

**An evaluation of the vaccine-vector potential of thymidine kinase-
disrupted recombinants of lumpy skin disease virus (South African
vaccine)**

by

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***Dedicated to the Lord,
May His will be done.***

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SUMMARY

An evaluation of the vaccine-vector potential of thymidine kinase-disrupted recombinants of lumpy skin disease virus (South African vaccine).

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The aim of this study was to investigate the feasibility of developing the South African vaccine strain of the capripoxvirus, lumpy skin disease virus (LSDV), as a vector for recombinant vaccines to various diseases of veterinary importance in Africa using the viral thymidine kinase (TK) gene as the site of foreign gene insertion.

The first part of the study involved the development of a DNA transfer vector (pLSTK7.5) specific for the South African vaccine strain of LSDV containing a multiple cloning site, viral promoter and viral flanking sequences for the insertion of foreign genes (initially visual reporter genes, and subsequently genes from pathogenic viruses which are immunogenic) into the viral TK gene and for the expression of these genes leading to a protective immune response.

In order to evaluate the proposed recombination strategy, a visual marker gene, the *Escherichia coli* β -galactosidase gene (*lacZ*), was inserted into the multiple cloning site in pLSTK7.5 and a TK-deficient cell line of bovine kidney cells (BU100) was obtained. However, using the TK-negative selection strategy commonly used for selecting other poxvirus recombinants, it was impossible to recover stable LSDV recombinants. The strategy was then modified to include the *E. coli* guanine phosphoribosyl transferase (*gpt*)

positive selectable marker gene, which resulted in the selection of stable, homogeneous recombinants.

In order to improve the cloning and selection process, the pLSTK7.5 transfer vector was streamlined by the removal of extraneous sequences and the enhanced green fluorescent protein (EGFP) visual marker gene was introduced, giving rise to the new transfer vector, pLSEG.

The structural glycoprotein genes of bovine ephemeral fever virus (BEFV) and Rift Valley fever virus (RVFV), that encode proteins that can elicit protective immunity, were inserted separately into the pLSEG transfer vector and recombinants were generated and selected for homogeneity.

Expression of the glycoproteins under control of the early/late vaccinia virus P7.5K promoter was shown using immunofluorescence and the ability of the recombinants to induce both humoral and cell-mediated immune responses was demonstrated.

In protection studies, the LSDV-BEFV recombinant construct was unable to provide effective protection to cattle against virulent BEFV challenge most probably due to an over-challenge of virulent virus, although high levels of neutralising antibodies were produced which serve as an indicator for protection, whereas the LSDV-RVFV recombinant conferred complete protection to mice and at least partial protection to sheep. An attempt to demonstrate the dual protective nature of the vaccine against sheeppox virus in sheep was unsuccessful as the sheep failed to react to the challenge strain of sheeppox virus.

The results of this study indicate that the South African vaccine strain of LSDV shows good potential as a vector for recombinant vaccines using the viral TK gene as the site for foreign gene insertion.

Keywords: poxvirus, recombinant, lumpy skin disease, capripoxvirus, vaccine vector, homogeneity, thymidine kinase, lacZ, selection



ABBREVIATIONS USED IN TEXT:

A	adenine
ATCC	American type cell collection
ATP	adenosine triphosphate
BEF	bovine ephemeral fever
BEFV	bovine ephemeral fever virus
BEM	Basal Eagle's Medium
bp	base pair
BTV	bluetongue virus
BUdR	5-bromo-2'-deoxy-uridine
C	cytosine
CAM	chorioallantoic membrane
°C	degrees Celsius
CEF	chicken embryo fibroblast
CFK	calf foetal kidney
cm	centimetre
CO ₂	carbon dioxide
cpe	cytopathic effect
Da	Dalton
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EDD	Exotic Diseases Department
EDTA	ethylene diamine tetra-acetic acid
EGFP	enhanced green fluorescent protein
ELISA	enzyme-linked immunosorbant assay
EtBr	ethidium bromide
EtOH	ethanol
F	fusion
FBT	foetal bovine testes
FCS	foetal calf serum
ffu	focus forming units
FITC	fluorescein isothiocyanate
g	gram or gravitational force
G	guanine
gfp	green fluorescent protein
GP	glycoprotein

gpt	guanine phosphoribosyl transferase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HI	haemagglutination inhibition
I-ELISA	indirect enzyme-linked immunosorbant assay
IF	immunofluorescence
IgG	immunoglobulin G
ID	intradermal
IM	intramuscular
IP	intraperitoneal
IV	intravenous
k	kilo
kbp	kilobase pair
kb	kilobase
KC	Kenya cattle
kDa	kiloDalton
kg	kilogram
KS	Kenya sheep
lacZ	β -galactosidase
LSD	lumpy skin disease
LSDV	lumpy skin disease virus
M	Molar
MCS	multiple cloning site
MDBK	Madin Darby bovine kidney
mg	milligram
μ g	microgram
μ l	microlitre
μ M	micromolar
mA	milliamperes
ml	millilitre
mM	millimolar
mmol	millimoles
MOI	multiplicity of infection
MPA	mycophenolic acid
mRNA	messenger RNA
MVA	modified vaccinia Ankara
MW	molecular weight
N	normal
nAb	neutralising antibody
NaCl	sodium chloride

ng	nanograms
nm	nanometer
NaOH	sodium hydroxide
OBP	Onderstepoort Biological Products
O/N	overnight
OD	optical density
ORF	open reading frame
OVI	Onderstepoort Veterinary Institute
PCR	polymerase chain reaction
PBMC	peripheral blood mononucleocytes
PBS	phosphate buffered saline
pfu	plaque forming units
pH	negative log ₁₀ of the hydrogen concentration
pi	post infection or post inoculation
PP	percentage positive
PPRV	peste des petits ruminants virus
R.E.	restriction enzyme
rLSDV	LSDV recombinant
RNA	ribonucleic acid
rpm	revolutions per minute
RPV	rinderpest virus
RR	ribonucleotide reductase
RT	room temperature
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SC	subcutaneous
SDS	sodium dodecyl sulphate
SI	stimulation index
Sn	supernatant
SN	serum neutralisation
T	thymine
TAE	Tris acetate EDTA
TE	Tris EDTA
TK	thymidine kinase
Tris	Tris-(hydroxymethyl)-aminomethane
tRNA	transfer RNA
U	units or uracil
UV	ultra-violet
V	volts



VN	virus neutralisation
VV	vaccinia virus
v/v	volume per volume ratio
wt	wild type
wtLSDV	wild type LSDV
w/v	weight per volume ratio
X-gal	5-bromo-4-chloro-3-indoyl- β -D-galactoside

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