DIAGNOSTIC TOOLS TO DETECT CAPRIPOXVIRUS INFECTIONS AND DIVA STRATEGIES

Charles Euloge LAMIEN
Animal Production and Health Laboratory, Joint FAO/IAEA Division

ANIMAL PRODUCTION AND HEALTH SECTION JOINT FAO/IAEA DIVISION

Our Mandate is to Assist MS to improve livestock productivity through:

- Efficient use of locally available feed resources,
- Adequate management practices and efficient reproductive / breeding programmes,
- Development of proactive disease prevention and control measures

using nuclear and nuclear-related technologies

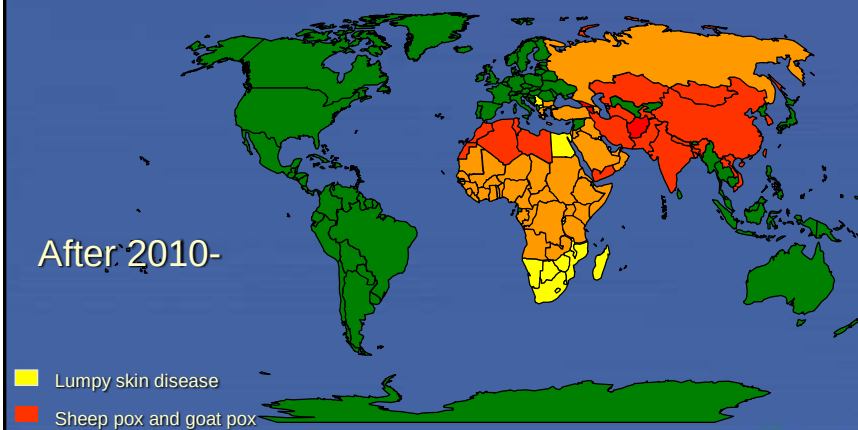
Examples: Irradiated vaccines / stable isotopes / early and rapid detection platforms

CAPRIPOXVIRUSES: NOMENCLATURE

- Nomenclature based on the host of origin
- Several reports indicate the involvement of both sheep and goats in the same outbreak

IAEA
Joint FAO/IAEA Division

GEOGRAPHICAL DISTRIBUTION OF CAPRIPOXVIRUSES



IAEA
Joint FAO/IAEA Division

GTPV, SPPV AND LSDV DISPLAY GENETIC DIFFERENCES

Serology

- Infections cannot be distinguished clinically or serologically

Experimental

- Some strains recovered from sheep can infect goats and some goat isolates can infect sheep and produce severe disease
- Experimentally each strain can cross infect all three species

Genetic

Comparison of their genomes show the existence of 3 genetically distinct species:

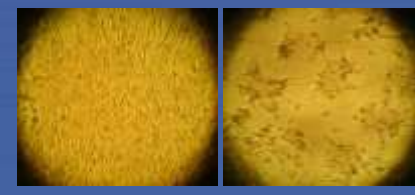
- SPPV
- GTPV
- LSDV

SPECIMENS FOR THE LABORATORY

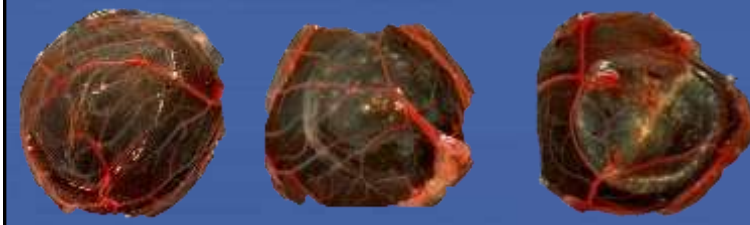
- Skin biopsies, swab samples for virus isolation, histopathology, and electron microscopy and molecular detection.
- Samples shipment: ice (within 2 days), or dry ice if a longer shipment time is required.
- Serum samples for serology: from acute and chronic cases and 2 to 3 weeks after the first appearance of skin lesions.

