Characterization of the putative wobbly possum disease virus

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Veterinary Virology

at Massey University, Manawatū, New Zealand.

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2017

Abstract

The objective of this PhD was to characterise a marsupial arterivirus, termed wobbly possum disease virus (WPDV), and to confirm aetiological involvement of the virus in the development of a neurological disease of the Australian brushtail possum (*Trichosurus vulpecula*), termed wobbly possum disease (WPD). An *in vitro* culture system supporting the growth of the virus, comprising primary possum macrophages was developed. Purified virus stock was prepared using iodixanol density gradient ultracentrifugation of infected cell culture lysates and the *in vitro* growth kinetics of WPDV in primary possum macrophages was investigated using a previously described WPDV-specific RT-qPCR. The steepest increase in the levels of intracellular viral RNA was observed between six and 12 hours post infection, followed by a gradual release of cell-free viral RNA between nine and 24 hours post infection. Maximum levels of intracellular and extracellular viral RNA levels occurred at 24 and 48 hours post infection respectively.

Aetiological involvement of the virus in the development of WPD was supported by induction of disease in healthy wild-caught possums following infection with the purified virus. The pathogenesis of viral infection was explored by characterisation of histological lesions and quantification of WPDV RNA in various tissues from experimentally infected possums. Mononuclear inflammatory cell infiltrates of variable size were consistently observed in the liver, kidney, salivary gland and brain. The highest viral RNA levels were found in lymphoid, splenic and liver tissues, suggesting virus tropism for cells of the immune origin, most likely of the monocyte-macrophage system. High levels of viral RNA in tissues and sera from possums euthanased nearly four weeks post-infection indicates that immune response was ineffective in clearing the virus in that time-frame.

To investigate the presence of the virus in wild possum populations in New Zealand, an indirect ELISA using *Escherichia coli*-expressed recombinant viral nucleocapsid (rN) protein as antigen was developed. Pre and post-infection sera from experimentally challenged possums was used for ELISA development. These sera were also characterised using Western-blot against rN antigen. A serological survey of archival possum serum samples that had been collected between the years 2000 and 2016 and from five different regions of New Zealand was also performed using indirect ELISA. Bayesian estimates of parameters for a model of the ELISA data were used to establish ELISA cut-offs for WPDV antibody positive and negative samples. Applying these cut-offs, 50/230 (22%) archival samples were seropositive by indirect ELISA. Altogether, our data suggest that WPDV has been circulating in wild possum populations in New Zealand. Five out of 14 (36%) of pre-infection sera from the challenge study were also seropositive by Western-blot. Development of WPD in these possums following challenge suggests that pre-existing immunity was insufficient for protection against the development of disease. As such, further exploration of virus, host and environmental factors that govern development of disease is required.

Acknowledgments

If I have seen further, it is by standing on the shoulders of giants.

- **Isaac Newton, 1675**

Science does not exist in a vacuum, and nowhere is the famous quote by Isaac Newton more apt than in the pursuit of advancing science. Scientists, researchers, academics, teachers, and technicians go to work each day with no more noble a goal than to make the world a better place for those inhabiting it. Whilst individually our contributions may look small, we are all in fact building up a massively interwoven fabric, becoming more and more connected in our understanding, growing larger and greater as every day goes by.

As members of the scientific community we all aspire to be giants for the next generation, and also in our personal lives we must all depend on our own community of giants to guide, support, and nurture us. It is therefore unfortunate that on the front of any one PhD thesis only one name may be present. This is not due to lack of respect or appreciation for the giants upon whose shoulders we stand. Similarly, our families, friends, and colleagues all leave indelible marks on us. We are formed by our scientific, societal, and familial connections, and it would be an impossibility to enumerate even a small fraction of them. To put any other name at the front of this PhD would only severely undermine the fact that so many people deserve so much of the recognition for enabling this research.

It is therefore my hope that this foreword will serve as an opportunity to identify and thank a few important people who have enabled this research to proceed. It can never be complete, so to everyone who has helped guide this journey, I thank you.

