DETECTION OF AVIAN LEUCOSIS VIRUS SUBGROUP J IN POULTRY TISSUE SAMPLES AND THEIR MOLECULAR CHARACTERIZATION

BY

DR. BALA RAM THAPA CHHETRI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science.

January 2004

Dedicated to
My Beloved Family: Parents, Wife Usha Thapa and Two Sons named
Ayush Bikram Thapa and Atish Bikram Thapa

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Veterinary Science

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Chairman: **Associate Professor Abdul Rahman Omar, Ph.D.**

Faculty: **Veterinary Medicine**

This study was carried out to diagnose and characterize avian leucosis virus subgroup J (ALV-J) specific sequence isolated from poultry organs with myelocytic infiltration. Archived tissues with and without myelocytic infiltration were examined by PCR followed by sequencing. Four ALV-J sequences identified and named as; UPM/A6, UPM/A10, UPM/A17 and UPM/A18 were characterized based on sequence and phylogenetic analysis. Different diagnostic tests (PCR, ELISA and Virus isolation) for ALV-J were also studied and compared. A total of 21 poultry tissue samples were examined by PCR using primers (H5/H7) and 16 samples were found positive for ALV-J proviral DNA. However, only 5 samples were found positive for ALV-J viral RNA. Sequence analysis indicated that the 4 sequences have significant homology (>90%) when compared to ALV-J from UK and USA. However, based on phylogenetic analysis, the sequences of the ALV-J were close to Houghton Poultry Research Station -103 (HPRS-103). In addition 3, 10, 3 and 8 amino acid substitutions were observed in sequences; UPM/A6, UPM/A10, UPM/A17 and UPM/A18, respectively. All these substitutions

were unique and have not been reported before from other ALV-J isolates. The importance of these substitutions requires further study especially in order to determine whether the sequences resemble variant ALV-J from UK or USA.

Different diagnostic techniques were also compared for the detection of ALV-J in a normal broiler breeder flock as the first isolation of ALV-J was made from normal meat-type chickens. PCR was found to be more sensitive than ELISA and virus isolation. However, even though the chickens were gp85 antibody positive, all the samples examined showed negative result for ALV-J proviral DNA and virus isolation. In addition, no virus was isolated from archived tissue samples with myelocytic infiltration. The actual explanation for this finding is not clear, but several probable factors were presented and discussed. In conclusion, ALV-J proviral DNA were detected in tissue samples obtained from chickens with and without myelocytic infiltration. However, no virus was isolated. The importance of PCR in detecting proviral DNA and viral RNA from chickens with gp85 antibody requires careful examination due to the complex nature of ALV-J infection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PENGESANAN VIRUS *AVIAN LEUCOSIS VIRUS SUBGROUP J* DALAM SAMPEL TISU AYAM DAN PENCIRIAN MOLEKUL

Oleh

DR. BALA RAM THAPA

January 2004

Pengerusi: Profesor Madya Abdul Rahman Omar, Ph.D.

Fakulti: Perubatan Veterinar

Kajian ini dijalankan untuk mendiagnosis dan mencirikan jujukan spesifik avian leucosis virus subgroup J (ALV-J) daripada sampel yang dipencilkan dari organ ayam dengan infiltrasi myelosit. Tisu arkib yang berinfiltrasi dan tanpa infiltrasi myelosit diperiksa menerusi PCR, diikuti dengan penjujukan. Empat jujukan ALV-J, telah dikenalpasti dan dinamakan sebagai UPM/A6, UPM/A10, UPM/A17 dan UPM/A18 telah dicirikan berdasarkan analisis jujukan dan filogenetik. Ujian diagnosis berlainan (PCR, ELISA and Virus pemencilan) juga dikaji dan dibandingkan. Sejumlah 21 sampel yang telah diperiksa menerusi PCR dengan menggunakan pencetus (H5/H7) dan 16 sampel didapati positif bagi proviral DNA ALV-J. Walau bagaimanapun, hanya 5 sampel yang didapati positif bagi RNA virus ALV-J. Analisis jujukan menunjukkan bahawa 4 jujukan tersebut mempunyai kesamaan ketara (> 90%) apabila dibandingkan dengan ALV-J dari UK dan USA. Walau bagaimanapun, berdasarkan analisis filogenetik, jujukan ALV-J tersebut didapati lebih hampir kepada Houghton

