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SHORT COMMUNICATION

SEROPREVALENCE OF RAT CYTOMEGALOVIRUS IN RICE-FIELD RATS (*RATTUS ARGENTIVENTER*)

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SUMMARY

An indirect enzyme-linked immunosorbent assay (ELISA) was developed to study the prevalence of antibody against rat cytomegalovirus (RCMV) in rice-field rats. The survey was conducted in five different geographic locations of Malaysia in order to determine the possible virus distribution. The results indicated that almost 50% of 295 rats had antibody against RCMV and the prevalence was consistent in all areas tested. This implies that even in different environmental conditions, RCMV can be successfully maintained in the rice-field rat populations. The high prevalence and persistency rates of RCMV infection within the rat populations have placed RCMV on the list of potential viral vector for immunocontraception. In this study, cross-reactivity was demonstrated among the strains of RCMV isolated from the different rat species.

Keywords: rat, cytomegalovirus, prevalence

INTRODUCTION

In recent years rat cytomegalovirus (RCMV) has received increasing attention because it provides important insights into the pathogenesis of human cytomegalovirus (HCMV) infections. The RCMV appears to be ubiquitous in wild rats when CMV-like inclusions were observed in wild rat tissues followed by successful isolation and characterisation of the virus (Bruggemen *et al.*, 1982; Priscott and Tyrrell, 1982). However, the actual data regarding the prevalence of RCMV infection in feral rat colonies is still lacking. In Malaysia, there is limited knowledge on RCMV infection and no survey has been conducted.

Prevalence data of RCMV in the present study would be used to decide its potential use for the development of immunocontraceptive vaccine to control rat populations. Although a survey of other common viruses in rice-field rats in Malaysia had been conducted (Lai *et al.*, 1997), none of these viruses seemed to fulfil the requirement as a vector to carry immunocontraceptive genes because their prevalence in the populations was too low. Therefore, the present study was carried out to obtain data regarding the geographic distribution of RCMV and its prevalence rate in rice-field rat, *Rattus argentiventer*.

MATERIALS AND METHODS

Preparation of antigen

The English strain of RCMV, obtained from Dr. G.R. Sandford (Medical College of Wisconsin, USA), was originally isolated from *Rattus norvegicus* (Priscott and Tyrrell, 1982). The virus was grown in rat embryonic fibroblast (REF) cells. The infection was allowed to advance until 90% CPE, which was normally observed within 6-8 days. The cells were then loosened from tissue culture flasks using sterile glass beads, collected in 50mL tubes and centrifuged at 3,000 rpm for 10 min. The cell pellet obtained was re-suspended in medium and stored at -70°C. The supematant was saved for use to isolate extracellular virions.

The extracellular virus was pelleted from the tissue culture supernatant by centrifugation at 40,000 rpm for 2 h at 4°C. The virus pellet was re-suspended in 1 mL TNE buffer (10mM Tris, 1 mM EDTA, 100mM NaCl, pH 7.2) and centrifuged at 3,000 rpm for 1 min. The concentrated virus suspension was layered onto a 10-60% (W/V) linear sucrose gradient and centrifuged at 25,000 rpm for 1.5 h at 4°C. The fraction containing virions was collected and diluted with 10 volumes of TNE buffer before being centrifuged at 40,000 rpm for 1 h at 4°C to pellet the purified viral particles. Finally, the pure virus was suspended in 100 µL TNE buffer and the protein concentration was determined by UV spectrophotometer.

Sampling procedure

A total of 295 rice-field rats were trapped at four different locations in Peninsular Malaysia; Parit, Seberang Prai, Tanjung Karang and Besut. Rats were anaesthetised with chloroform and 2-3 mL of blood was collected from each animal by cardiac puncture. Sera obtained from blood samples after centrifugation at 3,000 rpm were stored at -20°C until used.

ELISA procedure

An indirect ELISA was developed based on a well-established protocol (Lussier *et al.*, 1987). Each well of the microtiter plates (Immulon 2, Dynatech) was coated with 50µL of RCMV antigens in bicarbonate buffer, pH 9.6. After overnight incubation at 4°C, plates were washed three times with PBS containing 0.05% Tween 20 (PBST). Fifty µL of 2% SSA was added and incubated at 45°C for 2 h to block non-specific binding. The plates were washed three times with PBST before 50 µL of the test sera, diluted at 1:100 in PBST, were added. After 2 h of incubation at 37°C, the plates were washed three times with PBST. Fifty µL of 1:1000 pre-diluted goat anti-rat immunoglobulin peroxidase-conjugated (Sigma) was then added and allowed to react with antigen bound-rat antibodies. The plates were then incubated for 2 h. The washing step was repeated before 50 µL of freshly prepared substrate (10mg ABTS in citrate phosphate buffer, pH 5.0 containing 1µL of 30% H_2O_2) was added and incubated for 40 min at room temperature. Optical density was determined by ELISA reader (Dynatech MR7000) at dual wavelength mode, 410-490nm. Hyperimmune serum against the Dutch strain of RCMV obtained from Dr. C.A. Bruggemen (University Hospital Maastricht, Netherlands) and laboratory rat's serum were used as positive and negative controls respectively.

