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MURINE VIRUSES IN AN ISLAND POPULATION OF INTRODUCED HOUSE MICE AND ENDEMIC SHORT-TAILED MICE IN WESTERN AUSTRALIA

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ABSTRACT: House mice (*Mus domesticus*) were recently introduced to Thevenard Island, off the northwest coast of Western Australia. This island is also habitat for an endangered native rodent, the short-tailed mouse (*Leggadina lakedownensis*). Concerns have been raised that house mice may pose a threat to *L. lakedownensis* both through competition and as a source of infection. To assess the threat to *L. lakedownensis* posed by viral pathogens from *M. domesticus,* a serological survey was conducted from 1994 to 1996 of both species for evidence of infection with 14 common murine viruses (mouse hepatitis virus, murine cytomegalovirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus strains FL and K87, minute virus of mice, mouse parvovirus, reovirus type 3, Sendai virus, Theiler's mouse encephalomyelitis virus, polyoma virus, pneumonia virus of mice, and encephalomyocarditis virus) and *Mycoplasma pulmonis.* Despite previous evidence that populations of free-living *M. domesticus* from various locations on the Australian mainland were infected with up to eight viruses, *M. domesticus* on Thevenard Island were seropositive only to murine cytomegalovirus (MCMV). Antibodies to MCMV were detected in this species at all times of sampling, although seroprevalence varied. Infectious MCMV could be isolated in culture of salivary gland homogenates from seropositive mice. In contrast, *L. lakedownensis* on Thevenard Island showed no serological evidence of infection with MCMV, any of the other murine viruses, or *M. pulmonis,* and no virus could be isolated in culture from salivary gland homogenates. Although MCMV replicated to high titers in experimentally infected inbred BALB/c laboratory mice as expected, it did not replicate in the target organs of experimentally inoculated *L. lakedownensis,* indicating that the strict host specificity of MCMV may prevent its infection of *L. lakedownensis.* These results suggest that native mice on Thevenard Island are not at risk of MCMV infection from introduced house mice, and raise interesting questions about the possible selective survival of MCMV in small isolated populations of *M. domesticus.*

Key words: Island populations, house mouse, *Leggadina lakedownensis,* murine cytomegalovirus, murine viruses, *Mus domesticus,* serological survey, short-tailed mouse.

INTRODUCTION

Thevenard Island (Western Australia) is habitat to a rare and indigenous mammal, the short-tailed mouse (*Leggadina lakedownensis*). In 1986, house mice (*Mus domesticus*) were first reported on Thevenard Island. They subsequently became established, raising concerns about their impact on, and possible competition with, the native mice. Recently, a study was undertaken to understand the factors which allow the coexistence of *M. domesticus* and *L. lakedownensis* on Thevenard Island (Moro, 1997). Their coexistence is unusual because *M. domesticus* tends to colonize disturbed habitats where they occur in greater numbers than native murids (Chapman, 1981). Since *M. domesticus* are a known reservoir for a number of infectious agents (Sage, 1981; Singleton, 1985; Singleton and Redhead 1990; Singleton et al., 1993; Smith et al., 1993; Taylor et al., 1994), it seemed likely that *M. domesticus* could constitute a threat to *L. lakedownensis,* not only through competition for space and food, but also by acting as a reservoir of infection for the population of native short-tailed mice.

Previous serosurveys have shown that up to eight of 14 common murine viruses and *Mycoplasma pulmonis* may infect wild

M. domesticus in Australia (Singleton et al., 1993; Smith et al., 1993). Seroprevalence to murine cytomegalovirus (MCMV), mouse hepatitis virus, and murine rotavirus was high, but was low for mouse adenovirus strain K87, parvovirus, reovirus type 3, lymphocytic choriomeningitis virus (LCMV), and Sendai virus. Singleton et al. (1993) reported that the prevalence of antibodies to a number of murine viruses, particularly mouse adenovirus, reovirus type 3, and parvovirus in *M. domesticus* trapped at Walpeup (Victoria, Australia) varied over time and appeared to be dependent upon the density of the mouse population. Murine cytomegalovirus was present in all populations of *M. domesticus* tested across Australia and in up to 90% of individuals within a population (Shellam, 1994). The prevalence of antiviral antibodies to MCMV also varied with the habitat where *M. domesticus* were trapped (Singleton et al., 1993; Smith et al., 1993).

