UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF MALAYSIAN ISOLATES OF BOVINE HERPES VIRUS I OF BUFFALO ORIGIN

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By

LORETTA MARIE CHEOW

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Dedicated with love and gratitude to:

My husband Choo Teik:

Who taught me how to think independently, and was my source of encouragement and motivation whenever I got into a "brain rut"!

My parents Noel and Eunice:

Who started me all those years ago on the journey of knowledge which has brought me to where I am today.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

AO	Acridine orange
ATV	Antibiotic-Trypsin-Versene
Ab	Antibody
BHV-1	Bovine Herpes Virus I
CO2	Carbon dioxide
CPE	Cytopathic effect
DNA	Deoxyribonucleic acid
DNAase I	Deoxyribonuclease I
EDTA	Ethylene diamine tetra-acetate
FC	Final concentration
FBS	Foetal bovine serum
H&E	Hematoxylin and Eosin
IB	Inclusion body
IBR	Infectious Bovine Rhinotracheitis
IBRv	Infectious Bovine Rhinotracheitis virus
IP	Immunoperoxidase
IPV	Infectious Pustular Vulvovaginitis
IPVv	Infectious Pustular Vulvovaginitis virus
KT	Potassium tartrate
L-15	Leibovitz's L-15 media
L-15-S	Leibovitz's L-15 media with 5% foetal bovine serum
MDBK	Madin Darby bovine kidney
MW	Molecular weight
OD	Optical density

ODD	Ortho-dianisidine
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PFU	Plaque forming unit
PTA	Potassium phosphotungstate
RE	Restriction endonuclease
REA	Restriction endonuclease analysis
RNA	Ribonucleic acid
RNAase	Ribonuclease
SDS	Sodium dodecyl sulphate
TC	Tissue culture
TCID 50	50% Tissue culture infectious dose
TE	Tris-EDTA
TEN	Tris-EDTA-NaCl
UPM	Universiti Pertanian Malaysia
UV	Ultra violet
bp	base pairs
ds	double-stranded
kbp	kilobase pairs
mM	millimolar
nm	nanometre
p.i.	post-infection
rpm	revolutions per minute
ss	single-stranded
μl	microlitre

Abstract of thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

CHARACTERIZATION OF MALAYSIAN ISOLATES OF BOVINE HERPES VIRUS I OF BUFFALO ORIGIN

By

LORETTA MARIE CHEOW

April, 1992

Chairman : Professor Abdul Latif Ibrahim

Faculty : Veterinary Medicine and Animal Science

This research was carried out to investigate two isolates of bovine herpes virus I (BHV-1) isolated from buffaloes at Universiti Pertanian Malaysia, Serdang, Selangor Darul Ehsan, and compare them to a reference cattle strain of BHV-1 (Cooper strain).

The cytopathogenic and serologic properties of the three virus isolates (Buffalo respiratory, Buffalo genital, and the Cooper strain) were first studied. The cytopathogenicity of the three BHV-1 isolates was investigated using Acridine Orange staining (to compare localization of DNA), Hematoxylin and Eosin staining (to compare morphologic changes induced in cell culture), the Indirect Immunoperoxidase (IIP) staining method

(to compare localization of the virus antigen), plaque assay, and Negative Contrast Electron Microscopy. The cytopathogenic effects of the three BHV-1 isolates seen by staining were found to be similar. The viruses were also indistinguishable under the electron microscope, and formed plaques of similar morphology. The serologic properties of the BHV-1 isolates were investigated using the Serum Neutralization Test, Plaque Reduction Test, and the IIP test. Cytopathic effect in tissue culture and formation of plaques by the viruses were found to be inhibited by both homologous and heterologous antisera. Staining of the viral antigen was seen with both homologous and heterologous antisera in the IIP test. The results of these studies thus revealed that the three virus isolates were very similar antigenically and could not be differentiated by any of the above studies.

The viral isolates were then compared using Restriction Endonuclease Analysis (REA). The DNA of the BHV-1 isolates were cut with seven different REs, and compared using Agarose Gel Electrophoresis. The results showed that all three isolates were unambiguously differentiated with each RE used.

In conclusion, this study found that the three BHV-1 isolates were too similar antigenically to be differentiated by cytopathogenic and serologic studies. However, subsequent comparison of the viral DNAs using REA showed without a doubt that the three BHV-1 isolates were different from one another.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi keperluan Ijazah Master Sains.

