DETECTION OF VIRUSES THAT HAVE THE POTENTIAL TO ALTER THE PATHOGENICITY OF WILD TYPE MAREK'S DISEASE VIRUS

Thesis submitted by

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Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Statement of the Contribution of Others

This project was supervised by Dr. Graham W. Burgess, Dept. of Microbiology and Immunology, James Cook University, Townsville Australia. The laboratory procedural assistance was obtained from Mr. Ramon Layton, Dept. of Microbiology and Immunology, James Cook University, Townsville Australia. I am thankful to Dr. Graham Burgess for editorial and proofreading of the thesis.

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ABSTRACT

Marek's disease virus serotype one (MDV-1) causes neuropathic, cytolytic and lymphoproliferative disease in poultry. Chicken anaemia virus (CAV) is also a pathogen causing anaemia and immunosuppression in chicken. Marek's disease serotype two virus (MDV-2) is naturally occurring in chicken and apathogenic virus. Natural multiple infection of these three viruses is possible. Presence of CAV and MDV-2 in the poultry flocks is potential to change in the pathogenicity of MDV-1 and also the pathogenicity of the CAV when infected alone.

Three hundred and ten feather samples as three different panels were collected from representative broiler flocks throughout Australia. These broiler flocks had different vaccination history against MDV. These samples were examined to detect the status of MDV-2 and CAV. The results of nested polymerase chain reaction (PCR) demonstrated that these two viruses are present in the broiler flocks. The samples were also investigated for MDV-1 by Mr. Ramon Layton (JCU) and the data from the MDV-1 investigation was analysed together with these two viruses. The flocks, vaccinated with HVT (herpesvirus of turkey) were found to be free from both MDV-1 and MDV-2. However, after the cessation of vaccination, both MDV1 and MDV-2 reappeared, first MDV-1 and then MDV-2. Interestingly when the MDV-2 was increasing progressively, the MDV-1 was decreasing. Although the available data is not sufficient to draw a conclusion, it is strongly suggestive that the presence of MDV-2 in infected birds changes the pathogenicity of MDV-1.The MDV-2 possibly works as a vaccine reducing the clinical MDV despite up to 30% prevalence of wild type MDV-1.

The CAV was present in all the flocks tested with more or less similar pattern of the viral distribution ranged between 50%-100%. Two sets of nested primers were used for detection of the CAV variably reacted with samples. The difference in the reactivity of the primer was then assumed as primer sequence mismatch due to CAV strain differences. This project further aimed to determine the source of CAV in the broilers. The CAV can be transmitted through both horizontal and vertical route. Both parents can transmit the virus to the progeny. To control clinical disease in young chicks, breeding flocks are generally vaccinated with a live vaccine.

Possible source of CAV infection in the broiler flocks and the genetic divergence of the CAV detected in the samples were determined by sequencing the CAV field isolates selected on the basis of reactivity differences of two nested primer pairs and geographical location of the broiler farms. Eight field isolates, vaccine strain 3711 and a reference strain BF4 were sequenced generating 951 bp long sequences covering complete VP3 gene, 3' 86% of VP2 gene and starting 42% of VP1 gene including hypervariable region. The sequence alignment and evolutionary analysis of these data, only Australian isolates and along with other global CAV isolates demonstrated that the Australian CAV field isolates fitted in to three different groups. Vaccine (strain 3711), strain BF4 and one of the field isolates included in a group, six field isolates included in next group together with CIA-1, and remaining one field isolate included in the third group together with the Cux-1.

Although one of the field isolate is included in the group along with vaccines, the isolate had genetic differences sufficient to differentiate from the strain 3711. These results demonstrated that the CAV in the broilers is not the vaccine virus. In addition this group of virus have two non-synonymous mutations in the VP3 gene, not described to dates in the sequences of CAV global isolates except in the sequences of strain CAU/7 (an Australian isolate).

To differentiate possible vertical and horizontal transmission of CAV in the broiler flocks, weekly sequential samples from week one till slaughter from one of the previous test flock was collected and investigated by nested PCR. The nested PCR result demonstrated that the birds were infected from week one. Eighty percent of the samples tested contained CAV DNA in it. This result further indicated that the virus was transmitted vertically. However the infection was sustained to the flock with similar pattern of distribution in the subsequent week samples till slaughter.

