Partial characterisation of pilchard herpesvirus and the associated disease in pilchards

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

by

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Declaration

The experiments in this thesis constitute work carried out by the candidate unless otherwise stated. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliography and appendices, and complies with the stipulations set out for the degree of Doctor of Philosophy by Murdoch University, Western Australia.

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Abstract

In 1995 and again in 1998, millions of pilchards (*Sardinops sagax neopilchardus*) were found dead or dying off the coast of Australia and also in New Zealand. The epizootics moved progressively, at a rapid speed against the prevailing currents. A previously unrecognised herpesvirus, Pilchard herpesvirus (PHV), was identified as the causative agent.

Until recently, rapid and sensitive methods to detect PHV were not available and based on a previously identified and conserved 373 bp region of the genome, polymerase chain reaction (PCR), *in situ* hybridisation and real-time PCR methods were developed for the specific detection of PHV in formaldehyde-fixed and frozen tissues of pilchards. Real-time PCR was shown to have greater sensitivity than a conventional PCR and *in situ* hybridisation for the detection of PHV infection.

The PCR assay and sequence analysis of the amplification products was used to compare the 373 bp region of the genome from strains obtained during the 1995 and 1998 epidemics. Significant differences between the strains were not detected. Additional sequence data was obtained adjacent to the 373 bp of known PHV sequence, which did not match any sequence in any of the genetic databases, and this will be invaluable for further study of the pilchard herpesvirus and the development of improved detection methods.

The molecular-based methods of virus detection developed were applied to a re-examination of virus in paraffin-embedded tissues taken from fish during an attempt to transmit the virus to wild caught pilchards in 1999. The results obtained confirmed previously equivocal results that transmission of PHV to wild caught pilchards was achieved, although this experiment failed to demonstrate that transmission of the virus resulted in severe lesions typical of those seen in the epizootics.

Using formaldehyde-fixed samples from fish collected during the 1998 PHV epizootic, virus was detected in fish collected 4 days prior to the occurrence of the epizootic even though the fish then appeared clinically normal, during the epizootic, and 8 days after mortalities had ceased.

An investigation of wild pilchards collected from 4 Australian pilchard sub-populations using real-time PCR demonstrated that PHV was present in the gills of 13.75% of 800 fish sampled, indicating that the virus is now endemic in the Australian pilchard population. Variation in the prevalence of PHV infection in the 4

subpopulations was detected, higher in western and southern populations than in populations from the east coast. The endemic nature of PHV infection in the pilchard population explains why there have been no further epizootics with mass mortalities since 1998.

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Publications

Published

Crockford M. (2004) Australian and New Zealand Standard Diagnostic Procedure: White Spot Disease. PDF file on website: www.affa.gov.au/corporate_docs/publications/pdf/animalplanthealth/aquatic/whitespot_disease_sdt

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In Draft

Crockford M., McColl K., Jones J.B. and Wilcox G.E. Comparison of 3 molecular methods for the detection of Pilchard herpesvirus in archived paraffin-embedded tissue and frozen tissue. *Submitted to Diseases of Aquatic Organisms 2007; currently under peer review*

Whittington R.J., Crockford M., Jordan D. and Jones J.B. Survey for herpesvirus in pilchard, a marine pelagic finfish, in Australia. *Accepted for submission in Journal of Fish Diseases 2007*

Crockford M., Jones J.B., Hillier P. and Wilcox G.E. Molecular detection of Pilchard herpesvirus in archived pilchard (*Sardinops sagax neopilchardus*) paraffin-embedded tissues from a transmission trial conducted in 1999.

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Abbreviations and definitions

Abbreviation	Definition
AAHL	Australian Animal Health Laboratories
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
bp	base pair
CCO	Channel catfish ovary
CCV	Channel catfish virus
CHV1	Cyprinid herpesvirus 1
cm	centimetre
CMV	Cytomegalovirus
CNGV	Carp nephritis and gill necrosis virus
DAS	Downstream activating sequence
DBF	DAS binding factor
DNA	Deoxyribonucleic acid
E	Early (gene)
EBV	Epstein-Barr virus
E.coli	*Escherichia coli*
FHL	Fish Health Laboratory
FV4	Frog virus 4; RaHV2
g	times gravity
GFHNV	Goldfish haematopoietic necrosis virus
h	hour
HEPC	Herpesviral epidermal proliferation in carp
HSC	Herpesvirus septicaemia in carp
HSV1	Herpes simplex virus 1
HVEM	Herpesvirus entry mediators
HVHN	Herpesviral haematopoietic necrosis
IE	Immediate early (gene)
ISH	*in situ* Hybridisation
IU	International unit
kbp	kilo base pairs
kg	kilogram
KHV	Koi herpesvirus
km	kilometre
L	Late (gene)
LAT	Latency-associated transcript
LB	Luria-Bertani (broth or medium)
LTHV	Lucke tumour herpesvirus; RaHV1
M	molar
mg	milligram
MHC	Major histocompatibility complex

MHV	Murine herpesvirus
min	minute
mL	millilitre
mM	millimolar
MQ	Milli-Q (water)
mRNA	messenger RNA
MW	Molecular weight
ng	nanogram
nm	nanometre
nmol	nanomole
NSW	New South Wales
NZ	New Zealand
OMV	Oncorhynchus masou virus
ORF	open reading frame
Ori	Origin of replication
PCR	Polymerase Chain Reaction
PHV	Pilchard herpesvirus
pmol	picomole
RaHV1	Ranid herpesvirus 1; LTHV
RaHV2	Ranid herpesvirus 2; FV4
RNA	Ribonucleic acid
SA	South Australia
SaHV1	Salmonid herpesvirus 1
SaHV2	Salmonid herpesvirus 2
sec	second
TIF	Transcription initiation factor
TK	thymidine kinase
TNF	Tumour necrosis factor
U	Units of enzyme activity
UK	United Kingdom
µL	microlitre
UL	unique long (region of herpesvirus genome)
µM	micromolar
US	unique short (region of herpesvirus genome)
USA	Unites States of America
UV	ultra-violet
VHS	Viral host shutoff (protein)
v/v	volume per volume
VZV	Varicella-Zoster virus
WA	Western Australia
WSHV1	White sturgeon herpesvirus 1
WSHV2	White sturgeon herpesvirus 2