DETECTION AND CHARACTERIZATION OF LUMPY SKIN DISEASE VIRUS

Charles Euloge LAMIEN Animal Production and Health Laboratory, Joint FAO/IAEA Division c.lamien@iaea.org



ETIOLOGY AND HOST RANGE

- LSD is an acute to chronic viral disease of cattle characterized by skin nodules accompanied by a
 persistent fever
- LSD is caused by a capripoxvirus (CaPV): the Lumpy skin disease virus (LSDV).
- Others CaPVs, are sheep poxvirus (SPPV) and goat poxvirus (GTPV) affecting sheep and goats.
- LSD is primarily spread between animals by biting insects (vector), such as mosquitoes and biting flies.
- The movement of infected animal and animal products can also participate in LSD spread
- LSDV, SPPV and GTPV cannot be distinguished by virus neutralization or other serological tests.
- **Domestic animals:** Cattle
- Wildlife (Arabian oryx in Saudi Arabia and Asian water buffalo in Egypt)
 - Experimentally: Giraffe, Impala are susceptible
 - LSDV DNA was characterized in samples from Springbok antelope and Eland

CLINICAL SIGNS









In the field the incubation period is 2 to 5 weeks. In experimental infection by intradermal inoculation, a lesion develops at the inoculation site within 6 to 20 days

- Fever
- Nodular lesions in the skin followed by necrosis
- Generalized lymphadenitis and oedema of the limbs
- Lacrimation
- Nasal discharge
- Loss of appetite
- Ulcerated lesions may be present in the mouth and nares
- Morbidity in susceptible herds can be as high as 100%
- Mortality is rarely more than 1–2%





LABORATORY DIAGNOSIS

LABORATORY SPECIMEN: Skin biopsies, swabs for virus isolation, molecular detection; Serum samples for serology

VIRUS ISOLATION AND PROPAGATION: Primary cells from bovine/ovine Cell lines (VERO, MDBK, ESH-L, OA3.Ts) Chorioallantoic membrane (CAM)

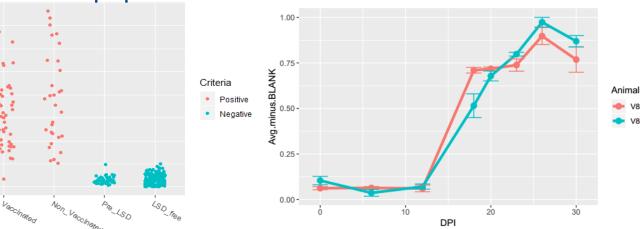
Serology

- Virus neutralization, Indirect fluorescent antibody test
- Immunoperoxidase Monolayer Assay
- ELISA)

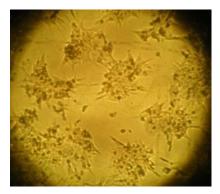
2.0

US 1.0

0.0



Capripoxvirus antibodies detection



infected Vero cell

LABORATORY DIAGNOSIS

MOLECULAR METHODS

GENERAL CAPRIPOXVIRUS DETECTION METHODS

- A gel-based PCR in the OIE manual LSD chapter (Ireland and Binepal 1998, Tuppurainen et al 2005)
- Real time PCR: 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.
- Kits (Techne); Genesig[®]; Tetracore; Biosellal

CAPRIPOXVIRUS SPECIES DIFFERENTIATION



Protocol

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

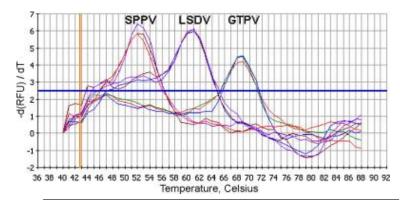
Charles Euloge Lamien^a, Mamadou Lelenta^a, Wilfried Goger^b, Roland Silber^c, Eeva Tuppurainen^d, Mirta Matijevic^e, Antony George Luckins^a, Adama Diallo^{a,*}

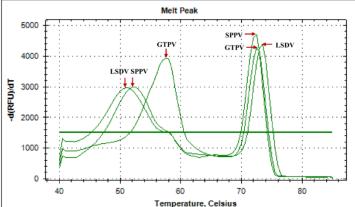
OPEN CACCESS Freely available online

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 Lamien¹*, Roland Silber², Eeva S. M. Tuppurainen³, Reingard Grabherr⁴, Adama Diallo¹