Poultry Research Station-103 (HPRS-103). Sejumlah 3, 10, 3 dan 8 perubahan asid amino masing-masing diperhatikan pada jujukan UPM/A6, UPM/A10, UPM/A17 dan UPM/A18. Kesemua perubahan ini adalah unik dan belum pernah dilaporkan daripada isolat ALV-J lain. Kepentingan perubahan ini memerlukan kajian lanjut terutamanya untuk menentukan sama ada jujukan tersebut menyerupai ALV-J varian dari UK atau USA. Teknik diagnosis yang berlainan juga diperbandingkan untuk mengesan ALV-J di dalam ayam pembiak pedaging normal kerana pemencilan pertama ALV-J dibuat melalui ayam pedaging yang normal. PCR didapati lebih sensitif daripada ELISA dan pemencilan virus. Walau bagaimanapun, bagi ayam yang didapati positif dengan antibodi gp85, kesemua sampel yang diperiksa menunjukkan keputusan negatif bagi DNA provirus ALV-J dan pemencilan virus. Tambahan pula, tiada virus dapat dipencilkan daripada arkib sampel tisu yang bermyelosit. Huraian sebenar bagi keputusan ini adalah tidak jelas, tetapi beberapa faktor telah diketengahkan dan dibincangkan. Kesimpulannya, DNA provirus ALV-J dapat dikesan dalam sampel tisu yang didapati daripada ayam yang berinfiltrasi dan tanpa infiltrasi myelosit. Namun begitu, tiada virus yang dipencilkan. Kepentingan PCR dalam pengesanan DNA provirus dan RNA virus tersebut daripada ayam yang berantibodi gp85 memerlukan pemeriksaan yang teliti, memandangkan sifat jangkitan ALV-J yang kompleks.

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I certify that an Examination Committee met on 28th January 2004 to conduct the final examination of Bala Ram Thapa on his Master of Veterinary Science thesis entitled "Detection of Avian Leucosis Virus Subgroup J in Poultry Tissue Samples and Their Molecular Characterization" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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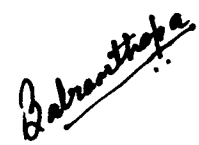
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.



DR. BALA RAM THAPA CHHETRI

Date: 9th feb. 2004

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%	percentage
µ	micro (10^{-6}x)
µg	microgram
µl	microlitre
A	adenine (nucleotide)
A	Alanine
A-	without neutralizing antibody
A+	with neutralizing antibody
ab	antibody
AEV	avian erythroblastosis virus
ag	antigen
ALSV(s)	avian leucosis sarcoma virus (es)
ALV (s)	avian leucosis virus (es)
AMV	avian myeloblastosis virus
AMV-RT	avian myeloblastosis virus reverse transcriptase
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
Bp	base pair
C	cysteine
C	cytosine (nucleotide)
CAV	chicken anaemia virus
cDNA	complementary DNA
CEF	chicken embryo fibroblast
CI	chloroform isoamylalcohol
cm	centimeter
cm^2	centimeter square
cm^3	centimeter cube
C-myc	cellular oncogene
CO_2	carbon dioxide
COFAL	compliment fixation test for avian leucosis virus
CPE	cytopathic effect
CRD	chronic respiratory disease
D	aspartic acid
Da	Dalton
DDBJ	DNA Data Bank of Japan
DEPC	diethyl pyrocarbonate
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acids
dNTP	deoxyribonucleotides
DR	direct repeat
DTT	dithiothretol
E	glutamic acid
EAV	endogenous avian virus
EAV-HP	endogenous avian retrovirus named Houghton Poultry
ed (s)	editor(s)
EDTA	ethylene diamine tetra acetic acid

ELISA	enzyme-linked immunosorbant assay
ELL	East Lansing Line
EMBL	European Molecular Biology Laboratory
env	envelope gene
ev	endogenous virus
F	phenylalanine
FBS	fetal bovine serum
FITC	fluorescein isothiocynate
G	glycine
g	gravity
G	guanidine (nucleotide)
gag	group-specific antigen gene
GCG	genetic computer group
gm	gram
gp37	glycoprotein 37 kDa protein
gp85	glycoprotein 85 kDa protein
GS	group specific
GSA	group specific antigen
H E	Haematoxilin and Eosin
H	histidine
HPRS	Houghton Poultry Research Station
hr	heterogeneous region
I	isoleucine
IBS	Institute of Bioscience
id	identification
IFAT	Indirect immunofluorescent antibody test
Ig G	Immunoglobulin G
K	lysine
kbp	kilobase (pair)
KCl	Potassium chloride
kDa	kilodalton
KH_2PO_4	potassium dihydrogen phosphate
L	leucine
l	litre
LL	lymphoid leucosis
LPDV	lymphoproliferative disease virus
LTR	long terminal repeat
M	methionine
M	Molar
M.W.	molecular weight
MC 29	avian myelocytomatosis virus MC 29
mg	milligram
$MgCl_2$	magnesium chloride
MH2	avian myelocytomatosis and carcinoma virus MH2
min	minute
ml	millilitre
ML	myeloid leucosis
mM	millimolar
mm^3	milimeter cube
mRNA	messenger RNA

