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Aspects of the molecular epidemiology of rabies in Zimbabwe and South Africa

by

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I certify that the thesis submitted to the University of Pretoria for the degree of PH. D. (Doctor of Philosophy) has not been previously submitted by me in respect of a degree at any other University.

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Date: 24/07/02

SUMMARY

ASPECTS OF THE MOLECULAR EPIDEMIOLOGY OF RABIES IN ZIMBABWE AND SOUTH AFRICA.

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Rabies, one of the oldest recognised viral zoonotic diseases, is a fatal encephalomyelitis transmitted to man via contact with infected animals. Even today, rabies still is a disease of public health concern with many potentially preventable deaths occurring mainly in Asia, Africa and Latin America. Rabies and rabies-related viruses are members of the Lyssavirus genus, which comprises the rabies virus (genotype 1), Lagos bat virus (genotype 2), Mokola virus (genotype 3), Duvenhage virus (genotype 4), European bat lyssaviruses 1 and 2 (genotypes 5 and 6) and the Australian bat lyssavirus (genotype 7).

Antigenic and genetic studies have shown that rabies virus strains circulating in particular host species tend to undergo genetic adaptation and evolve into distinct biotypes that differ in antigenicity and pathogenicity. Two biotypes of rabies virus are recognised in southern Africa. The first, called the canid viruses, infect carnivores of the family *Canidae* (dogs, jackals and bat-eared foxes) and the second, the viverrid

viruses, infect carnivores of the family *Herpestidae* (the yellow mongoose *Cynictis penicillata* and the slender mongoose *Galerella sanguinea*).

In an endeavour to better understand the molecular epidemiology of lyssaviruses in Zimbabwe and South Africa, we analysed nucleotide sequences of the glycoprotein and the G-L intergenic region (rabies viruses) and the nucleoprotein gene (Mokola viruses). The main aim of the studies described in this thesis was to characterise lyssaviruses (genotypes 1 and 3) from Zimbabwe and compare them to those present in South Africa. In addition, we wanted to establish the role of the various rabies variants in rabies epizootics in the southern African subcontinent.

It could be shown from this study that all the southern African canid viruses were closely related, with no general distinction between viruses from any of the canid species. Despite the general overall similarity between the canid viruses, certain phylogenetic groupings were apparent and by association with host species, geography and year of isolation, certain groups could be identified as particular epidemiological cycles. A high genetic diversity was evident amongst viverrid rabies viruses, the opposite of our observation for canid viruses. The viverrid virus groups corresponded to geographical pockets that were independent of host species. Mokola viruses from Zimbabwe were shown to be different from those from South Africa and phylogenetic relationships of these viruses were related to their geographical location of origin.

This study has demonstrated the value of multinational surveillance and investigation in understanding the epidemiology of lyssaviruses in southern Africa and elsewhere in Africa. The results presented here will serve as basis for future studies on lyssaviruses in Africa and will contribute to the improved surveillance and control programs of rabies and Mokola viruses in the region.

Key words: molecular epidemiology / rabies / Zimbabwe / South Africa / Lyssavirus / Mokola / phylogeny / canid/ viverrid/biotype

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LIST OF ABBREVIATIONS

CVS	Challenge virus standard
ERA	Evelyn-Rokitnicki-Abelseth strain
PV	Pasteur virus
RNP	Ribonucleoprotein
DNA	Deoxyribonucleic acid
cDNA	complementary DNA
RNA	Ribonucleic acid
mRNA	messenger RNA
MW	Molecular weight
SAG-2	SAD-Avirulent-Gift rabies vaccine strain
SAD	Street Alabama Dufferin rabies vaccine strain
V-RG	Vaccinia rabies glycoprotein
VNA	Virus neutralising antibodies
PET	post-exposure treatment
DMI	DNA-mediated immunisation
µl	microlitre
µg	microgram
ng	nanogram
WHO	World Health Organisation
ml	millilitre
mM	millimolar
pmol	picomoles
sp.	species
U	Units of enzyme activity

Rnase	Ribonuclease
dNTP	deoxynucleotide
bp	base pair
kb	kilobases
RT	room temperature/reverse transcription
PCR	polymerase chain reaction
RT-PCR	reverse trancription polymerase chain reaction
CNS	central nervous system
AIDS	Acquired immunodeficiency syndrome
VSV	vesiculostomatitis virus
Mab	monoclonal antibodies
FAT	fluorescent antibody test
ORF	open reading frame
NS	nonstructural
GT	genotype
Phylip	Phylogenetic inference package
PAUP	Phylogenetic inference using parsimony
ML	Maximum likelihood
MP	Maximum parsimony
NJ	Neighbour joining
UPGMA	Unweighted pair group method with arithmetic means
M-MuLV RT	Moloney Murine Leukemia Reverse Transcriptase
EBL	European bat lyssavirus
DNAdist	DNAdistance
eg	for example

^{o}C	degrees Celsius
viz	namely
Clustal	Cluster analysis
KDa	kilodalton
uv	ultraviolet
CMI	cell-mediated immunity
V-RN	Vaccinia recombinant nucleoprotein
GCG	Genetics computer group
GDE	Genetic data environment
ME	Minimum evolution

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