Since *M. domesticus* and *L. lakedownensis* occupy the same habitat on Thevenard Island, the presence or absence of viruses in these species could indicate how closely they interacted within the last 10 yr, and how persistent or latent viruses may survive population bottlenecks. As part of a wider study on the interactions between native and introduced rodents on Thevenard Island, we have investigated the occurrence of infection of *M. domesticus* and *L. lakedownensis* on Thevenard Island with murine viruses and *M. pulmonis* which are known to infect other Australian populations of *M. domesticus.* Since infection of *M. domesticus* by MCMV was common, an attempt was made to infect *L. lakedownensis* experimentally with MCMV in the laboratory.

MATERIALS AND METHODS

Study site

Thevenard Island is a nature reserve (21°28′S, 115°00′E) that is situated 20 km off the northwestern coast of Western Australia (Fig. 1). The island is a semi-arid sand cay of 550 ha, and was formed from the deposition of marine sediments. The floristic composition is relatively homogeneous across the island, consisting of low vegetation of *Acacia coriacea* with a grass and chenopod understorey. The region experiences an arid-subtropical climate. Rainfall is seasonal, falling mostly between January and June. Tropical cyclones can be a major factor influencing the rainfall and generally occur between the months of November and May, although January to March would be considered the peak cyclone season. There are only two mammals present on the island, *L. lakedownensis* and *M. domesticus.*

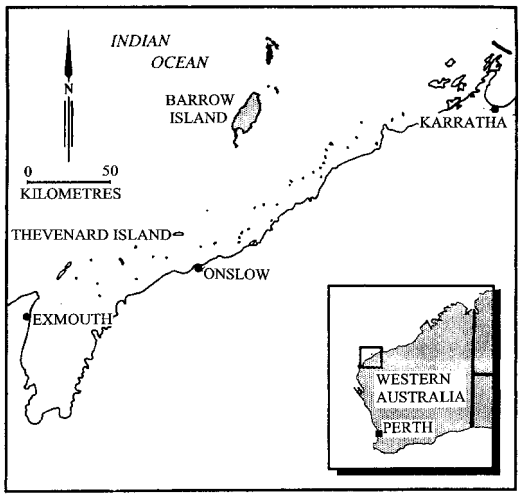

FIGURE 1. Location of study site at Thevenard Island off the northwestern coast of Western Australia.

Population census

This study was conducted between 1994 and 1996. Mice were live-trapped using standard Elliott (Elliott Scientific, Upwey, Victoria, Australia) and 20 L bucket pit traps (Rheem, Belmont, Western Australia, Australia) set in a grid network across the island. Trapping occurred every 8 to 12 wk, and information on the population dynamics, demographics, and breeding condition of mice was collected (D. Moro, unpubl. data). Mice were individually marked, and released at the site of capture after approximately 100 μl of blood was drawn into heparinised microhaematocrit tubes (Fortuna, Bildacker, Germany) from the infraorbital blood sinus of each mouse. Only adult mice (>9 g *M. domesticus,* >18 g *L. lakedownensis*) were bled because of the volume of blood required and to maintain a consistency in the age class sampled. The abundance of mice was estimated from numbers known-to-be-alive, and estimates were expressed as captures/100 trap-nights.

Sera and tissue collections

Blood samples were immediately centrifuged at 15,000 *g* for 5 min, and the plasma separated into fresh capillary tubes that were sealed and frozen at −70 C for later analysis of viral antibodies. Salivary glands collected from the mice immediately after a death in a trap were frozen at −70 C until they were assayed for the presence of virus.