LABORATORY DIAGNOSIS

MOLECULAR METHODS

6

DIFFERENTIAL DIAGNOSIS

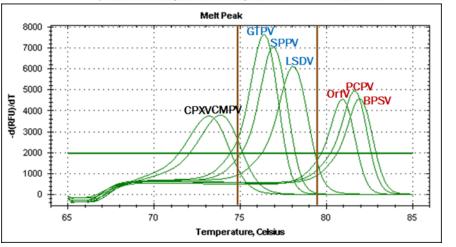
ww.nature.com/scientificreports

SCIENTIFIC REPORTS

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

Received: 15 September 2016 Accepted: 16 January 2017 Published: 20 February 2017

> Esayas Gelaye^{1,2,3}, Lukas Mach², Jolanta Kolodziejek⁴, Reingard Grabherr⁵, Angelika Loitsch⁶, Jenna E. Achenbach¹, Norbert Nowotny^{4,7}, Adama Diallo¹ & Charles Euloge Lamien¹





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

ISSUES WITH LIVE ATTENUATED CAPRIPOX VACCINES

To investigate lesions in cattle following vaccination using a live attenuated capripox vaccine we need:

7

- User-friendly tools to distinguish vaccine virus from field virus
- Accurate tools for quality control before vaccination

Ruling out vaccine involvement in LSD outbreak



Esayas Gelaye ^{a,b,c}, Alebachew Belay^c, Gelagay Ayelet^c, Shiferaw Jenberie^c, Martha Yami^c, Angelika Loitsch^d, Eeva Tuppurainen^e, Reingard Grabherr^f, Adama Diallo^a, Charles Euloge Lamien^{a,*}

^a Animal Production and Health Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria

Institute of Applied Genetics and Cell Biology. University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria Research and Diagnostic Laboratories, National Veterinary Institute, P.O. Box 19, Debre Zeit, Ethiopia

⁴Institute for Veterinary Disease Control, Austrian Agency for Health and Food Safety, Robert Koch-Gasse 17, A-2340 Mödling, Austria ⁶ Capripoxvirus Reference Laboratory, The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 ONF, United Kingdom

ute of Annlied Microbiology University of Natural Resources and Life Sciences, Mutheasse 11, 1190 Vienna, Austr

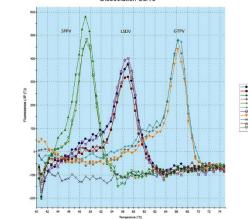
	90	100	110	120	130	140	150	160	
Akaki/001/2008	TATTAGCACAATT	CTCAGTGTAATT	TTAACAAAT	CAAAATAATGT	TACTACACCT	TCAACTTAT	AAAATACGA	CAACAA)
Assosa/G01/2010	••••••••••••••••••••••••••••••••••••••								
Chagni/G01/2012									Shee
Chagni/G02/2012									and
Chagni/G03/2012									isola
Chagni/006/2012	*************								ISOIA
Metekel/001/2010									
NVI/G01/2009	• • • • • • • • • • • • • • • •								
NVI/CaPV Vaccine									- Vacc
Adama/B01/2011	T								
Adama/B02/2011	·····T·····)
Andassa/B03/2012	T								
Andassa/B04/2012	T								
Andassa/B05/2012	T		.C		AG		G	G.	I
EDMTI Debre zeit/B01/2009	T		.C		AG		5A.	G.	
EIAR Debre zeit/B01/2009	T		.C		G		5A.	G.	Cattl
FairField Debre zeit/B01/2009	T		.C		AG		3A.	G.	isolat
Kajima Debre zeit/B01/2009	T		.C		AG		3A.	G.	13014
Mojo/B01/2011	T		.C		AG		5A.	G.	
Mojo/B02/2011	T		.c		AG		5A.	G.	
Wenji/B01/2011	T		.c		AG		3A.	G.	
Wenji/B02/2011	T		.c		AG		3A.	G.	
Wenji/B03/2011	T								/

