An Analysis of *Bovine immunodeficiency virus* and *Jembrana disease virus* Infections in *Bos javanicus*

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BSc (Hons)

This thesis is presented for the degree of Doctor of Philosophy

of Murdoch University.

April 2010

Abstract

Two closely related bovine lentiviruses have been described, *Jembrana disease virus* (JDV) and *Bovine immunodeficiency virus* (BIV), that produce very different clinical manifestations in infected cattle. JDV causes an acute disease with a case fatality rate of about 21% in *Bos javanicus* (Bali cattle) and is endemic in the cattle population of parts of Indonesia. BIV produces a subclinical infection in *Bos taurus* and buffalo and serological evidence has shown that this virus has a worldwide distribution, possibly including Indonesia.

Attempts were made to confirm a previous report that BIV was present in the *B. javanicus* population in Indonesia. BIV proviral DNA was not detected in any of the animals although JDV proviral DNA was detected in 12 of 171 animals, only one of which was seropositive.

To define the kinetics of BIV infection in *B. javanicus* and determine the optimal time for sampling to detect BIV infection, 13 animals were experimentally infected with the R29 strain of BIV. No clinical effects were detected but proviral DNA was detected from 4-60 days post-infection (dpi) with peak titres 20 days dpi, and a transient viraemia from 4 to 14 dpi. An antibody response to TM was detected 12 dpi but an anti-capsid (CA) antibody response was detected in one animal only and not until 34 dpi. The results indicated that detection of BIV in infected Bali cattle using PCR would have a greater chance of success soon after infection and prior to the onset of a CA antibody response.

To determine the effect of BIV infection on subsequent JDV infection in *B. javanicus*, 15 cattle were infected with BIV-R29 and 9 of these were subsequently infected 42 days later with JDV. The response to BIV was typical of that observed

previously but BIV infection did not markedly modify the response to subsequent infection with JDV. In response to JDV infection, all cattle previously infected with BIV still developed an acute disease process typical of Jembrana disease. The results suggested that despite the close genetic and antigenic relationship between BIV and JDV, BIV infection does not confer protection against subsequent JDV infection.

The close antigenic relationship between BIV and JDV is a problem in the development of specific serological tests and immunosurveillance of JDV infection. To develop reagents capable of differentiating between antibody to BIV and JDV infections, peptide mapping was used to define linear B cell epitopes on the matrix (MA), CA and surface unit (SU) proteins of JDV. Short overlapping peptides that spanned these regions were synthesised and used in an ELISA format to screen their reactivity with a panel of bovine sera from animals experimentally infected with JDV_{Tab87}, JDV_{Pul01} or BIV-R29. Peptides representing potential immunoreactive epitopes were identified that appeared to offer promise in the development of JDV-specific serological tests and need to be tested further with a panel of sera taken from naturally infected cattle.

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Tegan Josephine McNab.

Contents

Acknowledgements

First and foremost, I would like to thank my two supervisors, Dr. Moira Desport and Professor Graham Wilcox, for their help throughout my PhD. I really appreciate all the help they've given me. In particular, Moira for your input in designing my PhD, for helping me in the lab and for all the wisdom you've passed on to me. To Graham, for your help in editing my thesis and getting papers ready for submission. I hope you catch many fish in your retirement!

Thankyou to Robert Dobson for your help with all things statistical, I am sure I still owe you a few more cakes. Thanks must also be extended to other staff in the Veterinary and Biomedical Sciences building for your help over the past few years.

Thankyou to the Australian Centre for International Agricultural Research for providing the funding necessary to undertake this work and to the Australian Government for providing my scholarship.

Thankyou to the members of the laboratory in Denpasar, particularly Nining and Putu, for their help in running our animal trials and for the collection of samples. Without this, it would not have been possible to complete my thesis.

A big thankyou to past and present members of our office: Will, Mark, Andrew, Linda, Josh, Masa, Yudhi and honouree member Jill A. Thankyou so much for all your help and good times shared around cake.

To all my friends, Laura G, Jacqui, Gael, Genevieve, Sarah, Nikki, Bry, Celia, Laura H, Kendle, Michelle, and my entire family, thanks for all the good times. To everyone at kickboxing, thankyou for helping to keep the stress levels low by letting me punch and kick you on a regular basis.

