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# **Viruses of the common brushtail possum**

## ***(Trichosurus vulpecula)***

**Matthew Robert Finch Perrott**

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## ***Abstract***

A tissue culture survey was conducted to detect viruses in possums. Up to 14 tissues from 93 wild caught possums were inoculated (co-cultivation) onto three marsupial cell lines. Possum primary cell cultivation was also developed throughout the survey period and together these procedures sought to detect viral infections as overt clinical disease, as unapparent illnesses or present in a latent form. Three passages of seven days duration were routinely performed. Haemadsorption tests (chick, guinea pig and human "O" RBCs at 37°C) and examination of stained monolayers (chamber slides) were completed for the third passage. A few adenovirus-like particles were identified by electron microscopy in one of two possums' tissue cultures in which a non-sustainable cytopathic effect was detected. No haemadsorption or abnormal chamber slide cytology was demonstrated. Adenoviruses were identified by electron microscopy in faecal or intestinal contents samples from four of the survey possums.

**Wobbly possum disease (WPD)**, a newly described neurological disease of possums, was suggested to have a viral aetiology when filtered infectious material (clarified spleen suspension from a confirmed case of WPD passed through a 0.22 µm membrane) could transmit disease to susceptible possums following intra-peritoneal inoculation. Preliminary studies into routes of transmission of WPD used a standardised tissue suspension prepared from pooled infected liver, spleen and brain tissue. Titration of the tissue suspension *in vivo* demonstrated in excess of  $10^5$  possum infectious doses per ml. Clinical signs associated with neurological disease were confirmed by the presence of characteristic histological lesions and a scoring system was devised to assist diagnosis.

The tissue suspension was shown to cause disease when inoculated by the gastric, tracheal, intradermal (ID) and intraperitoneal (IP) routes. Blood and urine from infected possums were shown to be infectious when inoculated by the ID and IP routes respectively. Disease was transmitted by a suspension of blood feeding mites (*Trichosuirolaelaps crassipes*) injected intradermally however the transfer of live mites from an infected possum to a non-infected recipient failed to transmit WPD.

Sentinel control possums housed adjacent to experimentally infected possums did not develop WPD but when two inoculated possums were placed in group housing with five in contact controls, all possums became infected. The absence of aerosol transmission to non-contact control possums suggests that transmission requires direct contact between possums or contact with a contaminated environment. Naturally occurring transmission was demonstrated to be feasible by the cloacal / oral and intradermal routes. Further work is required to determine the relative importance of these routes under natural conditions.

A neurological disease syndrome was investigated in a wild possum population in the Rotorua district and determined to be almost identical to WPD. Comparison of the Rotorua syndrome and WPD together with histological evidence for more widespread distribution of similar disease processes suggest that WPD or variants may already be distributed throughout New Zealand. As such, WPD may be a newly recognised disease rather than a newly emergent disease.

Papillomavirus particles were detected in association with wart-like papillomas on the tail of a possum. A papillomavirus specific product was amplified by PCR and manually sequenced. Sequence comparisons and phylogenetic analysis determined a new papillomavirus type (possum papillomavirus). The survey methodology and the possum viruses described in this thesis are discussed in terms of identifying suitable viruses for use as biological control agents.

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## ***Abbreviations***

AGID	Agar gel immuno-diffusion
ATCC	American type culture collection
ATV	Antibiotic / trypsin / versene
BD	Borna disease
BLAST	Basic local alignment search tool
BPK	Baby possum kidney
BPV	Bovine papillomavirus
CAM	Chorio-allantoic membrane
CC	Co-cultivation
CC-I	Co-cultivation I
CC-II	Co-cultivation II
cDNA	Copy DNA
CMV	Cytomegalovirus
CNS	Central nervous system
CPE	Cytopathic effect
CRPV	Cotton-tail rabbit papillomavirus
DDBJ	DNA database of Japan
dNTPs	Dinucleotide triphosphates (dATP / dCTP / dGTP / dTTP)
dd NTPs	Dideoxy nucleotide triphosphates (ddATP / ddCTP / ddGTP / ddTTP)
DIC	Disseminated intravascular coagulation
DMSO	Dimethyl sulphoxide
EBV	Epstein Barr virus
EGTA	Ethylene glycol-bis ( $\beta$ -aminoethyl ether) N, N, N', N'-tetraacetic acid
EHV-1	Equine herpesvirus-1
ELH	Earles Lactalbumin hydrolysate
ELISA	Enzyme linked immunosorbant assay
EM	Electron microscopy
EMBL	Database run by European Bio-informatics Institute
EPP	Explant primary passage
EVPV	Epidermodysplasia verruciformis papillomavirus
FBS	Foetal bovine serum
FCM	Filtered conditioned medium
GenBank	Database (National Center for Bio-technology Information)
GIT	Gastrointestinal tract.
GM	Growth medium
H & E	Haematoxylin and eosin
HI	Haemagglutination inhibition
HPV	Human papillomavirus
IC	Intra-cerebral
IEM	Immune electron microscopy

IFAT	Immunofluorescent antibody test
IP	Intraperitoneal
MaHV	Macropodid herpesvirus
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MEM	Minimum essential medium
MM	Maintenance medium
MVE	Murray valley encephalitis
NCBI	National Center for Bio-technology Information (GenBank)
OPK	Opossum kidney
ORF	Open reading frame
PBL	Peripheral blood leukocytes
PAUP	Phylogenetic analysis using parsimony
PCEF	Primary chicken embryo fibroblast
PDB	Protein databank (Brookhaven)
PI	Post inoculation
PID	Possum infectious dose
PIR	Protein information resource (National Medical Research Foundation)
PM	Primary medium
PPK	Primary possum kidney
PPV	Possum papillomavirus
PRT	Possum reproductive tract
PSK	Penicillin streptomycin kanamycin
PTK2	Potoroo kidney
PV	Papillomavirus
PWHV	Parma wallaby herpesvirus
RBC	Red blood cell
RE	Restriction enzyme
RF	Restriction fragment
RT	Reverse transcriptase
SAPU	Small animal production unit
SNT	Serum neutralisation test
TCID	Tissue culture infective dose
TPB	Tryptose phosphate broth
WBC	White blood cell
WV	Whataroa virus