UNIVERSITI PUTRA MALAYSIA

DETECTION AND IDENTIFICATION OF JAAGSIEKTE RETROVIRUS ASSOCIATED WITH SHEEP PULMONARY ADENOMATOSIS

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By

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Chairman: **Prof. Dr. Mohd Zamri Saad**
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Sheep pulmonary adenomatosis (SPA) or jaagsiekte is a neoplastic disease of sheep, caused by jaagsiekte retrovirus (JSRV) infection in the lungs. In Malaysia, the disease was first observed in 1993, involving mainly the purebred imported sheep and their crossbreeds, and a long time after the first importation of sheep from Australia into Malaysia in 1987.

The JSRV was found to transform the alveolar type II cells in the lungs of sheep to become neoplastic cuboidal cells arranged in acinar or papillary patterns. The neoplastic cells were found to cover the entire alveolar wall with numerous macrophages filling the affected alveoli particularly the alveoli surrounding the neoplastic area.

The affected lung tissue and lung fluid were successfully processed to partially purify the materials before the JSRV was successfully detected under the

electron microscopy from several local SPA-affected sheep, which showed clinical signs typical of sheep pulmonary adenomatosis. Following the detection of JSRV under the electron microscopy, the virus was later confirmed as the JSRV by using the reverse-transcription polymerase chain reaction (RT-PCR) technique at the viral nucleic acid level.

The study, therefore, confirmed that JSRV is present in Malaysia and thus, should be considered in the diagnosis of sheep diseases and in controlling the spread of contagious diseases such as sheep pulmonary adenomatosis.

Following the confirmation of the jaagsiekte retrovirus in Malaysia, a RT-PCR diagnostic method was developed based on the U3 gene of the retrovirus. The method was able to identify the presence of retrovirus in the peripheral blood of sheep with and without clinical signs of sheep pulmonary adenomatosis. Thus, the technique can be used to identify subclinically infected animals for culling.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ljazah Doktor Falsafah

PENGESANAN DAN PENGENAL-PASTIAN VIRUS JAAGSIEKTE RETROVIRUS PENYAKIT ADENOMATOSIS PULMONARI BIRI-BIRI

Oleh

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Januari 2002

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Adenomatosis pulmonari bebiri (SPA) atau Jaagsiekte ialah sejenis penyakit neoplasia pada biri-biri akibat jangkitan peparu oleh jaagsiekte retrovirus (JSRV). Penyakit ini mula ditemui di Malaysia dalam tahun 1993 apabila malibatkan biri-biri baka tulen yang diimport dan kacukannya. Walau bagimanapun, penyakit ini dikesan setelah sekian lama pengimportan pertama dibuat dari Australia.

Kajian menunjukan virus JSRV menukar sel alveolus jenis II dalam paru-paru biri-biri menjadi sel epitelium kuboidal yang disusun bercorak asinus atau papilari. Sel-sel neoplasia ini di dapati menyelaputi keseluruhan bahagian dinding alveolus sedangkan makrofaj pula membanjiri rongga alveolus, terutama sekali di bahagian keliling kawasan berneoplasia.

Tisu peparu yang terlibat dan cecair telah diproses untuk penulenan separa. Hasil daripada penulenan separa ini, JSRV telah berjaya dikesan di dalam sampel

tersebut melalui pemeriksaan mikroskopi elektron. Pengesanan ini telah dilakukan ke atas beberapa ekor biri-biri Malaysia yang dikatakan telah dijangkiti penyakit tersebut serta menunjukkan petanda klinikal yang jelas. Setelah virus berjaya dikesan, virus ini kemudiannya telah dikenalpasti sebagai JSRV melalui ujian menggunakan teknik tindakbalas rangkaian polimerase pentranskripsian terbalik (RT-PCR) ke atas asid nukleus virus.

Maka, hasil kajian ini telah mengesahkan bahawa JSRV memang terdapat di Malaysia dan sepatutnya diambil-kira dalam membuat diagnosis penyakit biri-biri dan dalam menangani sebaran penyakit berjangkit seperti penyakit adenomatosis pulmonari biri-biri.

