



UNIVERSITI PUTRA MALAYSIA

**CHARACTERISATION OF NEW RAT CYTOMEGALOVIRUS
ISOLATES FROM RICE-FIELD RATS
(*RATTUS ARGENTIVENTER*)**

LAI KIT YEE

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**CHARACTERISATION OF NEW RAT CYTOMEGALOVIRUS ISOLATES
FROM RICE-FIELD RATS (*Rattus argentiventer*)**

By

LAI KIT YEE

**Thesis Submitted in Fulfilment of the Requirements for the
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Specially dedicated to

MUN YEE



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LIST OF ABBREVIATIONS

| | |
|-----------------|--|
| ATV | Antibiotic-Trypsin-Versene |
| CMV | Cytomegalovirus |
| CO ₂ | Carbon dioxide |
| CPE | Cytopathic effect |
| DAB | 3'-3' Diaminobenzidine |
| DNA | Deoxyribonucleic Acid |
| EDTA | Ethylene Diamine Tetra-Acetate |
| ELISA | Enzyme Linked Immunosorbent Assay |
| FBS | Foetal Bovine Serum |
| H&E | Hematoxylin-Eosin Staining |
| HCMV | Human Cytomegalovirus |
| HIS | Hyperimmune Serum |
| IIP | Indirect Immunoperoxidase |
| MCMV | Murine Cytomegalovirus |
| MEM | Minimum Essential Media |
| M_r | Relative Molecular Weight |
| OD | Optical Density |
| PBS | Phosphate Buffer Saline |
| PFU | Plaque Forming Unit |
| PVDF | Polyvinylidene Difluoride |
| SDS-PAGE | Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis |



| | |
|--------------------|------------------------------------|
| TCID ₅₀ | 50% Tissue Culture Infectious Dose |
| TE | Tris-EDTA |
| TEN | Tris-EDTA-NaCl |
| TEM | Transmission Electron Microscopy |
| Tg | Tanjung |
| UPM | Universiti Putra Malaysia |
| UV | Ultra Violet |
| bp | base pairs |
| ds | double stranded |
| kbp | kilobase pairs |
| kD | kilo delton |
| mM | millimolar |
| L | litre |
| p.i. | post-infection |
| rpm | revolutions per minute |
| μl | microlitre |

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

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By

LAI KIT YEE

May 1999

Chairperson: Mohd. Azmi Mohd. Lila, Ph.D.

Faculty: Veterinary Medicine

A serological survey for virus infections in rice field rats, *Rattus argentiventer* was conducted in five different geographic locations of Malaysia to identify viruses that have potential use in biological control of rat populations. The results indicated that rat cytomegalovirus (RCMV) was most prevalent, 50% of the rats had serum antibody to RCMV. Rat coronavirus was fairly common (16%) in these places, while relatively few (<5%) animals had antibodies against mouse adenoviruses, rat parvoviruses, Theiler's virus and Reovirus. None of the rat populations were found to have lymphocytic choriomeningitis virus, vaccinia virus, Hantaan virus, Seoul virus, pneumonia virus and Sendai virus implied that the local rice field rats were free from viruses that cause zoonotic diseases in human and livestock. Based on the present data, only RCMV fulfil the essential requirement to become an ideal virus for fertility control in rats since this virus has infected most of the population without causing lethal disease.



Rat cytomegaloviruses were successfully isolated from the kidneys and salivary glands of seropositive animals. The isolates had common herpesvirus morphology and produced typical cytomegalo-like cytopathic effects in rat embryonic fibroblasts that were comparable with those of the English strain of RCMV. Ultrastructural examination of the infected cells revealed two types of virus nucleocapsids: empty capsids and capsids with electron-dense core. Infected cells were characterised by chromatin margination, the presence of tubular structures and the formation of dense bodies. The cytopathogenicity of these viral agents was investigated by using indirect immunoperoxidase and indirect immunofluorescence assays. Staining of the English RCMV and the local isolates by both homologous and heterologous antisera indicated that these three RCMV isolates were antigenically similar. Both the isolates were host specific and relatively slow growing.

Since morphological and (cyto)pathological aspects failed to distinguish the two Malaysian isolates and the English RCMV, protein profiles of these viruses were compared. Protein compositions of extracellular virions were analysed by SDS-PAGE. Present finding showed that protein compositions among the RCMV isolates were slightly different in the ratios. Based on antigenic variation that exists among strains, Western blot successfully demonstrated that the three isolates were distinct from one another.

In conclusion, the study showed that RCMV is a suitable vector to carry immunocontraceptive gene because this virus has naturally infected the rats. The release of RCMV based contraceptive vaccine therefore would not introduce a new virus to the environment. Persistent and latent infection establishes by RCMV could

ensure long-lasting immune response. RCMV infection is species specific. It failed to replicate in non-rat cells thereby keeping other native species at low risk.