MV29	myeloblastosis virus 29
N	aspargine
Na_2HPO_4	di-sodium hydrogen phosphate anhydrous
NaOAc	sodium acetate
NDV	Newcastle disease virus
ng	nanogram
NJ	Neighbor-Joining
nm	nanometer
no.	number
NP	nonproducer
nr	non-redundant
nt	nucleotide
O.D.	optical density
^oC	degree celcius
ORF	open reading frame
P	proline
p.p.	pages
p27	protein 27
PBS	phosphate buffered saline
PCI	phenol:chloroform:isoamylalcohol
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
pg	picogram
PM	phenotypic mixing
pmole	picomole
pol	polymerase
pp^{60}	phosphoprotein 60 kilodalton
PRC	Prague subgroup C
Q	glutamine
QC-RT-PCR	Quantitative competitive reverse transcriptase Polymerase Chain Reaction
R	arginine
RAV-1	Rous-associated virus subgroup A
RAV-2	Rous-associated virus subgroup B
rc	replication-competent
rd	replication-defective
Ref. no.	reference number
REV	reticuloendothelial virus
RIF	resistance inducing factor
RNA	ribonucleic acid
rpm	revolution per minute
RSV	Rous sarcoma virus
RT	reverse transcriptase
RT	room temperature
rTM	redundant transmembrane
RT-PCR	reverse transcriptase polymerase chain reaction
S-	non-shedder
S	serine
S+	shedder
SDS	sodium dodecylsulphate

Sec	second (time)
src	viral oncogene
ss	single-stranded
SU	surface protein
T	threonine
T	thymine (nucleotide)
TBE Buffer	tris base EDTA Buffer
TCID	tissue culture infectious dose
TCIU	tissue culture infectious unit
TM37	transmembrane 37k Da protein
TMB	3,3',5'-tetramethyl-benzidine
U	unit
UK	United Kingdom
UPM	Unversiti Putra Malaysia
USA	United States of America
UV	ultraviolet
V- onc	viral oncogene
v-	no viremia
V	valine
V/V	volume/volume
V-erb	viral erb oncogene
V-myc	viral myc oncogene
VN	virus neutralization
vr	variable region
vvIBDV	very virulent infectious bursal disease virus
W	tryptophan
W/V	weight/volume
Y	tyrosine

CHAPTER I

GENERAL INTRODUCTION

Avian leucosis virus subgroup J (ALV-J) is an economically important pathogen of meat-type bird. ALV-J causes serious economic losses in poultry industry since significant growth suppression of an average 6-11% (Landman *et al.,* 2002) and mortality due to myeloid leucosis and related tumours was 22% (Witter *et al.,* 2000), which further leads to carcass condemnation. The virus was first reported in United Kingdom in 1989 (Payne *et al.*, 1991a, 1991b and Payne, 1992). Since then it has been reported from all over the world, which include USA (Smith *et al.,* 1998a), South America (Buscaglia *et al.,* 2000), Central America (Neuman *et al.,* 2000), Africa (Aly, 2000), Middle East (Banet *et al.,* 2000), Japan (Nakamura *et al.,* 2000), Australia (Bagust, 2000), Switzerland (Wunderwald *et al.,* 2001), the Netherlands (Landman *et al.,* 2002), Korea (Sung *et al.*, 2002a), Taiwan (Wang and Juan, 2002), Malaysia (Omar *et al.,* 2002) and China (Xu B. *et al.,* 2003).

The main clinical abnormality associated with ALV-J is myeloid leucosis (ML), which is characterized by multiple myelocytomas on the bone surfaces, commonly evident on the inner sternum, ribs and enlargement of visceral organs due to infiltration of myelocytes. The myelocytes are large round immature granulocytes with many eosinophilic granules in the cytoplasm normally found in bone marrow but detected in blood and other organs during certain diseases (Payne *et al.,* 1992b). However, histiocytic sarcomatosis, pulmonary sarcoma,