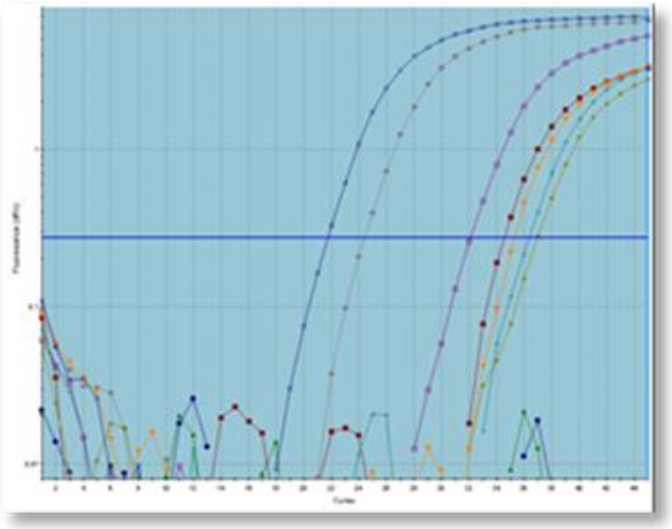
Timothy R. Bowden [a,*], Shawn L. Babiuk [b,c], Geoff R. Parkyn [b], John S. Copps [b], David B. Boyle [a]

Journal of Virological Methods 193 (2013) 446–451

Contents lists available at ScienceDirect

Journal of Virological Methods

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



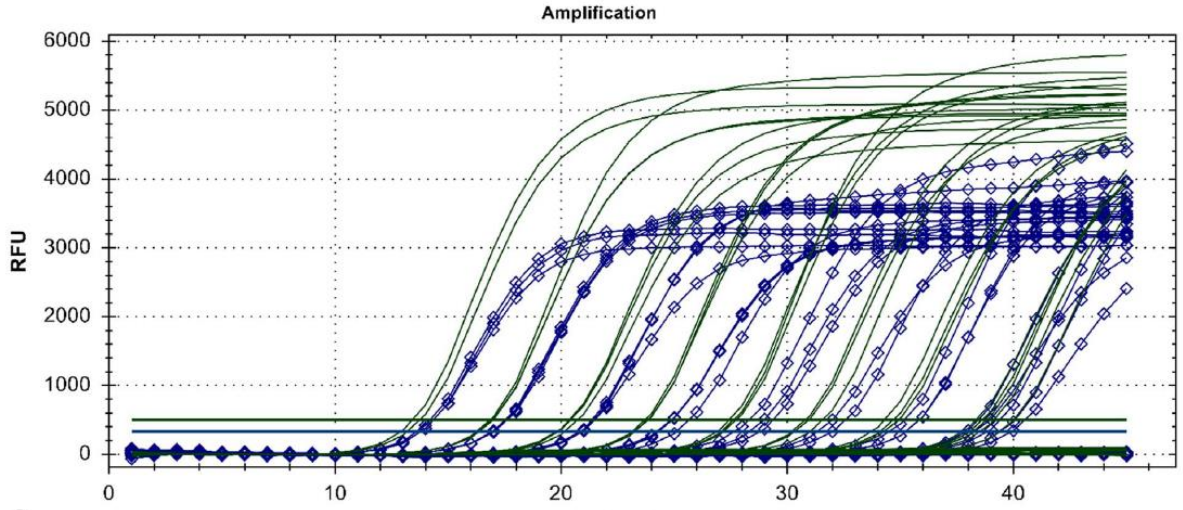
Development and validation of three Capripoxvirus real-time PCRs for parallel testing

A. Haegeman [a,*], K. Zro [b,c], F. Vandenbussche [d], L. Demeestere [a], W. Van Campe [e], M.M. Ennaji [b], K. De Clercq [a]

- Commercial real-time PCR kits for LSDV currently available

Molecular DIVA assays differentiating the LSD vaccine from the field strain

- A duplex quantitative real-time PCR: targeting the GPCR
- Simultaneous detection and differentiation of wild type and vaccine LSDV strains
- Based on a 12 bp deletion exists in all field LSDV strains, compared to SPPV, GTPV and the LSDV vaccine strains.



Amplification plots of two dilution series of linearized plasmid DNA copies of WT LSDV (FAM, blue, ◊) and vaccine LSDV (HEX, green). Ten-fold serial dilutions of each linearized plasmid, representing 10^8 to 10^1 LSDV DNA copies per reaction in three replicates

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Journal of Virological Methods

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

Development and validation of a TaqMan probe-based real-time PCR method for the differentiation of wild type lumpy skin disease virus from vaccine virus strains

Eirini I. Agianniotaki[a,b], Serafeim C. Chaintoutis[a], Andy Haegeman[c], Konstantia E. Tasioudi[b], Ilse De Leeuw[c], Panagiotis-Dimitrios Katsoulos[d], Achilleas Sachpatzidis[e], Kris De Clercq[c], Thomas Alexandropoulos[f], Zoe S. Polizopoulou[a], Eleni D. Chondrokouki[b], Chrysostomos I. Dovas[a,*]