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**PENCIRIAN RAT CYTOMEGALOVIRUS BARU YANG DIPENCIL DARI
TIKUS SAWAH PADI (*Rattus argentiventer*)**

Oleh

LAI KIT YEE

May 1999

Pengerusi: Mohd. Azmi Mohd. Lila, Ph.D.

Fakulti: Perubatan Veterinar

Satu kajian serologi terhadap jangkitan virus pada tikus sawah padi telah dijalankan di lima lokasi geografi yang berlainan di Malaysia untuk pencaman virus yang berpotensi bagi mengawal populasi tikus secara biologi. Keputusan menandakan bahawa “rat cytomegalovirus” (RCMV) adalah paling prevalens, 50% tikus mempunyai antibodi serum terhadap RCMV. Coronavirus tikus agak biasa (16%) di kawasan-kawasan ini, manakala tidak banyak (<5%) tikus mempunyai antibodi terhadap adenoviruses mencit, parvoviruses tikus, virus Theiler's dan Reovirus. Tiada populasi tikus dijumpai membawa virus lymphocytic choriomeningitis, virus vaccinia, virus Hantaan, virus Seoul, virus Pneumonia dan virus Sendai menunjukkan bahawa tikus sawah padi tempatan adalah bebas daripada virus yang menyebabkan penyakit zoonosis di kalangan manusia dan haiwan ternakan. Berasaskan data ini, hanya RCMV yang memenuhi keperluan sebagai virus sesuai bagi mengawal pembiakan tikus kerana virus ini menjangkiti kebanyakan populasi tanpa menyebabkan penyakit maut.



Cytomegalovirus tikus telah berjaya dipencil daripada ginjal dan kelenjar liur tikus seropositif. Pencilan mempunyai morfologi herpesvirus biasa dan menghasilkan kesan sitopati berupa “cytomegalo” pada fibroblas embrio tikus yang setanding dengan RCMV pencilan English. Dua jenis virus nukleokapsid dijumpai semasa pemeriksaan ultrastruktur sel yang dijangkiti: kapsid kosong dan kapsid yang mengandungi pusat elektron-padat. Sel yang terjangkit dicirikan marginasi kromatin, kehadiran struktur tubula dan pembentukan badan padat. Sitopatogenik virus diperiksa dengan teknik imunoperoksidase dan imunopendaflouran tak langsung. Perwarnaannya RCMV English dan pencilan tempatan dengan kedua-dua antiserum homolog dan heterolog menandakan ketiga-tiga pencilan RCMV adalah serupa dari segi antigen. Kedua-dua pencilan adalah perumah spesifik dan lambat membiak.

Disebabkan aspek morfologi dan (sito)patologi gagal membezakan kedua-dua pencilan Malaysia dan English RCMV, profil protin virus ini dibandingkan. Kandungan protin virus ekstrasel dianalisis dengan SDS-PAGE. Keputusan menandakan kandungan protin di kalangan pencilan RCMV hanya berbeza sedikit dalam nisbah mereka. Berasaskan kelainan antigen yang wujud di antara pencilan, teknik “Western blot” berjaya mempamerkan ketiga-tiga pencilan tersebut adalah berbeza daripada satu sama lain.

Kesimpulannya, kajian ini menunjukkan RCMV adalah vektor yang sesuai untuk membawa gen imunocegahhamil kerana virus ini menjangkiti tikus semulajadi. Pembebasan vaksin pencegahhamil berasaskan RCMV tidak akan memperkenalkan virus baru kepada alam sekitar. Jangkitan berterusan dan pendam RCMV boleh memastikan tindakbalas imun yang berpanjangan. Jangkitan RCMV adalah spesifik kepada spesis. Ia gagal membiak dalam sel yang bukan berasal daripada tikus, oleh itu ianya berisiko rendah ke atas haiwan lain.

CHAPTER I

INTRODUCTION

Rodents particularly rats are viewed as important agriculture pest by Malaysian Government. The main rat species found in Malaysia are *Rattus argentiventer* in rice, *Rattus rattus tiomanicus* in oil palm, *Rattus rattus diardii* in store grain and recently in oil palm too, also *Rattus exulans* in houses. Rats caused significant damage to crops. Malaysian Agricultural Research and Development Institute (MARDI) estimated an average annual losses caused by rats to rice crop alone is around 5%, equivalent to US\$17.3 million (Embi Yusof, Deputy Director MARDI, 1982). These losses are extremely patchy and often individual farmers suffer substantial losses of more than 50%. Unfortunately, little effort is given on rat pest management.

Chemical and mechanical methods are commonly used by farmers for rat control. These methods are costly and of limited usefulness as rats are well known for their high breeding rate. According to Semple (1982), “world-wide, 3.5 million rats are born everyday. In India, where the human population exceeds 600 million, rats outnumber man ten-fold.” The need for a biological control agent in manipulating rat populations is widely recognised following the successful use of myxoma virus to control European rabbits in Australia (Fenner and Ratcliffe, 1965).