Laboratory mice

Inbred, female BALB/c mice at 6 wk of age were obtained as specific pathogen free stock from the Animal Resources Centre (Canning Vale, Western Australia) and were barrier-maintained at the University of Western Australia (Perth, Western Australia). Routine serological surveys have revealed no evidence of viral infection in mice held in these conditions.

Enzyme-linked immunosorbent assay

The Enzyme-linked immunosorbent assay (ELISA) for detection of MCMV specific antibodies was a modification of the method used by Lawson et al. (1988). Serum dilutions began at 1:10 and consecutive doubling dilutions of sera were made in buffered saline adjusted to the osmolarity of mouse serum (MOBS) and supplemented with 1.0% bovine serum albumin and 0.05% Tween 20. The conjugate used was an alkaline phosphatase goat anti-mouse immunoglobulin (Biosource International, Camarillo, California, USA). The substrate solution consisted of 5 mg p-Nitrophenyl Phosphate tablets (Sigma, St. Louis, Missouri, USA) diluted in 5 ml diethanolamine buffer pH 9.8. The reaction was allowed to proceed and was read at 405 nm using a BioRad Model 3550 Microplate reader (BioRad, Hercules, California). Each test plate comprised six test sera and a positive and a negative control serum. The positive control was a hyperimmune serum produced in BALB/c mice by three intraperitoneal inoculations of 1×10^4 plaque forming units (pfu) of salivary gland derived K181 strain of MCMV. The mice were bled 2 wk after the last inoculation. The negative control was serum from normal, uninfected BALB/c mice. All serum samples from a trapping visit to Thevenard Island were tested in one run. Anti-MCMV titers were determined for each run by calculating the mean of all normal mouse sera ±3 standard deviations. The highest dilution with a reading greater than this value was designated the antibody titer to MCMV. A positive titer was considered to be >1:10. To determine whether the anti-immunoglobulin conjugate used detected immunoglobulin in *L. lakedownensis* serum, samples were spotted onto a Nitrocellulose membrane (BioRad), incubated with the goat anti-mouse conjugate and reacted with the chromogenic substrates 5-bromo-4-chloro-3-inodyl phosphate/ nitro blue tetrazolium (Sigma). The conjugate reacted equally well with serum from *M. domesticus* and *L. lakedownensis.* In contrast, the conjugate did not react with rabbit, marsupial mouse (dunnart), human, pig, and rat serum. This analysis was carried out on all new batches of conjugate used. Hyperimmune *L. lakedownensis* sera were made by inoculating two *L. lakedownensis* intraperitoneally three times over a 12 wk period with 2×10^4 pfu of the virulent K181 laboratory strain of MCMV derived by salivary gland passage in BALB/c mice. Two weeks after the final inoculation the animals were sacrificed and serum was collected. The *L. lakedownensis* sera were assayed for anti-MCMV antibodies using the ELISA described.

Immunofluorescence serology

Plasma samples from individual animals were titrated in Perth (Western Australia) by ELISA for antibodies to MCMV (see above), and the remaining samples were pooled, lyophilised and sent to Yale University (New Haven, Connecticut, USA), where screening for antibodies to 14 viruses, including MCMV, was undertaken. Concordance of seropositivity to MCMV in the sera tested by both laboratories suggested that lyophilisation and transport had not reduced antibody titers. After receipt, the samples were re-hydrated in sterile demineralized water and diluted 1:5 (*L. lakedownensis*) or 1:10 (*M. domesticus*) in phosphate buffered saline. Diluted samples from each pool were tested for reactivity with antigens by the anti-complement immunofluorescence (ACI) test (*L. lakedownensis*) or the indirect fluorescent antibody (IFA) test (*M. domesticus*). The ACI test is used for plasma samples derived from species for which there are no commercially available conjugates. Briefly, the diluted samples were heat-treated, and 10 μl of serum were added to 10 μl of 5% powdered nonfat milk 0.2% tween 20 solution on antigen-containing wells of glass slides. After 30 min at 37 C, the slides were washed three times and air dried. Fifteen μl of pre-titrated, diluted guinea pig complement were then added to the antigen-containing wells. Following a second 30 min incubation at 37 C, the slides were again washed three times and air dried. Fifteen μl of appropriately diluted FITC-conjugated anti-C3 were added for 15 min at 37 C, the slides were washed three times and air dried, cover-slipped and read with a fluorescence microscope. The

IFA test was performed exactly as described previously (Smith et al., 1993).