Berikutan pengenal-pastian jaagsiekte retrovirus di Malaysia, kaedah diagnosis mengguna tindakbalas rangkaian polimerase pentranskripsian terbalik (RT-PCR) telah dihasilkan berdasarkan gen U3 retrovirus. Kaedah ini boleh mengenal-pasti kehadiran retrovirus dalam sampel darah biri-biri sama ada yang menunjukkan petanda klinikal penyakit adenomatosis pulmonari biri-biri atau tidak. Maka, teknik ini boleh diguna untuk mengenal-pasti biri-biri yang dijangkiti secara subklinikal agar ia boleh ditakai.

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A	
α	alpha
Amp	ampocillin
ATP	adenosine triphosphate
B	
β	beta
bp	base pair
BLV	bovine leukemia virus
C	
C	cytosine/core
°C	degrees centigrade
cm	centimeter
cpm	counts per minute
D	
DNA	deoxy ribonucleic acid
DTT	1,4-Dithiothreitol
dsDNA	double stranded DNA
dsRNA	double stranded RNA
E	
EDTA	ethylenediaminetetra-acetic acid
ELSA	enzyme-linked immunosorbent assay
EM	electron microscopy
F	
Fig.	Figure
G	
g	gram
GA	glutaraldehyde
H	
H	hour
H & E	haematoxylin and eosin staining
I	
Ig G	immunoglobulin G
IPTG	isopropyl- ß-D-thiogalactopyranoside

J

JSRV	jaagsiekte retrovirus

K

kb	kilobase
kg	kilograms

L

L	liter
LB	Luria broth

M

μ	micro
μg	microgram (10^{-6} g)
μl	micro liter (10^{-6} L)
μM	micro molar (10^{-6} M)
M	Molar
mg	magnesium
min	minute
ml	millitre
mm	millimeter (10^{-3} L)
mmol	millimolar
MMTV	mouse mammary tumor virus
MPMV	Mason-Pfizer monkey virus
mRNA	messenger RNA
MuSV	mirine sarcoma virus
MVV	Maedi-Visna virus

N

Na	sodium
ng	nanogram (10^{-9} g)
nmol	nanmolar

O

OD	optical density
OH	hydroxyl radical
ORF	open reading frame

P

P	polymerase protein
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pH	Puissance hydrogene
pmole	picomole

R

RBC	red blood cell
RNA	ribonucleic acid
RT	reverse transcriptase,
rpm	revolutions per minute

S

s	second
SDS	sodium dodecyl sulfate
SDW	sterile distilled water
SMRV	squirrel monkey retrovirus
SPA	sheep pulmonary adenomatosis
ssDNA	single stranded DNA
ssRNA	single stranded RNA

T

TBE	Tris-boric acid-EDTA buffer
TBS	tris-buffered saline
TEM	transmission electron microscopy

U

U	unite
UPM	Universiti Putra Malaysia
UV	ultraviolet

V

V	volt
Vol	volume
v/v	volume / volume

W

w/v	weight / volume

X

Xg	centrifugal force
X-Gal	5-bromo-4-chloro-3-indolyl-ß-D-galactoside

CHAPTER 1

INTRODUCTION

Sheep pulmonary adenomatosis (SPA), ovine pulmonary carcinoma (OPC) and jaagsiekte are alternative terms describing a contagious lung neoplasm of sheep. It is caused by a jaagsiekte retrovirus (JSRV) that is transmitted by the aerosol route. The clinical signs include rapid breathing, moist rales, coughing and production of a pulmonary fluid that drains from the nostrils. The dyspnea is most evident when affected animals are being herded leading to the African term 'jaagsiekte' or driving sickness for the disease.

Sheep pulmonary adenomatosis is classified as a bronchioloalveolar carcinoma (Stunzi et al., 1974). It is a neoplastic disease of sheep in which the jaagsiekte retrovirus transforms the alveolar type II cells to become neoplastic cuboidal epithelial cells arranged in acinar or papillary patterns (Rosadio et al., 1988). The disease was first described in South Africa and since then in many parts of the world including Europe (Bassett et al., 1989; Houwers et al., 1984), the United States of America (Culip et al., 1982). In Malaysia, the disease was first observed in 1993 involving mainly the purebred imported sheep and their crossbreds (Zamri-Saad et al., 1996).

The first reported case in many countries has been associated with importation of susceptible sheep into the country (Houwers et al., 1984; Basset et al., 1989; Krishnan et al., 1994), and disease incidence usually increased

dramatically to involve many heads of animal and more sheep farms (Hunter et al., 1984; Zamri-Saad et al., 1996).