An effective way for controlling rats would be to target their reproduction. A new method known as ‘viral-vectored immunocontraception’ is being developed by



the Vertebrate Biocontrol Centre in Australia. In this approach, the target species is induced to become infertile by mounting an immune response to the proteins involved in its own reproduction which are delivered via a recombinant virus (Shellam, 1994; Chambers, 1997). Sperm antigens (Shagli *et al.*, 1990) and zone pellucida peptides (Millar *et al.*, 1989) have been shown to cause contraception in rats and mice.

Murine cytomegalovirus (MCMV) has been revealed as a potential viral vector for immunocontraception in mice (Shellam, 1994). Recently, two new rat cytomegaloviruses (RCMV) were isolated from rice-field rats (Lai *et al.*, 1998). It appears that RCMV has many similar features common to MCMV. The virus could possibly be a suitable candidate for the same purpose in controlling the rat populations.

Human cytomegalovirus (HCMV) has been recognised as an important pathogen of man. It is believed to be one of the major causes of human birth defects including mental retardation, microcephaly, blindness and deafness. It is also the most frequent viral cause of morbidity and mortality in patients receiving immunosuppressive therapy after organ transplantation. Therefore, RCMV has been used to provide an excellent model for the pathogenesis study of HCMV.

Cytomegaloviruses produce cytomegalic structures in infected cells. Intranuclear inclusions had been recorded in the salivary glands and kidneys of wild rats long before the causative agent was identified (Thompson, 1932; Kuttner and Wang, 1934; Syverton and Larson, 1947; Lyon *et al.*, 1959; Rabson *et al.*, 1969). Later, the virus was also detected in livers, spleens, brains, lungs, skin and even hearts (Priscott and Tyrrell, 1982). Two RCMV strains have been isolated from wild brown rats, *R. norvegicus* and both have been well characterised (Priscott and

Tyrrell, 1982; Bruggemen *et al.*, 1982). The RCMV is a species specific herpesvirus which establishes acute, latent and persistent infection in rats (Bruggeman *et al.*, 1983). Similar to HCMV, RCMV infection is harmless and symptomless in most animals that are immunocompetent. Mortality however has been reported in newborn rats and immunosuppressed animals.

Species specificity, capability of establishing latent and persistent infection, having large DNA genome, are some of the main features that make RCMV an attractive vector for fertility associated genes. Previous workers have demonstrated that RCMV only grew on rat cells. It fails to replicate or infect human and mouse cells (Bruggemen *et al.*, 1982) thereby keeping the native rodent and other species at minimum risk. Latent and persistent infection in addition will provide a reservoir of virus for restimulation of the immune response, ensuring long-lasting immunity towards the encoded fertility proteins. However, the pathogenesis of CMV infection, the basis and exact site of latency, as well as the reactivation process are still poorly understood.

Before MCMV is recognised as potential viral vector, a survey of murine viruses in mice, *Mus domesticus* (Smith *et al.*, 1993) and a study of the dynamics of the seroprevalence of six of these viruses in wild mouse population (Singleton *et al.*, 1993) were conducted to identify the best candidate for the above purpose. Similar survey was carried out in rice-field rat populations (Lai *et al.*, 1997) to determine if there were other candidates more suitable than RCMV since RCMV had not been reported yet in rice-field rats.

The objectives of this study are therefore:

1. to determine the status of common viruses in rice-field rats.
2. to determine the prevalence of RCMV infection in rice-field rats including its geographic distribution in Malaysia.
3. to isolate and characterise RCMV isolated from rice-field rats.

CHAPTER II

LITERATURE REVIEW

Many natural viruses of rodents are of value to researchers whereby they provide experimental system of interest in themselves like lactic dehydrogenase virus in mice or as models for diseases of man or animals of veterinary importance. Examples of such models are the use of lymphocytic choriomeningitis virus to study immunopathology, mousepox to study the pathogenesis of generalised infection as well as the role of cell-mediated immunity in the recovery process and the use of murine cytomegalovirus as model of human disease.

Common Viruses of Rats

Rats (*R. rattus*, *R. norvegicus*, *R. diardii*, *Mus musculus*, etc.) carry a wide variety of viruses. Those frequently found are lymphocytic choriomeningitis virus (LCMV), Hantaan virus, cytomegalovirus (CMV), pneumonia virus, Sendai virus, Theiler's virus, mouse adenovirus, coronavirus, parvovirus and reovirus. Viruses that can cross-infect human and livestock such as Hantaan virus, LCMV are always the main concern.

Hantaan virus causes haemorrhagic fever with renal syndrome in human, a syndrome often characterised by acute high fever, shock, hemorrhage and renal failure. An outbreak occurred during the Korean War, thousands of cases were reported and caused the death of 10 to 15% of those infected. Rats have been the

