Development of methods for detection and eradication of mouse parvovirus from a laboratory mouse colony

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Declaration

I declare that this is my own account of my research and contains work that has not been submitted for a degree at any tertiary institution

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Abstract

The mouse parvovirus designated MPV can infect laboratory mice and affect the humoral and cellular immune response of infected mice, reducing their value for biomedical and medical research. The development and maintenance of MPV-free mouse colonies for biomedical research is therefore essential and requires routine monitoring of the infection status of mice, using serological surveillance procedures.

Recent experience in the Animal Resources Centre (ARC), a major supplier of mice to the medical research community in Australia, was that MPV infection was present but was not detectable with the serological tests that were then in routine use.

This thesis reports the development of a polymerase chain reaction (PCR) assay for the detection of the MPV in the ARC mouse colonies, the genetic characteristics of the strain of MPV detected, the development of a recombinant virus protein that provided a suitable antigen for enzyme-linked immunosorbent assay (ELISA) and a Western immunoblot (WIB) assay for the detection of MPV antibodies, and use of these various assays to determine aspects of the epidemiology and pathogenicity of the infection that were critical to the eradication of virus infection and future immunological surveillance to ensure the absence of infection.

The recombinant protein produced as an antigen was a biotinylated fusion protein, a truncated capsid protein of the strain of MPV detected in the ARC, and was produced using the Pinpoint^{TM} vector and with expression in *Escherichia coli*. The protein was produced as an insoluble intracellular product within inclusion bodies and was solubilised using urea and purified. The purified protein was utilised as an antigen for ELISA and the WIB assays to detect virus antibody in infected mice.

The outbreak of MPV infection in the ARC was used as an unique opportunity for assessment of the seroprevalence of MPV-1 infection in a large laboratory mouse colony and to utilise this data to determine the sampling size needed to reliably detect MPV-1 infection within such large laboratory mouse colonies. An overall seroprevalence of 16.5% was detected using the developed serological tests, but considerable variation in prevalence was detected in different mouse strains.

The response to MPV infection of 4 different but common strains of mice was determined as a basis for developing appropriate surveillance procedures and the selection of appropriate sentinel animals. The effect of infection of these strains at different ages was also investigated. Virus replication was detected in tissues of all the mice strains infected (outbred ARC(s) and inbred C57BL/6JArc, BALB/c and BALB/c-*Foxn1*^{nu}/Arc) as juveniles and adults, with the exception of C57BL/6JArc inoculated as adults. However, while seroconversion in mice inoculated as juveniles and adults was detected in ARC(s) and C57BL/6JArc mice, it was not detected in BALB/c mice. The high rate of seroconversion to MPV, the early and prolonged development of an immune response, and the lack of age differences in their susceptibility indicated that ARC(s) mice would provide reliable sentinels for the detection of MPV.

The genomic nucleotide sequence of the ARC strain, excluding the terminal palindromic regions and the predicted amino acid sequences of the non-structural and structural proteins was determined. This strain was very similar (98-99% nucleotide identity) to the previously described MPV strains MPV-1a, MPV-1b and MPV -1c. The similarity suggested there were unlikely to be significant antigenic differences in the proteins of the ARC strain and those strains of MPV reported previously.

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To my son Emil

Abbreviations used in thesis

aa	Amino acid
Ab	Antibody
ARC	Animal Resources Centre
ATP	Adenosine triphosphate
BCCP	Biotin carboxyl carrier protein
C DNA strand	Complementary DNA strand
CI	Confidence intervals based on the binomial distributions
cRF	Covalently closed replicative form
Crm1	Chromosome region maintenance
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
dNTPs	Deoxynucleoside triphosphates (dATP, dCTP, dGTP, dTTP)
ds DNA	Double-stranded DNA
ds RF	Double-stranded replicative form
E. coli	*Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
EDTA	Ethylenediamide tetra acetic acid
eRF	Extended replicative form
FN	False negative
FP	False positive
HAI	Haemagglutination inhibition test
HaPV	Hamster parvovirus
HRP	Horseradish peroxidase
IL-2	Interleukin 2
IFA	Immunofluorescence antibody assay
IPTG	Isopropyl-β-thiogalactopyranoside

ISH	*In situ* hybridisation assay
LB	Luria broth
MPV	Mouse Parvovirus
MPV-1arc	ARC isolate of MPV-1
mRF DNA	Monomer replicative form DNA
mRNA	Messenger ribonucleic acid
m.p.	Map units
MVM	Minute virus of mice
NS	Non structural proteins
nt	nucleotide
ORF/ORFs	Open reading frame/s
OPV/OPVs	Orphan parvovirus
P	Prevalence
PID	Post infection/inoculation day
PIF	Parvovirus initiation factor
PCR	Polymerase chain reaction
PV/PVs	Parvovirus/Parvoviruses
PMSF	Phenyl methyl sulfonyl fluoride
PV+	Positive predictive value
PV-	Negative predictive value
RNA	Ribonucleic acid
rNS	Recombinant non-structural protein
rVP	Recombinant structural protein
RPV	Rat parvovirus
RV	Rat virus
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPF	Specific pathogen free

StDev	Standard deviation
TP	True positive
TN	True negative
TBS	Tris-buffered saline
TEMED	N, N, N', N'-tetra methyl ethylene diamine
Tris	Tris (hydroxymethyl) aminomethane
VAF	Virus antibody free
V strand DNA	Virion/parental DNA
VLP	Virus-like particles
VP	Virion/capsid proteins
WIB	Western immunoblotting

List of units used in thesis

bp	base pair
°C	degrees Celsius
g	gram
h	hours
MID_{50}	50% mouse infectious dose
min	minutes
mg	milligrams
mM	millimolar
µg	micrograms
µl	microliter
µM	micromolar
ng	nanogram
OD	optical density
rpm	revolutions per minute
s	seconds
V	volts
v/v	volume per volume
w/v	weight per volume