VIRUS ISOLATION AND PROPAGATION

- **Primary cells from bovine/ovine origin**
  - Foetal bovine testicle or Lamb testicle, foetal bovine kidney or lamb kidney, foetal bovine dermis cell cultures
- **Cell lines:**
  - VERO, MDBK, ESH-L, OA3.Ts
- **Chorioallantoic membrane (CAM)**



infected Vero cell



Non Infected nLSDV Herbivac

IAEA

Joint FAO/IAEA Division

VIRUS ISOLATION AND PROPAGATION

Comparison of some primary cells (Binepal, 2001, OVRJ)

TABLE 2 Titration of viruses carried out in the various cell lines (average of three titrations)

	FBS	FBM	FBK	BT	LT
KS-1	10^{-4}	10^{-3}	10^{-1}	10^{-1}	10^{-5}
2490	$10^{-2.5}$	10^{-3}	$10^{-1.5}$	10^{-1}	10^{-3}
O40	10^{-2}	10^{-2}	Foci	$10^{-0.5}$	10^{-3}
O181	10^{-3}	10^{-4}	Foci	10^{-1}	10^{-3}
Neoth.	10^{-2}	10^{-4}	DNG	10^{-1}	10^{-4}
CPV/RPV	10^{-3}	10^{-4}	10^{-2}	10^{-1}	10^{-4}
Kedong	10^{-3}	$10^{-2.5}$	DNG	10^{-1}	10^{-4}
Isiolo	$10^{-2.5}$	$10^{-1.5}$	DNG	Foci	10^{-3}
F1	$10^{-2.5}$	10^{-3}	DNG	DNG	$10^{-3.5}$
199	10^{-3}	10^{-3}	DNG	DNG	10^{-3}
275	10^{-1}	10^{-1}	DNG	DNG	10^{-3}
257	10^{-3}	10^{-3}	Foci	DNG	$10^{-3.5}$
G38	10^{-3}	$10^{-1.5}$	Foci	DNG	10^{-3}
B156	10^{-1}	$10^{-1.5}$	DNG	DNG	10^{-3}

TABLE 3 Primary isolation of LSD virus using three different cell lines (CPE at 80 %)

Sample no.	LT	FBS	FBM
199	1st passage—6 days	1st passage—8 days	2nd passage—5 days
257	1st passage—6 days	1st passage—6 days	1st passage—8 days
275	1st passage—5 days	1st passage—6 days	2nd passage—4 days

SEROLOGY

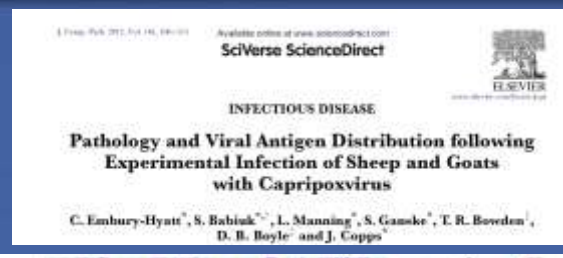
- Virus neutralization
- Indirect fluorescent antibody test (Gari et al 2008)
- Capripox antibody ELISA (not currently recommended by OIE)

Few assays under investigation (Heine et al., 1999; Tian et al., 2010; Babiuk et al., 2009; Bowden et al., 2009;)

- Western blot
- Agar gel immunodiffusion tests: not very sensitive (Mangana-Vougiouka, 2000)

Limitation: not optimal for large scale screening

HISTOPATHOLOGY


SciVerse ScienceDirect

INFECTIOUS DISEASE

Pathology and Viral Antigen Distribution following Experimental Infection of Sheep and Goats with Capripoxvirus

C. Embury-Hyatt, S. Babiuk, L. Manning, S. Ganske, T. R. Bowden, D. B. Boyle and J. Copps

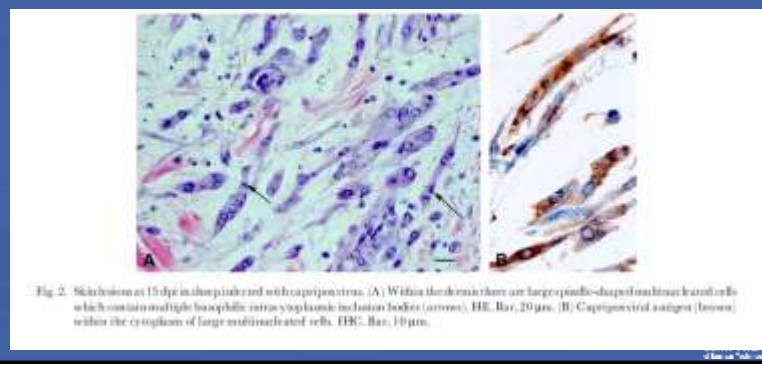


Fig. 2. Skin lesion at 15 dpi in sheep infected with capripoxvirus. (A) Within the dermis there are large spindle-shaped multinucleated cells which contain multiple basophilic intracytoplasmic inclusion bodies (arrows). HE. Bar, 20 μm. (B) Capripoxviral antigen (brown) within the cytoplasm of large multinucleated cells. IHC. Bar, 10 μm.