Viral antigens used in serologic assays

The antigens used were MCMV, mouse hepatitis virus, rotavirus, lymphocytic choriomeningitis virus, vaccinia virus (used to test for ectromelia virus antibody), mouse adenovirus strains FL and K87, parvoviruses (minute virus of mice and mouse parvovirus), reovirus type 3, Sendai virus, Theiler's mouse encephalomyelitis virus, polyoma virus, pneumonia virus of mice, encephalomyocarditis virus (EMCV), and *Mycoplasma pulmonis.* All antigens except EMCV were obtained and prepared as previously described (Smith et al., 1993). EMCV was obtained from the American Type Culture Collection (Rockville, Maryland, USA) and was grown in Vero (African green monkey) cells. Antiviral antibodies were scored as either present or absent.

Detection of MCMV in wild mice

Salivary glands from *M. domesticus* or *L. lakedownensis* were homogenised in 2 ml Minimal Essential Medium (MEM) (GibcoBRL, Life Technologies, Grand Island, New York, USA) plus 2% Newborn Calf Serum (NCS) (GibcoBRL, Life Technologies, Auckland, New Zealand). The homogenates were clarified by centrifugation at 4 C, 2,000 *g* for 20 min, and the supernatants were divided into aliquots and stored at −70 C until required. Mouse embryo fibroblast (MEF) cultures were made as described elsewhere (Lawson et al., 1987). Confluent MEF monolayers were inoculated with a 1:10 dilution of 10% (wt/vol) salivary gland homogenates and covered with 1.75% carboxymethyl cellulose (Sigma) in MEM with 2% NCS. After five days, viral plaques exhibiting a typical MCMV-induced cytomegaly were stained using an immunoperoxidase-conjugated goat anti-mouse immunoglobulin reagent (Biosource International) as described by Bartholomaeus et al. (1988).

Experimental infection of short-tailed mice and BALB/c mice

Eleven *L. lakedownensis* and 24 BALB/c mice were inoculated with 2×10^4 pfu K181 virulent Laboratory strain MCMV salivary gland virus by the intraperitoneal route. Animals were killed on days 3, 7 and 20 post inoculation; serum, salivary glands, spleen and liver were removed and stored at −70 C. For BALB/c mice, eight animals were used per time point, while for *L. lakedownensis,* three, four, and four animals were killed at days 3, 7 and 20 post inoculation, respectively. Sera were tested by ELISA to detect anti-MCMV antibodies, and organs were tested by plaque assay to detect replicating virus.

Plaque assay

The method used was a modification of that described previously by Lawson et al. (1987). Briefly, organs were homogenised in 1 mL MEM with 2% NCS using disposable tissue grinders (Quantum Scientific Pty. Ltd., Queensland, Australia). Tissue homogenates were clarified by centrifugation at 2,000 *g.* Dilutions of clarified homogenate were made in MEM with 2% NCS and allowed to incubate on 24 well trays of confluent MEF at 37 C for 1 hr. The inoculum was removed and 1.75% methylcarboxy cellulose (Sigma) in MEM with 2% NCS overlay was applied. Cells were incubated for 5 days, and then stained overnight with 0.5% methylene blue containing 10% formalin.

Statistical analyses

Comparisons of seroprevalence with trip, sex, breeding condition, and location were performed using Chi-square analysis (Sokal and Rohlf, 1981). Variables were transformed for normality before Pearson correlation analyses using SYSTAT (Wilkinson, 1988).