Firstly, thank you to Dr Magdalena Dunowska, Dr Matthew Perrott, and Dr Wendi Roe for being wonderful, knowledgeable, and passionate mentors as they undertook the role of PhD supervisor in my research. They all truly took me under their wing. When I began my PhD all those years ago, my knowledge of virology was rudimentary compared to where it stands today. I came into this PhD as simply a veterinarian, but I leave as so much more – I am a scientist in the most fundamental sense. My knowledge of virology, my research and lab skills, and my approach to scientific methodology have been brought forward so significantly that it is hard to believe, and I owe that entirely to their dedication. Thank you.

I must also thank the support I received from Gaya Gopakumar, a lab technician in the early stages of my PhD who helped me develop laboratory skills that this PhD required.

Being a PhD student is not an easy proposition – it requires multiple years of dedication, and there is the eternal struggle of needing financial support without impacting the pace at which research can proceed. When I started this PhD, I was very fortunate to receive multiple scholarships, and without these my ability to pursue my PhD would have been severely hampered. I am indebted to their kindness, generosity, and support. In particular, I would like to show appreciation to the following scholarships for supporting me early in my PhD:

- Massey University, for the Massey University Doctoral Scholarship
- The Colin Holmes Dairy Scholarship
- The George Mason Sustainable Land Use Scholarship

I would also like to thank the following grants for supporting my research:

- Massey University Research Fund
- IVABS Postgraduate Research Fund
- McGeorge Research Fund

Early on in this work I was in desperate need of possums. It was through the generosity of Professor Kevin Stafford (Massey University) and Dr Paul Chambers (Massey University) that I received the possums that formed the basis for this research. Thanks also to Stephan van Haren for building a dozen possum nest boxes for me at very short notice, and to Landcare Research for providing live animal traps and housing cages for the possums. I would also like to thank Dr Laryssa Howe (Massey University) for Western-blot and ELISA tips, and for letting me use her ELISA plate washer and ELISA plate reader. Thanks also to Dr Bryce Buddle (AgResearch) for providing a large number of archival possum sera for a serological survey. Finally, thank you to Dr Patrick Reading (University of Melbourne) for providing mouse macrophage cell lines.

Multiple researchers contributed to the papers presented in this thesis. The contributions that these individuals have made toward the paper have also been stated at the bottom of each chapter, but I would like to thank them here also:

- Dr Magdalena Dunowska (Massey University), for attempts to grow wobbly possum disease virus in a range of commercial cell lines and in primary kidney cells.
- Dr Matthew Perrott (Massey University), for preparing histological slides from tissues of experimental possums, and also for developing immunohistochemistry to detect viral antigen in these tissues.
- Dr Matthew Perrott, Dr Wendi Roe (Massey University) and Dr Dani Aberdeen (Massey University), for their help in interpreting histological lesions from experimental possums.
- Dr Patrick Morel (Massey University), for providing statistical analysis of histological and molecular data in chapter 4.

- Dr Wesley Johnson (University of California, Irvine), Dr Geoff Jones (Massey University) and Dr Cord Heuer (Massey University), for performing Bayesian modelling of ELISA data.

Last, but certainly not least, I want to thank my family for their support. To my parents Ron and Ingrid, thank you for raising me to be inquisitive. To my husband Jonathan, thank you for making it possible for me to pursue this PhD (and for putting in countless hours of thankless copy-editing support!). Without your support and encouragement, this PhD would have been far more difficult. To my son Henry and daughter Pippa – both of whom didn't even exist when I started this PhD – and who are now my special nearly-four-year-old and seven-month-old: you bring a constant joy and happiness into my world. My pursuit of this PhD was counterbalanced – and made so much better – by the fact that I knew I was always coming home to play with you.

To Susan Giles – thank you for your support in taking such great care of Henry and Pippa, and enabling me to conclude this research when it never seemed like I would. Without your availability, I don't think I would have ever managed to find the time to write everything that needed to be written.

To everyone - the thesis you are about to read is something I have immense pride in. I have been working towards the results presented here since mid 2012, and as I sit here writing this in mid 2017, I feel a massive sense of accomplishment, pride, and excitement. Here ends a chapter in my life, and onwards I must go! I hope that in doing so, my shoulders become a little bit wider, and my legs a little bit taller, and I too become a giant for someone else.