MOLECULAR DIAGNOSIS : GENERAL CAPRIPOXVIRUS DETECTION METHODS

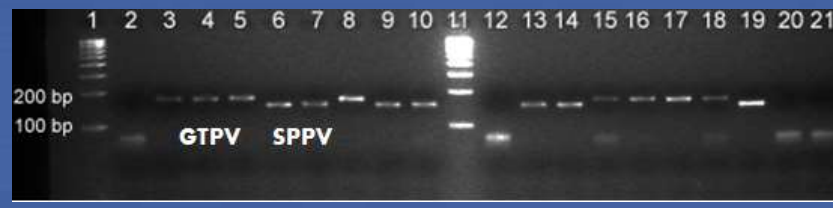
- A gel-based PCR is described in the OIE manual LSD chapter (Ireland and Binepal 1998, Tuppurainen et al 2005)
- Real time PCR: Balinsky et al., 2008; Bowden et al 2008, validated by Stubbs et al 2010;, Haegeman et al 2013.
- LAMP PCR: Murray et al 2013 and Das et al 2013.
- Field-Ready Nucleic Acid Extraction and Real-Time PCR Platform (Amson, 2015).
- Only LSDV based detection (Stram, 2008)

MOLECULAR DIAGNOSIS : GENERAL CAPRIPOXVIRUS DETECTION METHODS

Commercial Kits:

- Techne: with and internal control (IC)
- Genesig® Standard Kit
- Genesig® Advance Kit (LSDV116 RNA polymerase subunit), IC
- Tetracore
- Biosellal

MOLECULAR DIAGNOSIS: CAPRIPOXVIRUS SPECIES DIFFERENTIATION

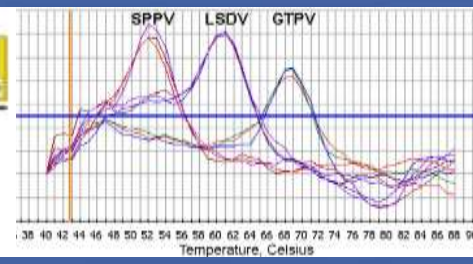


Classical PCR:
Can differentiate sheep poxvirus from goat poxvirus

IAEA
Joint FAO/IAEA Division

MOLECULAR DIAGNOSIS: CAPRIPOXVIRUS SPECIES DIFFERENTIATION

DUAL HYBRIDIZATION PROBES ASSAY (FRET)



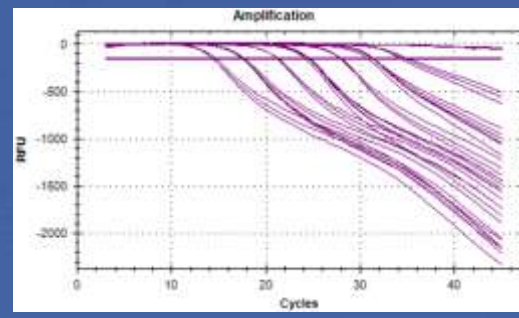
Compatibility
LC480
LC2
Rotor Gene 6000
iQ5

Differentiates all three capripoxviruses

IAEA
Joint FAO/IAEA Division

MOLECULAR DIAGNOSIS: CAPRIPOXVIRUS SPECIES DIFFERENTIATION

INVERTED DUAL HYBRIDIZATION PROBES ASSAY



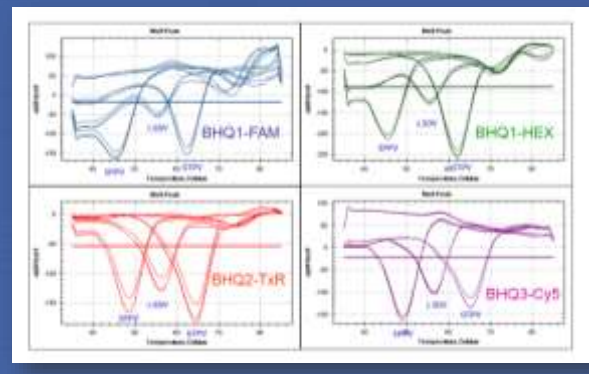
The accumulation of the PCR products results in a decrease of fluorescence.