Genotype the viral strain in the vaccine Antiviral Research 109 (2014) 1-i

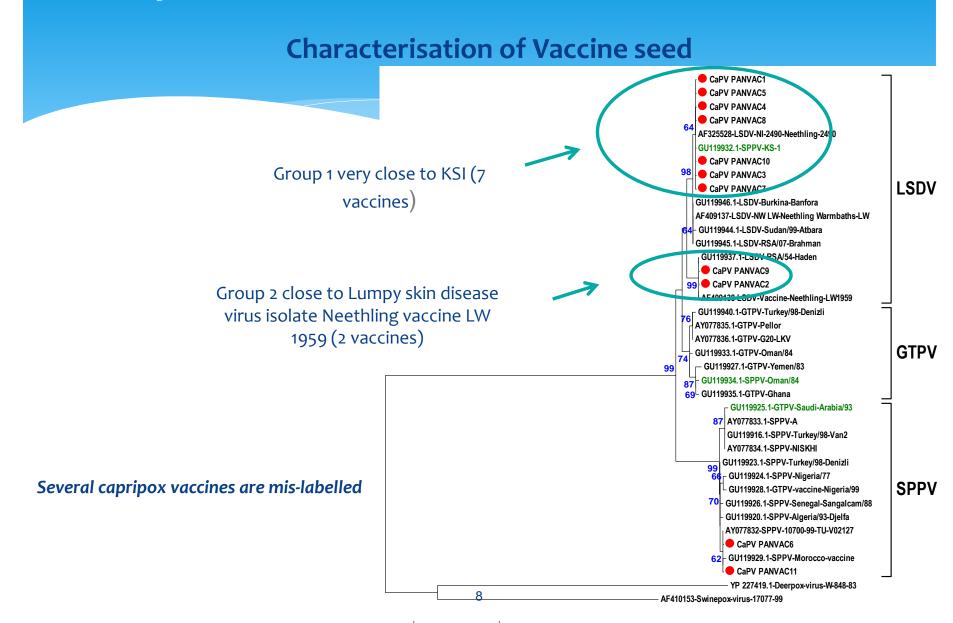


Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus

Eeva S.M. Tuppurainen^{a,*}, Caroline R. Pearson^a, Katarzyna Bachanek-Bankowska^a, Nick J. Knowles^a, Shadi Amareen^b, Lorraine Frost^a, Mark R. Henstock^a, Charles E. Lamien^c, Adama Diallo^c Peter P.C. Mertens **Dissociation** Curve



QUALITY CONTROL OF CAPRIPOX VACCINES



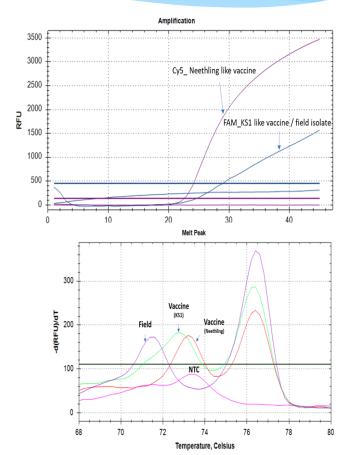
QUALITY CONTROL OF CAPRIPOX VACCINES

Cross contamination (KS1/Neethling vaccine)

* When both Neethling (for cattle) and KS1 (for small ruminants) are produced by the same manufacturer, there is a high risk for cross contamination







MOLECULAR EPIDEMIOLOGY

A multi-targets approach to detect a vaccine-like field isolate of LSDV in Kenya

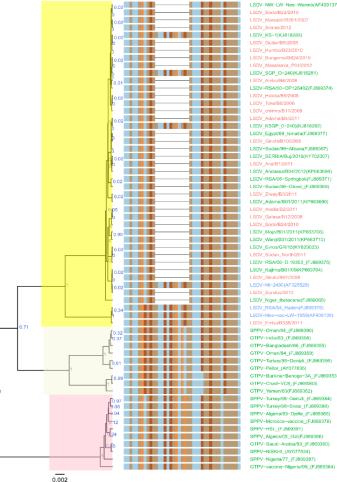
Partial B22R gene

30 40 50 60 70 80 LSDV Egypt/89 Ismailia GTTACAACATTACAAACATGACAACGATGGCCACACCGCCACCGCCTACAGTAATGACAACACCGCTATATTTAGAAAAATAAAAATAACACCCAATGATAT LSDV SIS-Lumpyvax KX764643 LSDV_Neeth_LW_1959_AF409138

PANVAC-7 SPPV_KS-1 LSDV Marsabit/B291/2007 LSDV Arsi/B1/2011 LSDV_Sudan_North/2011 LSDV Embu/B338/2011* LSDV Sundus/2012 LSDV Toke/B6/2008 LSDV Ziway/B3/2011 LSDV_155920/2012_KX894508 LSDV_Russia/D/2015_MH893760 LSDV Evros/GR/15 KY829023 LSDV SERBIA/B/2016 KY702007 LSDV KSGP 0240 KX683219 LSDV NT-2490 AF325528 LSDV NW-LW AF409137 LSDV Herbivac LS B008 MK44183 LSDV Russia/S/2017 MH646674 LSDV Cro2016 MG972412 LSDV Neeth OBP KX764645 LSDV Neeth Herbivac KX764644