Nearly full length genomic identification of the vaccine virus was determined (19 bp shorter). In attempts to sequence full length genome of one field isolates from three genetically different groups of isolates, five different sequence data were obtained. Three overlapping primer pairs were designed to complete the sequences of the remaining 1347/1368 bp long DNA. The same primers were used for amplification

of vaccine strain as well. Two sequences, 723 bp and 438 bp long were identified as chicken genome, one 563 bp long as *Psychrobacter arcticus* 273-4 and two sequences, 1.28 kb and 499 bp long do not have sequence identification more than twenty base pair except primer sequence as CAV at both end of the sequence. These sequence data demonstrated that chicken/other bacteria also have the primer sequence similarity in their genome enough to initiate DNA amplification in that given condition. In addition, these sequences data of chicken genomes or would be chicken genome or the bacterial genome further indicates reliability of the single round PCR results if used as diagnostic tool.

The results from this project demonstrated that MDV-2 and CAV are present in Australian broilers. The presence of MDV-2 infection in the broiler flock prior to MDV-1 infection is likely to decrease the pathogenicity of MDV-1. Every broiler flocks is likely to have CAV infection. At lease three different strains of CAV are circulating in the present Australian commercial broilers. The CAV strains were not the vaccine virus administered to the parental flocks. The CAV strains were wild type virus most likely vertically transmitted to the broilers and they were maintained in the broiler shed environment.

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LIST OF ABBREVIATIONS

µ	Microns
µg	Microgram
µl	Microlitre
A260	Absorbance at 260 nanometres
A280	Absorbance at 280 nanometres
B-cell	B -lymphocyte
Bcl-2	Cancer expressing protein
BFDV	Beak and feather disease virus
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CAV	Chicken anaemia virus
CD	Cluster of differentiation
CEF	Chicken embryo fibroblasts
CIA-1	Chicken infectious anaemia-1
CKC	Chicken kidney cells
CO_2	Carbon dioxide
CPE	Cytopathic effect
CTL	Cytotoxic T lymphocytes
Cux-1	Cuxhaven-1
DEF	Duck embryo fibroblast
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide 5'-triphosphates
dsDNA	Double stranded DNA
EDTA	Ethylene diamine tetra-acetic acid
EGTA	Ethylene glycol tetra-acetic Acid
ELISA	Enzyme-linked immunosorbent assay
ERE	Estrogens response element
FAPP	Filtered-air, positive-pressure
FBS	Foetal bovine serum

FFE	Feather follicle epithelium
FREIT	Fluorescence resonance energy transfer
g	Unit of acceleration
g	Gram
GaHV-3	*Gallid Herpesvirus* Type 3
G-C	Guanine and cytosine
HVT	Herpes virus of turkey
IBDV	Infectious bursal disease virus
IFAT	Indirect immunofluorescent antibody test
IFNγ	Interferon gamma
IPTG	Isopropyl-beta-D-thiogalactopyranoside
IL-6	Interleukin 6
IL-18	Interleukin 18
IR_L	Long internal repeat
IR_S	Short internal repeat
IU	International unit
kb	Kilo base
kDa	Kilo Dalton
LB	Luria-Bertani
M	Molar
MATSA	Marek's disease tumour-associated surface antigen
MBP	Maltose-binding protein
MD	Marek’s disease
MDV	Marek's disease virus
MDV-1	Marek's disease virus serotype one
MDV-2	Marek's disease virus serotype two
MSB1	Marek’s disease lymphoblastoid cell lines
mg	Milligram
Mg^{+2}	Magnesium ion
MHC	Major histocompatility complex
ml	Millilitre
mM	Millimolar
NDV	Newcastle disease virus

ng	Nanogram
NO	Nitric oxide
P53	Cancer expressing protein
PBS	Phosphate buffer saline
PBS A	Phosphate buffer saline A
PCR	Polymerase chain reaction
PFU	Plaque formation unit
pH	The negative logarithm of the hydrogen ion concentration
qPCR	Real-time quantitative PCR based serum neutralisation
RECC-CU205	Reticuloendotheliosis virus transformed T-cell line
rpm	Revolutions per minute
RPMI	Rosewell Park Memorial Institute
RNase	Ribonuclease
SN	Serum neutralisation
SOgE-QM7	Recombinant quail muscle cell line
SPF	Specific pathogen free
ssDNA	Single stranded DNA
TAE	Tris acetate EDTA
T-cells	T- lymphocyte
$TCIC_{50}$	Tissue cytotoxic infective dose
TCR+	T-cell antigen receptor
Th	T-helper cell
T_m	Melting temperatures
TR_L	Long terminal repeat
TR_S	Short terminal repeat
U_L	Unique-long
U_S	Unique-short
UV	Ultra violet
vIL8	Viral interleukin-8
VP	Viral protein
vvMDV	Very virulent pathogenic strains of MDV
vv+ MDV	Very virulent plus strains of MDV
w/v	Weight per volume

X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
°C	Degree Celsius