mx3005p, CFX, Chromo4, miniopticon, iQ5, Rotor gene 6000, ABI 7500, ABI QuantStudio 6, LC480

IAEA
Joint FAO/IAEA Division

MOLECULAR DIAGNOSIS: CAPRIPOXVIRUS SPECIES DIFFERENTIATION

INVERTED DUAL HYBRIDIZATION PROBES ASSAY

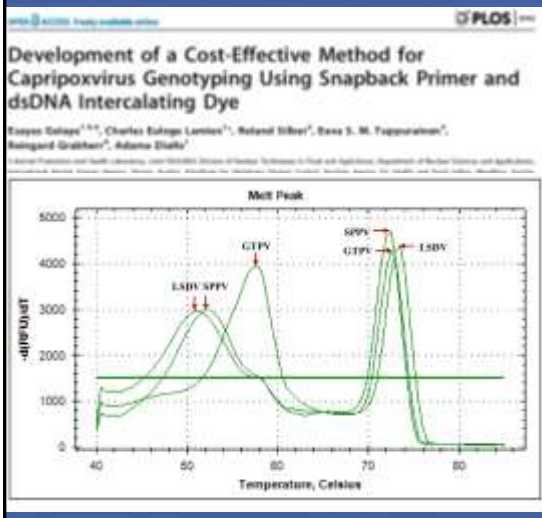


Tm values of the negative melting peaks obtained are used to determine CaPVs genotype.

Differentiates all three capripoxviruses

IAEA
Joint FAO/IAEA Division

MOLECULAR DIAGNOSIS: CAPRIPOXVIRUS SPECIES DIFFERENTIATION



Compatibility

- **CFX**
- **RotorGene 6000,**
- **LC480,**
- **ABI QuantStudio 6**

The melting of the probe element of the snapback hairpin provides targeted genotyping.

Differentiates all three capripoxviruses GTPV (58.00, 72.50), SPPV (52.50, 72.50) and LSDV (51.50, 73.50)

IAEA
Joint FAO/IAEA Division

DIFFERENTIAL DIAGNOSIS

LSD

- Pseudo lumpy skin disease
- Bovine papular stomatitis
- Pseudocowpox
- Vaccinia and Cowpox
- Dermatophilosis
- Insect or tick bites
- Hypoderma bovis infection
- Photosensitisation
- Cutaneous tuberculosis

Sheep pox and goat pox

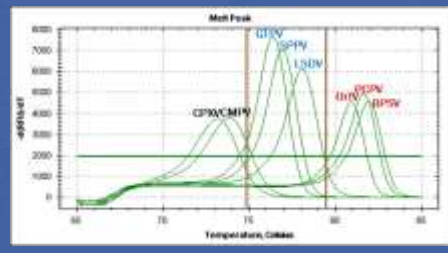
- Peste des petits ruminants
- Parasitic pneumonia
- Caseous lymphadenitis
- Insect bites
- Contagious exthyma
- Bluetongue
- Mycotic dermatitis
- Sheep scab
- Mange
- Photsensitization

IAEA
Joint FAO/IAEA Division

DIFFERENTIAL DIAGNOSIS

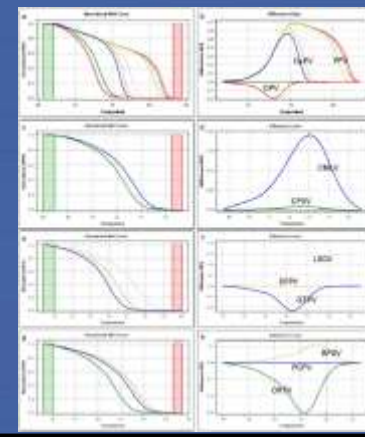

SCIENTIFIC REPORTS

OPEN A novel HRM assay for the simultaneous detection and differentiation of eight poxviruses of medical and veterinary importance