Julia Giles,

BVSc, MVM, PhD (Candidate),

September 2017

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List of Abbreviations

$3CL^{pro}$	3C-like protease
Ab	Antibody
ACTB	β-actin gene
aPTT	Activated partial thromboplastin time
AUC	Area under the curve
BSA	Bovine serum albumin
C-terminus	Carboxy-terminus
cDNA	Complimentary DNA
cELISA	Competitive ELISA
CI	Control inoculum
CM	Culture medium
CMI	Cell-mediated immune
CNS	Central nervous system
CoV	Coronavirus
CPE	Cytopathic effects
Cq	Quantification cycle
CTL	Cytotoxic lymphocyte
CV	Coefficient of variation
DAPI	4',6-diamidino-2-phenylindole
DIC	Disseminated intravascular coagulation
DMEM	Dulbecco’s modified eagle medium
DMV	Double-membrane vesicle
DNA	Deoxyribonucleic acid
DOC	Department of Conservation
DUB	Deubiquitinating
E	Envelope

E.Coli	*Escherichia coli*
EAV	Equine arteritis virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme linked immunospot
EM	Electron microscopy
ER	Endoplasmic reticulum
EVA	Equine viral arteritis
ExoN	Exonuclease
FA	Fluorescent antibody
FDP	Fibrin degradation product
FP	False positive
GBSS	Gey's balanced salt solution
GM	Growth medium
GP	Glycoprotein
HCT	Haematocrit
HEL	RNA helicase
HRP	Horseradish peroxidase
ICL	Infected cell culture lysate
ICTV	International Committee on Taxonomy of Viruses
IF	Immunostaining
IFA	Immunofluorescent antibody
IFN	Interferon
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL	Interleukin
IP	Intraperitoneal
ISH	*In situ* hybridisation

LDH	Lactate dehydrogenase
LDV	Lactate dehydrogenase-elevating virus
M	Membrane
mAB	Monoclonal antibody
MHV	Mouse hepatitis virus
MLV	Modified live vaccine
moi	Multiplicity of infection
mRNA	Messenger RNA
N	Nucleocapsid
N-terminus	Amino-terminus
NendoU	Uridylate-specific endoribonuclease
nsp	Non-structural protein
OIE	World Organisation for Animal Health
OMT	Ribose-2'-O-methyltransferase
ORF	Open reading frame
PBS	Phosphate buffered sodium
PBST	Phosphate buffered sodium + Tween 20
PCR	Polymerase chain reaction
pi	Prevalence
PI	Percentage inhibition
PID	Possum infectious doses
PLP	Papain-like protease
pp	Polyprotein
PPM	Primary possum macrophages
PRRS	Porcine reproductive and respiratory syndrome
PRRSV	Porcine reproductive and respiratory syndrome virus
PT	Prothrombin time
qPCR	Quantitative PCR

RdRp	RNA-dependent RNA polymerase
RFS	Ribosomal frameshift site
rN	Recombinant nucleocapsid protein
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RPMI	Roswell park memorial institute medium
RT-PCR	Reverse transcriptase PCR
RT-qPCR	Quantitative reverse transcriptase PCR
RTC	Replication and transcription complex
RVN	Reticulovesicular network
S	Spike
SARS	Severe acute respiratory syndrome
SD	Standard deviation
sg	Subgenomic
sg MRNA	Subgenomic-length mRNA
SHF	Simian haemorrhagic fever
SHFV	Simian haemorrhagic fever virus
SI	Standard inoculum
SPF	Specific pathogen free
TB	Tuberculosis
$TCID_{50}$	Tissue culture infectious dose 50%
TMD	Transmembrane domain
TNF	Tumour necrosis factor
TP	True positive
TRS	Transcription regulating sequence
USG	Urine specific gravity
VNT	Virus neutralization test
WPD	Wobbly possum disease

WPDV	Wobbly possum disease virus
ZBD	Zinc-binding domain