PENCIRIAN ISOLAT-ISOLAT MALAYSIA HERPES VIRUS BOVIN I YANG BERASAL KERBAU

Oleh

LORETTA MARIE CHEOW

April, 1992

Pengerusi: Profesor Abdul Latif Ibrahim

Fakulti : Kedoktoran Veterinar dan Sains Peternakan

Penyelidikan ini dijalankan untuk menyiasat dua isolat Herpesvirus Bovin I (HVB-1) yang diasingkan daripada kerbau di Universiti Pertanian Malaysia, Serdang, Selangor Darul Ehsan, dan bandingkan isolat-isolat ini dengan strain lembu rujukan HVB-1 (strain Cooper).

Mula-mula, sifat-sifat saitopatogenik dan serologik ketiga-tiga isolat virus ini dikaji. Tiga strain virus ini adalah isolat pernafasan kerbau, isolat genital kerbau, dan strain Cooper. Kesitopatogenikan isolat-isolat HVB-1 ini disiasat melalui pewarnaan dengan Acridine Orange (untuk membanding penempatan asid deoksiribonuklik (ADN), pewarnaan dengan Hematoksilin dan Eosin (untuk membanding penukaran-

penukaran morfologik yang teraruh dalam kultur sel), kaedah pewarnaan Imunoperoksidas Tak Langsung (ITL) (untuk membanding penempatan antigen virus), assai plak, dan Mikroskopi Elektron Kontrast Negatif. Kesan-kesan sitopatogenik ketiga-tiga isolat HVB-1 yang dilihat melalui pewarnaan didapati serupa. Virus-virus ini juga tidak dapat dibezakan dengan menggunakan mikroskop elektron, dan membentuk plak-plak bermorfologi serupa. Sifat-sifat serologik isolat-isolat HVB-1 disiasat dengan menggunakan Ujian Peneutralan Serum, Ujian Penurunan Plak, dan Ujian ITL. Kesan saitopati dalam kultur tisu, dan pembentukan plak, direncat oleh antiserum homolog dan juga antiserum heterolog. Dalam Ujian ITL, pewarnaan antigen virus dilihat dengan antiserum homolog dan antiserum heterolog. Keputusan ujian-ujian ini menunjukkan bahawa ketiga-tiga isolat virus ini adalah sangat serupa secara antigenik dan tidak dapat dibezakan melalui sebarang ujian yang tersebut di atas.

Isolat-isolat virus ini kemudiannya dibandingkan dengan menggunakan Analisis Endonukleas Pembatas (AEP). ADN isolat-isolat HVB-1 dipotong dengan tujuh enzim pembatas (EP) yang berlainan, dan dibandingkan dengan menggunakan Elektroforesis Gel Agaros. Keputusannya adalah bahawa ketiga-tiga isolat ini dapat dibezakan dengan setiap EP yang digunakan.

Kesimpulannya, ujian ini telah mendapati bahawa ketiga-tiga isolat HVB-1 ini adalah terlalu serupa secara antigenik untuk dibezakan melalui ujian-ujian sitopatogenik dan serologik. Walau bagaimanapun, perbandingan ADN virus-virus

ini dengan AEP menunjukkan dengan tidak syak lagi bahawa isolat-isolat HVB-1 ini memang berlainan.

CHAPTER I

INTRODUCTION

Bovine herpes virus 1 (BHV-1) is a major pathogen of cattle. It is associated with a variety of clinical manifestations which include respiratory disease, genital disease, abortion, balanoposthitis, conjunctivitis and encephalitis. Among these, the two most prominent clinical entities are respiratory disease and genital disease.

The occurrence of BHV-1 in various clinical forms suggests that strains with differing tissue affinities may exist in the field (Kahrs, 1977). The strain causing respiratory disease is known as infectious bovine rhinotracheitis virus (IBRv), and that causing genital disease is known as infectious pustular vulvovaginitis virus (IPVv). However, the disease is commonly referred to as IBR.

Generally, BHV-1 infections of the IBR-like type seem to be of greater importance and significance than the IPV-like type (Ludwig, 1984). IBR is known worldwide as an acute, contagious viral disease of bovines which primarily affects the nasal and tracheal turbinates. However, abortion in infected females, meningoencephalitis (predominantly in young calves), conjunctivitis, mastitis, and enteritis may be observed. IPVv, on the other hand, does not exhibit such a high virulence as the IBRv does, and abortions due to IPVv are rare or non-existent (Wyler *et al.*, 1989).

