

An investigation into the status of porcine circovirus in Australia

Warren Sean Raye BSc (Hons)

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University 2004

DECLARATION

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

Warren Sean Raye

Abstract

This thesis reports for the first time the detection of porcine circovirus virus (PCV) in the Australian pig herd.

PCV DNA was detected in the tissues of pigs from several Australian states using a multiplex polymerase chain reaction (PCR) assay, the primers for which were based on the sequence of PCV1 and PCV2 strains detected in North America and Europe. PCV type 1 or 2 was detected in 80 of 367 (21.7%) pigs tested. In the 80 positives, both PCV1 and PCV2 were detected in 14 samples. Virus was detected in pigs from all states from which samples were obtained: Western Australia, South Australia, New South Wales and Queensland.

The complete genomes of 13 strains of Australian PCV were sequenced. Analysis of the data indicated there was extremely high homology between the Australian strains of PCV1 and PCV2 and previously published sequences of PCV1 and PCV2 strains from North America and Europe. There were no consistent differences between the genome of the Australian strains and strains in North America and Europe

The widespread occurrence of PCV in the tissues of pigs was confirmed by a small scale serological study of the Western Australian pig herd using an immunofluorescence assay, which did not discriminate antibody to PCV1 and PCV2. This assay detected PCV antibody in 11 of 14 pig herds in Western Australia, with a prevalence rate in positive herds varying from 25 to 47%, but it was unable to differentiate antibody to PCV1 and PCV2.

A PCV2-specific recombinant viral capsid protein was produced in insect cells with a baculovirus expression system and this was used to develop a PCV2-specific ELISA and a Western immunoblotting assay. These assays were applied to samples from a national pig serum bank and detected PCV2 antibody in 33% of 3933 serum samples. The highest seroprevalence to the recombinant PCV2 capsid antigen was detected in the samples from Victoria where there was a 51.3% seroprevalence rate, and the lowest in Western Australia where there was an 11.4% seroprevalence rate.

An *in situ* hybridisation (ISH) technique was developed for the detection of PCV in tissues of infected pigs and infected cell cultures. A monoclonal antibody specific for the capsid protein of PCV2 was also produced and has application for the development of immunocytochemical procedures for the detection of PCV2 in tissues and cell cultures.

The high prevalence of PCV in the Australian pig herd and the absence of reports of PMWS suggested that the Australian strains of PMWS detected may have been of low virulence. To examine the pathogenicity of Australian strains, two animal experiments were conducted where the type species of PCV1 present in persistently-infected PK15 pig kidney cells and an Australian PCV2 strain were cultured *in vitro* in cell cultures and inoculated into weaner pigs. As expected, the PCV1 replicated well in pigs but did not result in the induction of clinical signs or lesions in the inoculated pigs. The inoculation into weaner pigs of cell

culture replicated PCV2 with an apparent virus titre of 10^3 virus particles/mL resulted in infection of only some of the inoculated pigs and it was concluded that the PCV2 inoculum contained insufficient virus to infect all pigs into which it was inoculated. The PCV2 did not induce any disease syndrome and could not be visualised in tissue sections of infected pigs using immunohistochemical techniques.

In conclusion, techniques were developed for the detection of PCV in the Australian pig herd. PCV of both genetic types were detected at prevalence rates similar to those reported in other countries where PMWS has occurred, and the widespread occurrence of PCV was confirmed by serological assays. The PCV strains present were genetically indistinguishable from those present in North America and Europe. The reason for the absence of PMWS in Australia is most likely not due to differences in the characteristics of the PCV strains present.

