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Molecular epidemiology and pathogenesis of Lagos bat virus, a rabies-related virus specific to Africa

by

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Supervisor: Professor Louis H. Nel

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I declare that the thesis, which I hereby submit for the degree PhD at the University of Pretoria, South Africa, is my own work and has not been submitted by me for a degree at another university

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SUMMARY

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For the degree PhD (Microbiology)

Lagos bat virus (LBV) belongs to genotype (gt) 2 of the lyssavirus genus in the family *Rhabdoviridae*, order *Mononegavirales.* This virus causes fatal rabies encephalitis in vertebrate animals and has only been reported from the African continent except for an imported case from African origin identified in France. The prototype lyssavirus is in fact rabies virus (gt 1) for which a variety of different vaccines are commercially available. These vaccines, however, do not provide protection against the gt 2 viruses. Genotype 2 viruses have not been well studied to date and the true risk for humans and animals is uncertain. The aim of this study was to investigate the epidemiology and pathogenicity of this uniquely African virus. In this project, our surveillance in South Africa reported six new LBV cases after this virus was not reported for the previous 12 years prior to this study. These results indicated that the incidence of this virus is greatly underestimated due to lack or absence of surveillance or ineffective diagnostic abilities of laboratories in Africa. Molecular epidemiological analysis of previously identified and new gt 2 isolates from this study indicated a high intragenotypic nucleotide and amino acid sequence diversity with respect to the Nucleo-, Phospho-, Matrix- and Glycoprotein genes. Based on these analyses, it has been proposed that two virus isolates that were previously reported as gt 2 LBV, may in fact constitute a new lyssavirus genotype. These findings emphasize the need to investigate different criteria for lyssavirus classification. As more lyssaviruses are discovered and with rapid progress in full genome sequencing, diversity becomes accentuated and challenges the criteria upon which lyssavirus

taxonomy is based. As a compliment to these genetic findings, our study of viral pathogenicity in a murine model, identified that the pathogenicity of phylogroup II viruses has previously been underestimated. LBV poses a potential risk to humans and animals and future vaccine strategies should ideally include protection against phylogroup II viruses.

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LIST OF ABBREVATIONS

aa	Amino acid
ABLV	Australian bat lyssavirus
BBB	Blood brain barrier
bp	Basepair
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CVS	Challenge virus strain
ddNTP	Dideoxynucleotide triphosphate
DEPC	Diethylpyrocarbonate
dNTP	Deoxynucleotide triphosphate
DUVV	Duvenhage virus
EBLV	European bat virus
ELISA	Enzyme linked immunosorbent assay
ERA	Evelyn Rokitniki Abelseth
FAT	Fluorescent antibody test
FAVN	Fluorescent antibody virus neutralization test
FFD	Focus forming dose
FITC	Fluorescein isothiocyanate
G	Glyco
gt	Genotype
HEP	High egg passage
HRIG	Human rabies immunoglobulin
i.c.	Intracerebral inoculation
i.m.	Intramuscular inoculation
i.p.	Interperitonial
IHC	Immunohistochemistry
L	Polymerase
LBV	Lagos Bat virus
LD	Lethal dose
LEP	Low egg passage
M	Molar
M	Matrix
mg	Milligram
MIT	Mouse inoculation test
ml	Milliliter

MNA	Murine neuroblastoma
MOKV	Mokola virus
MP	Maximum parsimony
N	Nucleo
NJ	Neighbor-joining
nt	Nucleotide
P	Phospho
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PM	Pittman Moore
PV	Pasteur virus
RABV	Rabies virus
RFFIT	Rapid Fluorescent Focus Inhibition Test
RTCIT	Rabies tissue culture infection test
s.c.	Subcutaneous
SAD	Street Alabama Dufferin
SD	Standard deviation
USA	United States of America
UV	Ultra-violet
VNA	Virus neutralizing antibodies
VSV	Vesicular Stomatitis virus
WCBV	West Caucasian Bat virus
WHO	World Health Organization