Finally, a big thankyou to my parents, Marg and Ross, for being fantastic and so supportive all the time.

To Bong for just being you.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
AGID	Agar gel immunodiffusion
APOBEC	Apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like
AUC	Area under the curve
BFL	Bovine foetal lung
BIV	*Bovine immunodeficiency virus*
BVDV	*Bovine viral diarrhoea virus*
CA	Capsid
CAEV	Caprine arthritis encephalitis virus
CE	Cell equivalents
CNS	Central nervous system
CCR5	C-C (beta) chemokine receptor 5
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CTL	Cytotoxic T-lymphocyte
CXCR4	C-X-C (alpha) chemokine receptor 4
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide Triphosphate
DMSO	Dimethyl sulfoxide
dpi	Days post-infection
ELISA	Enzyme linked immunosorbent assay
EIAV	*Equine infectious anaemia virus*
EDTA	Ethylenediamine tetra-acetic acid
FIV	*Feline immunodeficiency virus*
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HRP	Horse radish peroxidase
HIV	*Human immunodeficiency virus*
HTLV-1	*Human T-cell lymphotropic virus type 1*
ID	Immunodominant
IgG	Immunoglobulin G
IR	Immunoreactive
IN	Integrase
JDV	*Jembrana disease virus*
LTR	Long terminal repeat
M-tropic	Macrophage tropic
VMV	*Visna maedi virus*
MHR	Major homology region
MA	Matrix
NC	Nucleocapsid
Nef	Negative factor
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PBS-T	Phosphate-buffered saline-Tween 20
PCR	Polymerase chain reaction
pi	Post-infection
PR	Protease

qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
Rev	Regulator of expression of virion proteins
RT	Reverse transcriptase
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
SIV	*Simian immunodeficiency virus*
SRLV	Small ruminant lentivirus
SU	Surface unit
T-tropic	T-lymphocyte-tropic
$TCID_{50}$	Median tissue culture infective dose
Tat	*Trans*-activator of transcription protein
TM	Transmembrane glycoprotein
U3	3’ Untranslated region
U5	5’ Untranslated region
Vif	Viral infectivity protein
VL	Viral load
Vpr	Viral protein R
Vpu	Viral protein U
Vpx	Viral protein X
WIB	Western immunoblotting
YT	Yeast tryptone broth

Publication and International Conference Presentations

Publications arising from this thesis

McNab, T., Desport, M., Tenaya, W. M., Hartaningsih, N., and Wilcox, G. E. (2010). Bovine immunodeficiency virus produces a transient viraemic phase soon after infection in Bos javanicus. *Vet. Microbiol.* 141**,** 216-223.

Desport, M., Ditcham, W. G., Lewis, J. R., McNab, T. J., Stewart, M. E., Hartaningsih, N., and Wilcox, G. E. (2009). Analysis of Jembrana disease virus replication dynamics in vivo reveals strain variation and atypical responses to infection. *Virology.* 386(2)**,** 310-6.

Lewis, J., McNab, T., Tenaya, M., Hartaningsih, N., Wilcox, G., and Desport, M. (2009). Comparison of immunoassay and real-time PCR methods for the detection of Jembrana disease virus infection in Bali cattle. *J Virol Methods.* 159(1)**,** 81-6.

Manuscripts submitted for publication

McNab, T., Desprt, M., Dobson, R., Tenaya, I.W.M., Hartaningsih, N., Wilcox, G.E. (2010) Prior Bovine immunodeficiency virus infection does not inhibit subsequent superinfection by the acutely pathogenic Jembrana disease virus. *Virology*.

Oral presentations

"Bovine immunodeficiency virus infection fails to provide protection against subsequent Jembrana disease virus infection" Presented at the European Society for Veterinary Virology Conference in Budapest, Hungary 2009.

Poster presentations

"Towards a Jembrana disease virus specific diagnostic immunoassay- peptide mapping of gag and env proteins of bovine lentiviruses" Presented at the European Society for Veterinary Virology Conference in Budapest, Hungary 2009.