Thirty-five out of the 295 serum samples were randomly selected and tested against two local RCMV strains (UPM/kn and UPM/sg) recovered from rice-field rats, *R. argentiventer*. The microtiter plates were divided evenly and coated with the above mentioned three isolates of RCMV to minimise the standard errors.

RESULTS

Prevalence of RCMV

As shown in Table 1, RCMV was found in all selected locations. Out of 295 serum samples collected, approximately 49% of the samples were seropositive for RCMV. The prevalence of RCMV in Seberang Prai was highest (61.5%), followed by Parit (49.0%), Tanjung Karang (35.0%) and Besut (26.9%).

The 35 serum samples that were tested against two local RCMV strains revealed cross reactivity. There was no significant difference ($p > 0.05$) between the antibody titres to the heterologous and homologous antigens (Fig. 1).

Table 1. Prevalence of RCMV infection in rice-field rats at various locations in Peninsular Malaysia

Location	No. rat	Strong[a] +tive	Positive[b]	% +tive	% -tive
Parit	100	12	37	49	51
Sbrg Perai	109	33	34	62	38
Besut	26	1	6	27	73
Tg Karang	60	0	21	35	65
Total	295	46	98	49	51

[a]OD $\geq$ mean of negative sera $\pm$10SD
[b]OD $<$ mean of negative sera $\pm$10SD

DISCUSSION

To identify the rats with RCMV infection, serological assays have been developed, which include the serum neutralisation test, the indirect

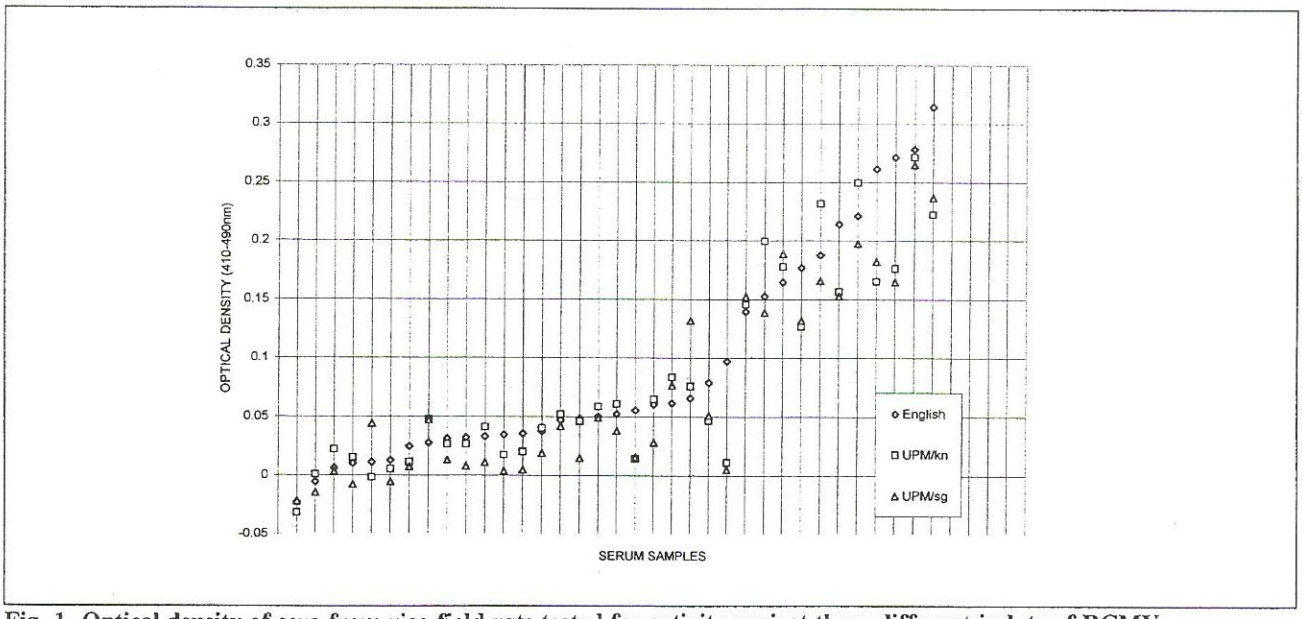

Fig. 1. Optical density of sera from rice-field rats tested for activity against three different isolate of RCMV.