- Commercial DIVA real-time PCR kits for LSDV currently available

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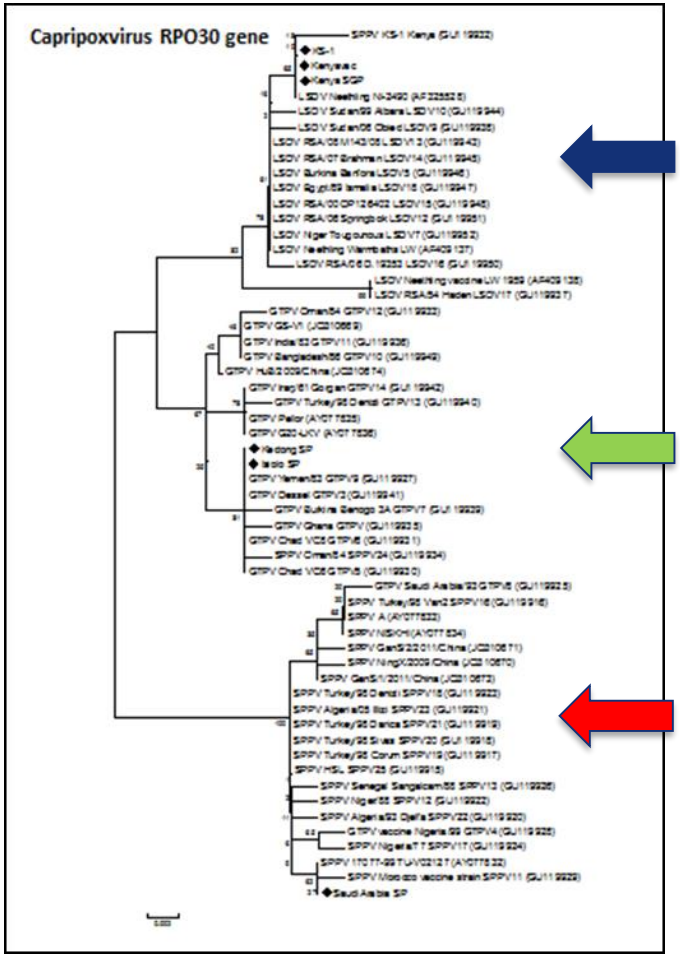
Sequencing

- Phylogenetic grouping based on host range genes
  - RNA polymerase subunit (RPO30) (Gelaye et al 2015)
  - G-protein coupled chemokine receptor (GPCR gene) (Le Goff et al., 2009)
  - LSDV126 putative extracellular enveloped virus (EEV) and LSDV127 hypothetical glycoprotein genes (Menasherow et al., 2014)

- Full Genome Sequencing
  - Full genomes are required for molecular tracing but due to the limited variation (and the small number of WGS reference data available) this may only be possible on a large scale distance-wise and time-wise.
  - Full genome sequences should be highly accurate (low variation: weight of 1 wrong mutation is very high)



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Serology: VNT and ELISA

- 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)

- Commercial available ELISA
  - Evaluation of the performance of a novel ELISA (ID-Vet) has been carried out using a large number of serum samples
  - Detects antibodies approximately five months post-vaccination
  - Performs well on herd/flock level
  - Sensitivity is clearly better than VNT
  - Vaccinated animals and individuals with mild disease show low antibody levels may not be detected

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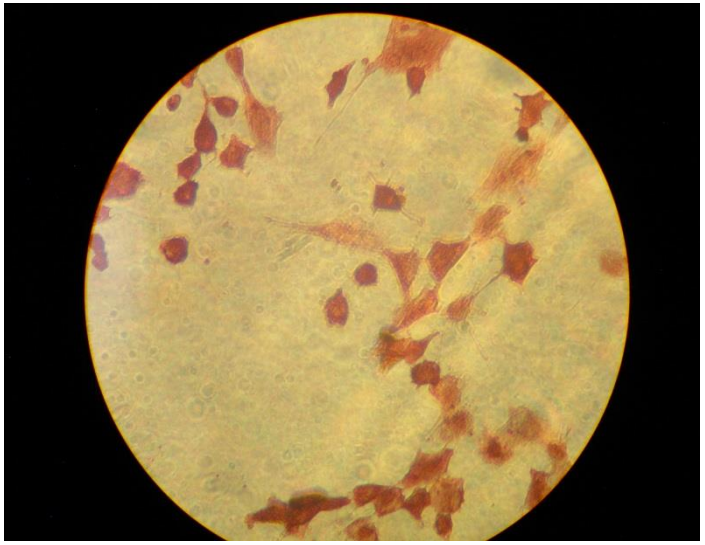
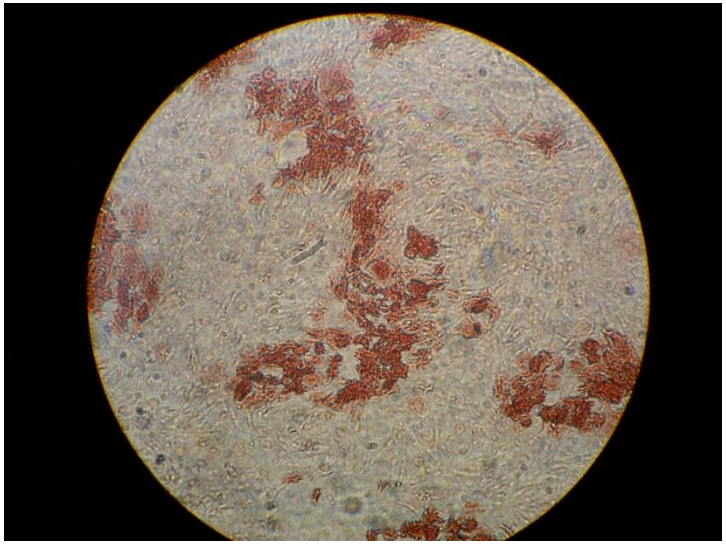
Serology: IPMA
Immuno peroxydase monolayer assay