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List of abbreviations used throughout thesis

2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABST)

4-chloro-naphthol (4-CN)

5-bromo-4-chloro-3-indolyl phosphate toluidine salt (BCIP)

5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal)

Australian National Genome Information Service (ANGIS – www.angis.org.au)

Autographa californica nuclear polyhedrosis virus (AcNPV)

banana bunchy top virus (BBTV)

beak and feather disease virus (BFDV)

bean golden yellow mosaic virus (BGMV)

beet curly top virus (BCTV)

bovine serum albumin (BSA)

calf intestinal alkaline phosphatase (CIAP)

chicken anaemia virus (CAV)

competitive ELISA (c-ELISA)

congenital tremor (CT)

cytopathic effect (CPE)

diaminobenzidine (DAB)

digoxigenin (DIG)

dimethylsulfoxide (DMSO)

dinucleotide triphosphate (dNTP)

distilled water (dH₂O)

double stranded (ds)

double-distilled deionised water (ddH₂O)

Dulbecco's modified essential medium (DMEM)

enzyme linked immunosorbent assay (ELISA)

fluorescent *in situ* hybridisation (FISH)

glutathione-S-transferase (GST)

hexahistidine (6xHIS)

histidine-tagged (HIS)

horseradish peroxidase (HrP)

human immunodeficiency virus type 1 (HIV-1)

immunohistochemistry (IHC)

immunoperoxidase monolayer assay (IPMA)

in situ hybridisation (ISH)

incomplete Freund's adjuvant (ICFA)

indirect immunofluorescence (IIF)

International Committee for the Taxonomy of Viruses (ICTV)

intraperitoneal (IP)

isopropyl- β -D-thiogalactopyranoside (IPTG)

Jembrana disease virus (JDV)

keyhole limpet hemocyanin (KLH)

maize streak virus (MSV)

monoclonal antibody (MAb)

multiplicity of infection (MOI)

National Centre for Biotechnology Information (NCBI)

new-born calf serum (NBCS)

nitro blue tetrazolium chloride (NBT)

non-structural (NS)

open reading frame (ORF)

phenylmethylsulfonyl fluoride (PMSF)

phosphate buffered saline (PBS)

pig kidney cell line 15 (PK15)

pigeon circovirus (PiCV)

polyethylene glycol (PEG)

polymerase chain reaction (PCR)

porcine circovirus (PCV)

porcine circovirus type 1 (PCV1)

porcine circovirus type 2 (PCV2)

porcine dermatitis and nephropathy syndrome (PDNS)
porcine parvovirus (PPV)
porcine reproductive and respiratory syndrome (PRRS)
porcine reproductive and respiratory syndrome virus (PRRSV)
post-infection (p.i.)
post-inoculation (PI)
post-weaning multisystemic wasting syndrome (PMWS)
psittacine beak and feather disease (PBFD)
replicative form (RF)
rolling circle replication (RCR)
single stranded (ss)
sodium dodecyl sulphate (SDS)
sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)
subterranean clover stunt virus (SCSV)
tomato golden mosaic virus (TGMV)
tomato pseudo-curly top virus (TPCTV)
TT virus (TTV)
Western immunoblot (WIB)

List of unit abbreviations used throughout thesis

°C – degrees Celsius

μg – micrograms

ng – nanograms

μL – microlitre

μm – micrometre

μM – micromolar

nm – nanometre

pmol – picomoles

bp – base pairs

CCID₅₀ – 50% cell culture infectious dose

d – days

g – grams

h – hours

kb – kilobases

kDa – kiloDalton

M – molar

mg – milligrams

min – minutes

mL – millilitre

mm – millimetre

mM – millimolar

nt - nucleotides

OD – optical density

pfu/mL – plaque forming units per millilitre

rpm – revolutions per minute

s – seconds

TCID₅₀ – 50% tissue culture infectious dose

U – enzyme units

V – volts

v/v – volume per volume

w/v – weight per volume

x g – times gravity

Publications arising from this thesis

Presented at scientific meetings:

Buddle JR, Muhling J, Raye W, Raidal SR, Wilcox GE. 2003. Porcine circovirus in Australia. *Proceedings of the Australian Association of Pig Veterinarians 2003, Cairns Conference*, AAPV Indooroopilly, p.67-77.

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