immunofluorescence (IFA) test (Bruggemen *et al.*, 1982; Priscott and Tyrrell, 1982) and the ELISA (Schmitz *et al.*, 1977; Bruggemen *et al.*, 1983). A good correlation was found when the ELISA antibody titres were compared with the neutralising antibody titres (Bruggemen *et al.*, 1983). Lussier *et al.* (1987) on the other hand, compared the relative sensitivity of complement fixation (CF), IFA and ELISA tests for detection of MCMV antibody. They found that ELISA was less sensitive for detection of early antibody in acute infection but was useful for detection of antibody in chronically infected colonies. ELISA was preferred in this study due to the large number of samples involved.

The results of present study indicate that RCMV has consistent distribution in rice-field rat populations throughout Peninsular Malaysia. Approximately 49% of the rats tested at different places were seropositive for RCMV. This is in agreement with the previous finding that reported that nearly 50% of trapped wild rats, *R. rattus* in Panama had histologic evidence of RCMV infection (Rabson *et al.*, 1969). The widespread RCMV infection was not surprising because once an animal becomes infected, the virus remains alive within its body for life and transmission occurs readily when the rats are in close contact. The existence of RCMV infection in all selected locations in this study suggests that this virus is capable of maintaining itself in the rat population even under different geographical areas. Persistent infection was proven by successful virus isolation from the kidney and the salivary gland of seropositive animals (unpublished data).

A large variation in antibody titres among the rats in this study was observed. The OD readings ranged from 0.2 to 0.9. However, a study on MCMV revealed no clear correlation between the virus titres and the antibody level (Booth *et al.*, 1993). Similarly, the antibody titre does not reflect the level of CMV infection. The ELISA data, however, can be used to determine the stage of CMV infection if paired tests are performed (CDC, 1998). Paired tests showing a four-fold rise in IgG antibody and a significant level of IgM (equal to at least 30% of IgG value), indicate an active CMV infection.

In order to investigate to what extent the result would be affected, two partially characterised RCMVs (UPM/kn and UPM/sq strains) infecting *R. argentiventer* (unpublished) were used in place of the English strain. The results indicate that there were no significant changes in the overall results. One of the possible reasons could be due to the antibody cross react in a heterospecific binding manner.

There were some doubts that the ELISA data were not RCMV strain specific since there was a possibility for other isolates to co-exist. Infection by multiple isolates was reported in murine cytornegalovirus (MCMV) infection of wild mice. Four distinct strains of MCMV were identified from a seropositive wild mouse by restriction enzyme analysis and RFLP (Booth *et al.*, 1993). The same phenomenon may also occur with RCMV in rats; thereby there is possibility for all three separate isolates to co-exit in the presence of heterologous antiviral antibodies.

It must be emphasised that seronegative rats were not necessarily free from CMV infection. These rats might be latently infected. By using the nucleic acid hybridisation techniques, Cheung *et al.* (1980) demonstrated the presence of MCMV genome in specific pathogen free (SPF) mice that were both seronegative and virus negative. Therefore, the data collected in this study give only a gross estimation of the true prevalence of RCMV in rice-field rats.

A widespread occurrence and persistence in the population and the capability to establish latent infection have made RCMV as an ideal vector for immunocontraceptive protein delivery. These properties could provide a reservoir of virus for re-stimulation of immune response, thereby ensuring the encoded fertility proteins capable to evoke long-lasting immunity in the population (Shellam, 1994).

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RINGKASAN

SEROPREVALENS CYTOMEGALOVIRUS TIKUS PADA TIKUS BENDANG (RATTUS ARGENIVENTER)

Asai imunoserap terangkai-enzim (ELISA) tak langsung telah dibentuk bagi mengkaji prevalens antibodi terhadap cytomegalovirus tikus (RCMV) pada tikus bendang. Bagi menentukan kemungkinan taburan virus, kajian dijalankan di lima kawasan di Malaysia. Keputusan menunjukan hampir 50% tikus yang dikaji mempunyai antibodi terhadap RCMV dengan prevalens yang konsisten di semua kawasan yang diuji. Ini mengambarkan bahawa RCMV boleh tersenggara pada populasi tikus bendang dalam mana-mana keadaan persekitaran. Kadar prevalens dan kekalan jangkitan RCMV yang tinggi dalam populasi tikus menyenaraikan RCMV sebagai vector virus berpotensi untuk kegunaan pencegah-hamil-imuno.