- In house IPMA (Sciensano)
  - OAT cells + IPMA staining





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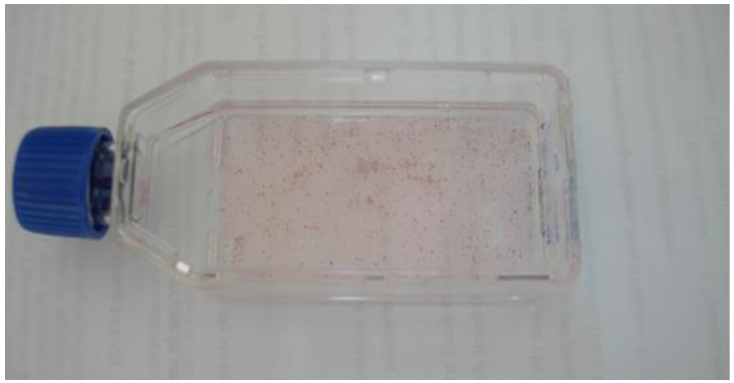
Detection infectious virus: Virus isolation

- Lamb testis or Bovine dermis cells: primary and secondary
- Cytopathic effect (3–14 days) and intracytoplasmic inclusion bodies
- Ovine testis secondary cell line (OA3.Ts) (Babiuk et al., 2007)
- Virus Titration
- The antigen of capripoxvirus can be demonstrated in tissue culture using immunoperoxidase or immunofluorescent staining



OAT cells + IPMA staining

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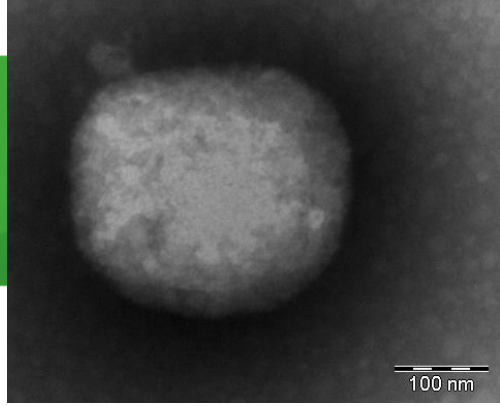
Detection virus: Electron Microscopy



Brick-Like Structure Typical of Poxviridae Viruses

- Demonstration of typical capripox virions in biopsy material or desiccated crusts using the transmission electron microscope
- Capripoxvirus is distinct from parapoxvirus, which causes bovine papular stomatitis and pseudocowpox
- Cannot be distinguished morphologically from cowpox and vaccinia virus, both orthopoxvirus infections of cattle

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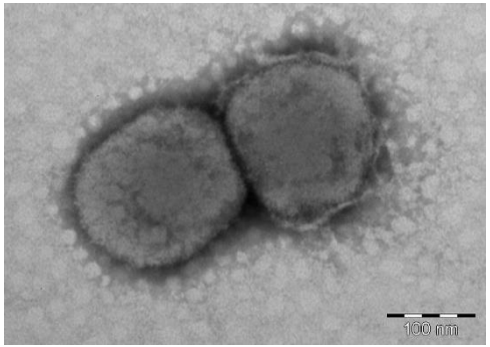

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EM Photo courtesy Dr Jan Mast

EU Reference Laboratory for Peste des Petits Ruminants

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toute une vie en bonne santé

COMBINED

PESTE DES PETITS RUMINANTS / CAPRIPOX VIRUS

NATIONAL REFERENCE LABORATORIES

WORKSHOP

Capripox Proficiency Testing 2019

Brussels, Belgium 3 October 2019

Thank you for your attention!

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