RESULTS

Population dynamics

House mice and short-tailed mice were regularly captured together within the same pit traps. The population of *M. domesticus* was low during 1994, and corresponded to an abundance of 5 to 8 mice/100 trap-nights (Fig. 2a). Numbers increased during 1995 to an average abundance of 17.3 mice/100 trap-nights. A population crash during April 1995 was believed to be the consequence of a severe tropical cyclone passing close to Thevenard Island during mid-February 1995. Mouse abundance remained high during 1996; another cyclone affected Thevenard Island in April 1996 and the abundance of *M. domesticus* was again reduced from >28 to 16 adult mice/100 trap-nights. Short-tailed mice showed a similar trend in population abundance as house mice, but at a lower abundance (Fig. 2a). Population demographic data for mice will be

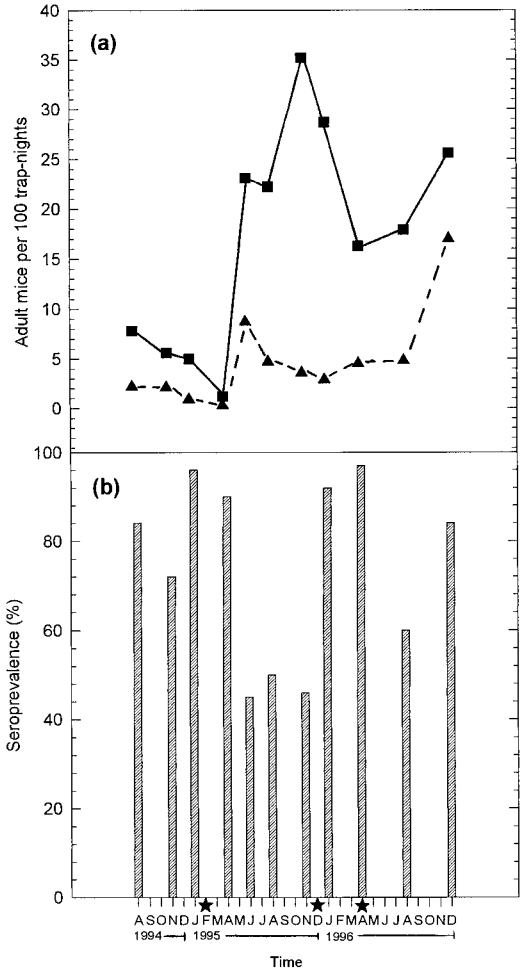

FIGURE 2. (a) Numbers of adult *Mus domesticus* (■) and *Leggadina lakedownensis* (▲) captured/100 trap-nights, and (b) seropositivity to murine cytomegalovirus in *M. domesticus,* on Thevenard Island. A star (★) indicates the occurrence of a tropical cyclone. Abbreviations on horizontal scale represent consecutive months beginning with August and ending in December.

presented elsewhere (D. Moro, unpubl. data).

Viral serology

Sera of *M. domesticus* and *L. lakedownensis* were screened for the prevalence of antibodies to 14 murine viruses and to *M. pulmonis.* Only antibodies to MCMV were detected in sera from *M. domesticus.* No antiviral antibodies were detected in the *L. lakedownensis* sera assayed. Data for experimentally inoculated *L. lakedownensis* (see below) gave support for an efficacious assay.

TABLE 1. Change in seroprevalence to murine cytomegalovirus in *Mus domesticus* on Thevenard Island, Western Australia.

Year	Time of sampling	n	ELISA titer[a] ≤1/10	>1/10 to <1/160	≥1/160
1994	August	6	17	66	17
	November	11	27	36	36
1995	January	22	4	37	59
	April	11	9	45	45
	June	20	55	35	10
	August	35	48	45	5
	November	29	55	46	0
1996	January	41	7	24	68
	April	45	2	31	66
	August	50	40	30	30
	December	39	16	26	58
	Total	309	25.6	38.6	35.9

[a] Values represent percentage of individuals within a titer range.