EEV glycoprotein amino acids sequence

	110 .	120	130 1	40 150	160	170	180
LSDV KSGP 0240(KX683219)	NNNVLENGCTTNTGEE	NLIWDDNNVYC	LPPNDSVYDLPP	NDLSCNNDCVYTLP	DDNVSNIEEKIT	KLMHKNNSESNY	YNCC*
LSDV 155920/2012(KX894508)							*
LSDV Russia/Dag/2015(MH893760)							*
LSDV Evros/GR/15 (KY829023)						I	*
LSDV SERBIA/Buj/2016 (KY70200)	, 					I	*
LSDV ALB2016 (MH639082)	P					I	*
LSDV ISR_EZ06(MH639091)						I	*
LSDV BLG172 (MH639084)						I	*
LSDV SNGL75 (MH639094)							*
LSDV ISR197 (MH639088)							*
LSDV NW-LW(AF409137)							*
LSDV NI-2490 (AF325528)							*
LSDV Embu/B338/2011							*
LSDV Russia/Sar/2017(MH646674)	· · · · · · · · · · · · · · · · · · ·				• • • • • • • • • • • •		*
LSDV Cro2016 (MG972412)			· · · · .		• • • • • • • • • • • •		*
LSDV Neethling-OBP(KX764645)			· · · · <mark>·</mark>		• • • • • • • • • • • • •		*
LSDV Neethling-Her(KX764644)			· · · · <mark>·</mark>		• • • • • • • • • • • •		*
LSDV SIS-Lumpyvax(KX764643)			· · · · <mark>·</mark>		• • • • • • • • • • • •		••••*
LSDV Neethling_LW_1959(AF40913	3				• • • • • • • • • • • • •		••••*
LSDV ISR317 (MH639090)							••••*



GPCR gene

MOLECULAR EPIDEMIOLOGY

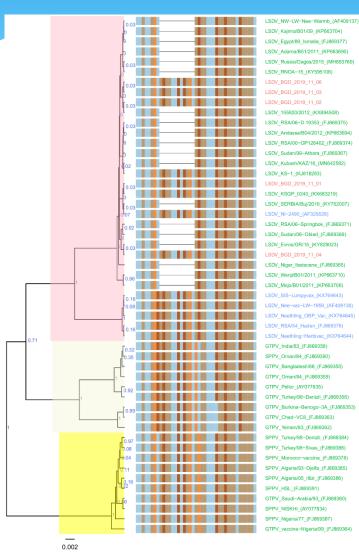
A multi-targets approach shows NI240/KS1 like virus in Bangladesh

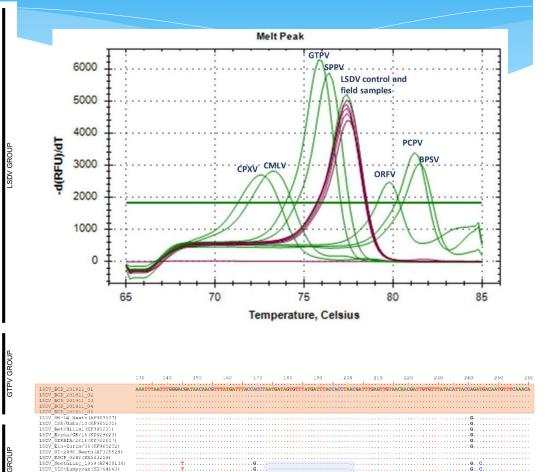
LSDV Cro2016 (MG972412)

LSDV_Neeth-Eerbivac(KX764644) LSDV_Neeth-LSD-OBP(KX764645)

LSDV_Dergachevskyi (KE077561)

LSDV Russia/Saratev/2017(MH646





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CAPACITY BUILDING AND TECHNOLOGY TRANSFER (VETLAB NETWORK)

Group trainings



Fellowship and long term training



Field support



TEAM AND COLLABORATIONS

JOINT FAO/IAEA DIVISION

- Gerrit VILJOEN
- Giovanni Cattoli
- Bharani Settypalli
- Francisco Berguido
- William Dundon
- Tesfaye Chibssa
- Esayas Gelaye
- Jenna Achenbach
- Adama Diallo

AGES

- Christian Hollub
- Angelika Loitsch
- Prof. Dr. Friedrich Schmoll
- All the team

VETLAB Network partners in R&D

- NAHDIC, Ethiopia
- NVI, Ethiopia
- CVRI, Zambia
- CVL, Namibia
- IRVT, Tunisia
- LNERV, Senegal
- CVL, Mozambique
- BNVL, Botswana
- CVL, Kenya
- CDIL, Bangladesh
- SCVL, Mongolia
- LCV, Mali
- NCVD, Vietnam

Others

- BOKU University
- AU-PANVAC, Ethiopia
- Faculty of Veterinary Medicine, University, Skopje
- NRL of CSF, ASF, Sheep pox and LSD, Sofia, Bulgaria

FAO/IAEA Agriculture and Biotechnology Laboratory