Based on earlier studies on the etiology, clinical and histopathology of sheep pulmonary adenomatosis, studies reported in this thesis were conducted with the following hypotheses:

Jaagsiekte retrovirus is present in Malaysia, and it would be confirm by using electron microscopy and the reverse-transcription polymerase chain reaction (RT-PCR) technique at the viral nucleic acid level.

The genomic RNA of the jaagsiekte retrovirus contains a unique nucleotide sequence, which is not available in the genome of other organisms or the host cells. If such area can be defined, specific DNA primers could be used to amplify the target region in the virus-specific manner by the hemi-nested reverse transcription polymerase chain reaction. Thus will enable the development of a diagnostic tool.

Since the disease was fairly new in this country (Zamri-Saad et al., 1996), the disease prevalence, transmission and development were unknown. Thus the objectives of this study were:

1. To study the tissue changes caused by jaagsiekte retrovirus following naturally occurring sheep pulmonary adenomatosis.
2. To describe sheep pulmonary adenomatosis and jaagsiekte retrovirus characteristics by electron microscopy.

3. To detect and confirm the presence of jaagsiekte retrovirus from several case of naturally occurring sheep pulmonary adenomatosis.
4. To develop the hemi-nested reverse-transcription polymerase chain reaction for detection of the jaagsiekte retrovirus. Cloning and sequencing of the suitable jaagsiekte retrovirus gene.
5. To determine and statistical analysis of the prevalence of the jaagsiekte retrovirus associated sheep pulmonary adenomatosis in Malaysia.

CHAPTER 2

LITERRATURE REVIEW

2.1. The Disease

Sheep pulmonary adenomatosis (SPA), jaagsiekte and ovine pulmonary carcinoma (OPC) are alternative terms describing a contagious lung neoplasm of sheep, apparently caused by a retrovirus known as the jaagsiekte retrovirus (JSRV). The virus is a slow virus with long incubation period, transmitted from the carrier ewes to the lambs by aerosol. There are occasional metastases to regional lymph nodes and other organs. The clinical signs of sheep pulmonary adenomatosis include rapid breathing, moist rallies, coughing, and over-production of a pulmonary fluid that drains from the nostrils. The dyspnea is most evident when affected animals are being herded leading to the Afrikaans terms for the disease of jaagsiekte, or 'driving sickness'.

Sheep pulmonary adenomatosis was first described in South Africa and since then in many parts of the world including Europe, the United States of America and Africa. In Malaysia, the disease was first observed in 1993 involving mainly the purebred imported sheep and their crossbreed (Krishnan et al., 1994). The first reported case in many countries has always been associated with importation of susceptible sheep into the country (Krishnan et al., 1994). Following the first incidence, the disease incidence usually increases dramatically to involve many more heads of animal and more sheep farms (Shah et al., 1997).

Sheep pulmonary adenomatosis is classified as bronchiole-alveolar carcinoma (Stunzi et al., 1974) with morphological similarities to human bronchiole-alveolar carcinoma (BAC), which constitutes between 3-5% of human pulmonary neoplasm (Sing et al., 1981; Perk et al., 1982). Both are relatively well-differentiated tumors of alveolar type 2 or non-ciliated bronchiolar (Clara) cells that exhibit malignant growth characteristics and have a peak occurrence in adults. Furthermore, the etiologies of both sheep pulmonary adenomatosis and bronchiole-alveolar carcinoma have been associated with a genetic factor (Wu et al., 1988). Studies on naturally occurring retrovirus-induced neoplasm, primarily lymphomas, have contributed immensely to the understanding of this disease.

2.2. Occurrence

Sheep pulmonary adenomatosis was first recognized in South Africa in the 19th century as a cause of dyspnea in sheep being driven. Hence the origin of the Afrikaans name 'jaagsiekte', meaning driving sickness (Verwoerd et al, 1985). Subsequent reports documented the occurrence of sheep pulmonary adenomatosis as a sporadic or endemic disease of sheep and occasionally goats (Sharma et al., 1975) in all continents of the world except Australia (Verwoerd et al., 1985). Sheep pulmonary adenomatosis has been recognized as a common disease of sheep in Peru (Cuba Caparo et al., 1961; Snyder et al., 1983) and Chile (Shultz et al., 1965). Based on gross examination of lungs, the rate of diagnosis of sheep pulmonary adenomatosis among male was 1.5 times more than that of female, but the differences in management of the sexes could not be excluded as a cause of the