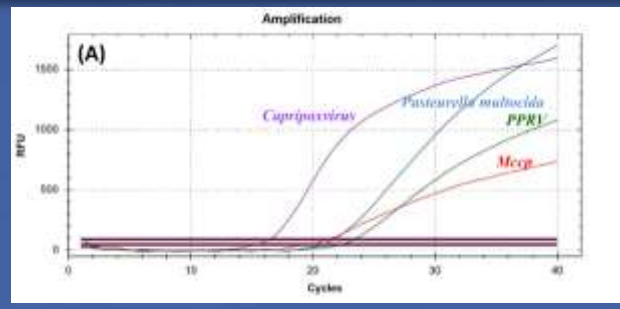


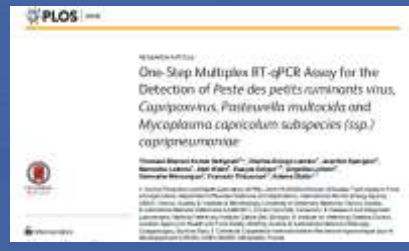
Compatibility

- **CFX**
- **RotorGene 6000,**
- **LC480,**
- **ABI QuantStudio 6**



DIFFERENTIAL DIAGNOSIS




PLOS

One-Step Multiplex RT-qPCR Assay for the Detection of *Peste des petits ruminants virus*, *Capripoxvirus*, *Pasteurella multocida* and *Mycoplasma capricolum subspecies (ssp.) capripneumoniae*

Assay targeting the main respiratory pathogens of small ruminants

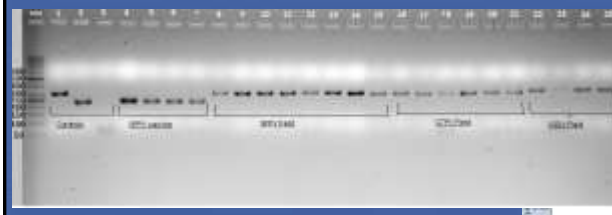
Joint FAO/IAEA Division

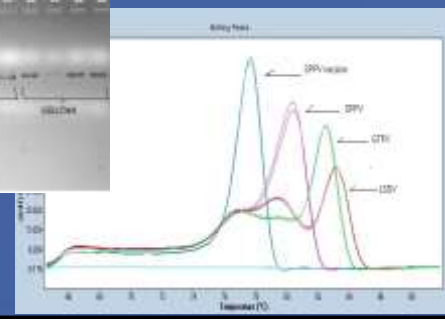
MOLECULAR DIVA

SPPV vaccine/SPPV Field

1: Haegeman et al., 2016 (real time PCR and gel based PCR to differentiate SPPV wild-type in Morocco from/SPPV vaccine

2: In preparation (IAEA) real time PCR and gel based to differentiate SPPV field isolates from SPPV vaccines

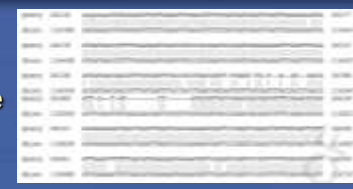


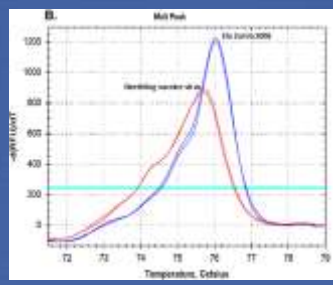


MOLECULAR DIVA

LSDV vaccine/LSDV Field

1. LSDV126 EEV gene: 27 nucleotides difference





Menasherow et al., 2014 gel based PCR
Israeli wild type versus neethling vaccine

Menasherow et al., 2016: qPCR, HRM based
Israeli wild type versus neethling vaccine

Dejan et al., 2016, qPCR, probes-based
European isolates versus Neethling

IAEA

MOLECULAR DIVA

LSDV vaccine/LSDV Field

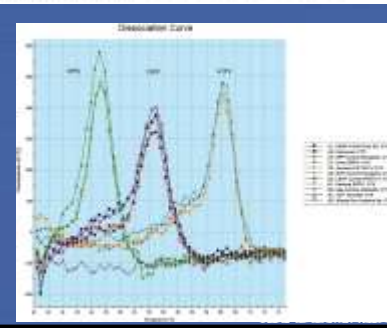
Gelaye et al., 2015: Sequencing based LSDV KS1 vaccine/ Ethiopian isolates
Works for LSDV Neethling vaccines
Versus European isolates