BHV-1 is an economically important pathogen of cattle (Misra *et al.*, 1983). Serious economic losses are caused all over the world due to loss of animals, abortions, decreased milk production and loss of weight (Wyler *et al.*, 1989). In an investigation carried out by Wiseman *et al.* (1979), the most severe incident of IBR resulted in the loss of 19 out of 280 (7%) 12 - 18-month old bullocks. They estimated the total losses from deaths, considerable weight loss, extra feeding and treatment to approximate 20,000 pounds sterling.

IBR appears to be a naturally occurring disease of cattle only (McKercher, 1959), although goats (Mohanty *et al.*, 1972; Wafula *et al.*, 1985), swine (Derbyshire and Caplan, 1976) and water buffalo (Ibrahim *et al.*, 1983) can be infected. Cattle appear to be the principal reservoir of the virus (Kahrs,1977) with the virus apparently being widely distributed in cattle populations in all parts of the world.

Latency is defined as the silent persistence of the virus in the body, not detectable by conventional virological procedures, with subsequent intermittent episodes of reexcretion (Thiry *et al.*, 1986). As with all herpesviruses, BHV-1 has the ability to establish latency in an animal following natural infection or vaccination (Bitsch, 1984; Pastoret *et al.*, 1984; Straub, 1984; Conraths and Ludwig, 1988). Once in latency, the dormant virus can be reactivated (Wyler *et al.*, 1989). The virus may be reactivated by natural stimuli such as stress from disease, socialization, movement, transport, estrus, and parturition (Kahrs, 1977) or experimentally by the administration of

glucocorticoids such as dexamethasone (Pastoret *et al.*, 1984). This ability of the virus to lie latent is one reason why McKercher (1959) concluded that cattle themselves, either as subclinically infected animals or convalescent carriers or both, maintain the virus and serve as the chief means of spread.

In 1987, the number of cattle and buffaloes in Peninsular Malaysia stood at 579,726 and 143,766 respectively. While the cattle population is estimated to have increased to 623,900 in 1989, the buffalo population has slowly declined over the years to an estimate of 141,600 in 1989. The production of beef has been largely static, where in 1989, beef production remained at the level of about 13,800 metric tonnes which is equivalent to about 37% self-sufficiency (Department of Veterinary Services, 1990). Although local production of fresh milk has been able to meet 100% of the home demand, this is only because local demand of fresh milk forms only 4.5% of the total demand for all forms of milk. Malaysia's import bill for milk powder and other milk products is still high, and amounted to $605.26 million in 1985 (Mustaffa, 1988).

One way in which local dairy and beef production can be increased is by improving the buffalo population. Thus, the buffalo will be able to play an increasingly important role as an additional source of milk and meat.

There is no doubt that good management is essential towards the development of the dairy and beef industries.

However, there is no denying the debilitating effect that disease can have on animal productivity. As such, a constant surveillance on economically important diseases such as IBR-IPV, must be kept.

With this in mind, serological surveys to assess the status of IBR in Peninsular Malaysia have been carried out. Saw (1983) found serum neutralizing antibody (Ab) to IBRv in 52.52% of cattle and 65.07% of buffalo in Peninsular Malaysia. He found no significant difference in the susceptibility of cattle and buffaloes to IBRv infection ($P>0.01$). The survey carried out by Lo and Syed Hassan (1989) revealed that 49.68% to 64.49% of the cattle surveyed possessed antibodies to IBRv. This included 57 buffaloes which were found positive out of 63 tested. Since vaccination against IBRv is not practiced in Malaysia, we can presume that all the animals with positive titres to IBRv have been infected in the past with the field virus.

The buffalo has a good average daily gain, food conversion efficiency and carcass yield, while the high fat content in its milk enables the total fat yield per lactation to compare favourably with that of improved breeds of dairy cattle (Hilmi, 1984). Hence, the buffalo has the potential to be a good meat and milk producer. However, the true potential of the buffalo has never been realised due to two major problems afflicting the buffalo, ie. low reproductivity and high calf mortality. In view of the large number of buffaloes with positive Ab titres to IBRv, further research should be carried