MOLECULAR DIVA

SPPV vaccine/LSDV Field

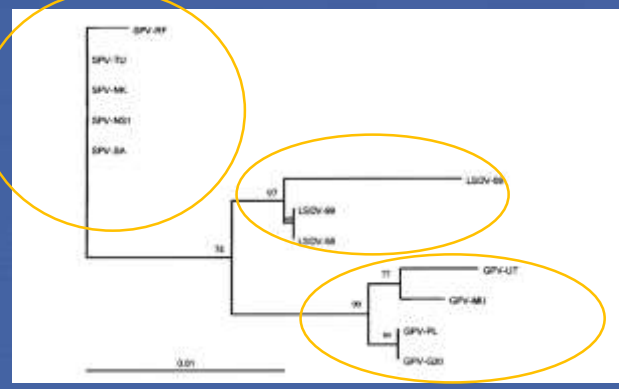
Tuppurainen et al., 2014:
qPCR for genotyping

Kenyavac (KSGP O-240) = LSDV.
The Jovivac RM65 strain = SPPV
Romanian strain in the Saudi Arabian Sheep Pox Vaccine = SPPV



MOLECULAR EPIDEMIOLOGY

Capripoxvirus P32 gene, Hosamani et al., 2004



Amplification of the fragment of about 1025 bp containing the full P32 gene
Size of the gene: 969 for GTPV and LSDV, 972 for SPPV
Gene sequence extracted for phylogeny

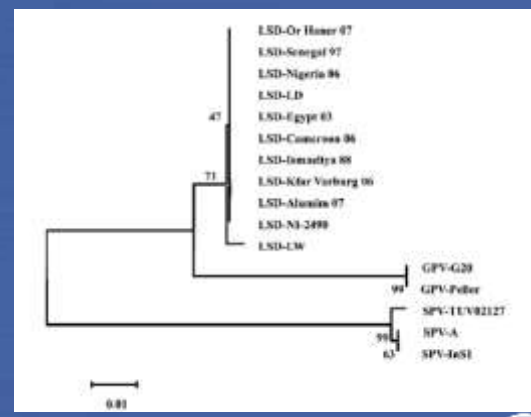
IAEA
Joint FAO/IAEA Division

MOLECULAR EPIDEMIOLOGY

LSDV002 gene: unknown function, Stram et al., 2008

Truncated in SPPV and GTPV

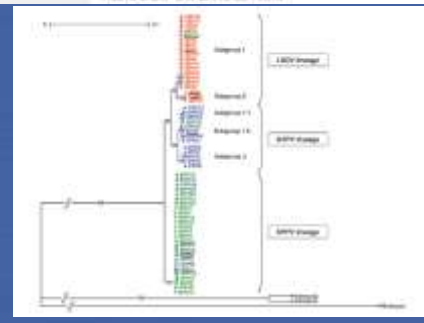
A 466 is used for phylogenetic reconstruction



IAEA
Joint FAO/IAEA Division

MOLECULAR EPIDEMIOLOGY

Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination



Size of the gene:

1046 for GTPV and LSDV

1025 for SPPV and some GTPVs

Gene sequence extracted for phylogeny

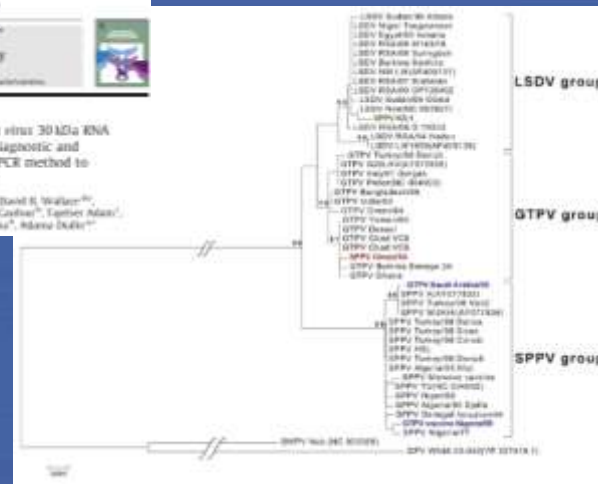
IAEA

Joint FAO/IAEA Division

MOLECULAR EPIDEMIOLOGY

Veterinary Microbiology

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



Size of the Gene:

606 for GTPV and LSDV

585 for SPPV

IAEA

Joint FAO/IAEA Division

PRACTICAL APPLICATIONS

CHARACTERISATION OF OUTBREAK ISOLATES

Strain name	Origin	Species of origin	Genotyping result
GTPV Saudi Arabia/93	Saudi Arabia	Goat	SPPV
SPPV OMAN/84	Oman	Sheep	GTPV
SPPV KS-1	Kenya	Sheep	LSDV
LSDV RSA 06 Springbok	South Africa	Springbok	LSDV
LSDV RSA/00 OP126402	South Africa	Springbok	LSDV
GTPV Nigeria goat vaccine	Nigeria	Goat	SPPV
058/2011	Kenya	Sheep	GTPV
059/2011	Kenya	Sheep	GTPV
Akaki/2008	Ethiopia	Sheep	GTPV
Metekel/2010	Ethiopia	Sheep	GTPV
Chagni O06/2012	Ethiopia	Sheep	GTPV

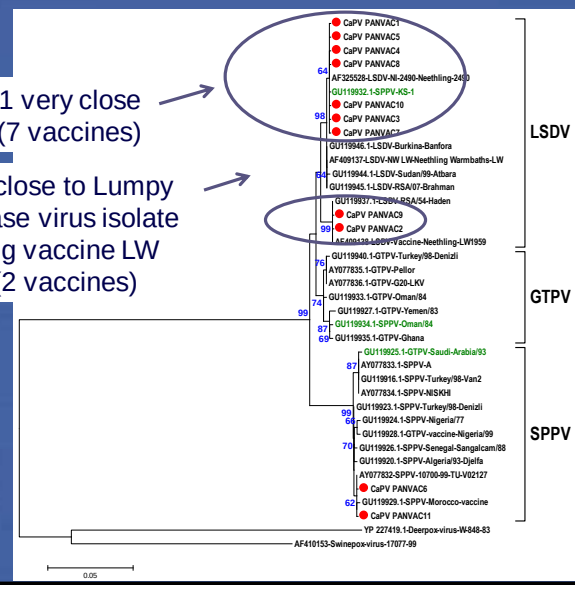
Evidence of cross infections by capripoxviruses

PRACTICAL APPLICATIONS

CHARACTERISATION OF VACCINE SEED

Group 1 very close to KSI (7 vaccines)

Group 2 close to Lumpy skin disease virus isolate Neethling vaccine LW 1959 (2 vaccines)



PRACTICAL APPLICATIONS

OUTBREAK INVESTIGATION IN VACCINATED FLOCKS

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Adverse Reactions to Field Vaccination Against Lumpy Skin Disease in Jordan

S. M. Abutarbush[1,2], W. M. Hananeh[3], W. Ramadan[1], O. M. Al Shayab[1], A. R. Alnajjar[1], I. G. Al Zoubi[1], N. J. Knowles[4], K. Bachanek-Bankowska[4] and E. S. M. Tuppurainen[4]

Generalised lesions caused by an unlabelled vaccine

Species-specific PCR and sequencing of the CaPV RPO30 and GPCR genes showed the vaccine to be a LSDV

IAEA

Joint FAO/IAEA Division

DECISION TREE FOR CAPRIPOX SCREENING

Real time PCR

GTPV SPPV LSDV

STOP

GPCR or RPO30 sequencing

SPPV GTPV LSDV

STOP

GPCR or RPO30 sequencing

LSDV GTPV or SPPV

STOP

Full genome sequencing

Capacity building

- 22 countries supported
- 36 Laboratory Experts trained on laboratory tests and procedures specific for LSD and Capripox detection and typing (IAEA / APHIS)
- A Regional workshop on LSD (November 2016)

Technology transfer

- One protocol for DNA extraction
- Two laboratory PCR test SOPs selected for virus detection, evaluated and distributed to affected and at-risk MS
- Three rapid Capripox genotyping SOPs developed and validated in APHL distributed to MS
- One SOP for purification of PCR products (to be sequenced) distributed to MS
- Two sequencing protocols to genetically characterize and trace LSD virus standardized and presented to MS

CONCLUSIONS

- Viral isolation can be done in primary cells of bovine and ovine origin
- Serology is mainly VNT but time consuming, ELISA highly needed.
- It is possible to quickly assess the genotype of CaPV outbreak isolates
- It is important to genotype both field isolates and vaccines strains
- Gene sequencing is needed to rule to out vaccine involvement when an outbreak occurs in a vaccinated herd

THANK YOU