Analysis of anti-MCMV titers

Antibody titers to MCMV in the sera of *M. domesticus* were tabulated according to the month of collection, and are shown in Table 1. The average (±SE) number of *M. domesticus* that had antibody titers above 1:10 was 74.2 ± 6.2%. There were no significant differences between seropositivity and sex, location or breeding condition of house mice. The frequency of seropositivity in *M. domesticus* fluctuated significantly between months ($\chi^2 = 80.7$, df = 10, $P < 0.0001$) and varied from as low as 45% in June 1995 to as high as 97% in April 1996 (Figure 2b). Seropositivity averaged 78%, 65%, and 83% during 1994, 1995, and 1996, respectively. Seroprevalence to MCMV was lowest during mid-to-late 1995 in the sampled population of *M. domesticus,* and corresponded to a high rainfall and a high host density year compared with 1994 and 1996. However, these correlations were not statistically significant. There were no anti-MCMV antibodies de-

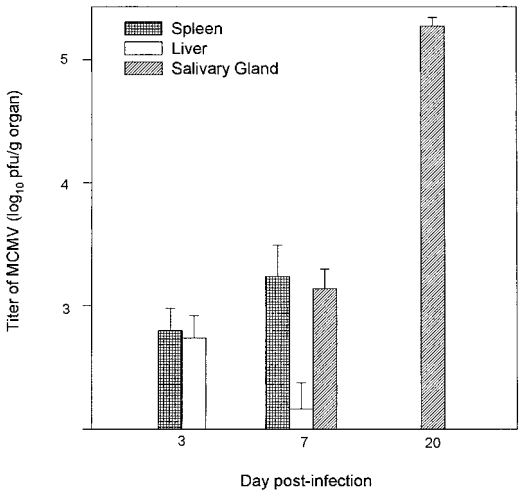

FIGURE 3. Titer of murine cytomegalovirus (MCMV) in BALB/c laboratory mice inoculated with 2×10^4 pfu of MCMV intraperitoneally. Values are mean ± standard error of titers in individual organs.

tected in the sera of 159 *L. lakedownensis* collected over this period.

Replication of MCMV in experimentally infected mice

As expected, MCMV replicated to high titers within a few days in the spleen and liver of BALB/c mice inoculated intraperitoneally with the virus, and reached high titers in the salivary glands, which is the site of viral persistence at later times (Fig. 3). However, for similarly inoculated *L. lakedownensis,* no MCMV was detected in the spleen, liver or salivary glands over the 3 wk period post-inoculation except in one plaque. Here, MCMV was detected in one of three replicates of a 1:10 dilution of spleen homogenate at day 3 from one animal. No plaques were observed in homogenates from any other organs or other animals at any time point, and could indicate that replication has merely occurred in the cell line and not necessarily in the organs. As a corollary to these data, 7 of 8 infected BALB/c mice developed antibodies to MCMV by day 7 post-inoculation, and by day 20, titers of 1:40 to ≥1:80 were recorded. In contrast, no *L. lakedownensis* had developed antibodies by day 7 post-inoculation, and the antibody response remained lower at day 20 than in BALB/c mice (Table 2). When *L. lakedownensis* received three intraperitoneal inoculations with 2×10^4 pfu MCMV over a 3 wk period, antibody titers of 1:1,280 were recorded 2 wk after the last inoculation. These titers are comparable to those of hyperimmune sera raised in BALB/c mice in a similar manner, and demonstrate that *L. lakedownensis* can respond immunologically to MCMV antigens. These data also establish that the ELISA was capable of detecting antibodies to MCMV in free living *L. lakedownensis* on Thevenard Island, if present.

Isolation of MCMV from salivary glands

Mouse embryo fibroblasts incubated with salivary gland homogenates from individual *M. domesticus* developed cyto-

TABLE 2. Change in prevalence of murine cytomegalovirus (MCMV) antibodies in BALB/c laboratory mice and *Leggadina lakedownensis* inoculated with MCMV.

Species	Day post-infection[a]	n	MCMV ELISA titer[b]			
			≤1/10	1/20	1/40	≥1/80
L. lakedownensis	3	3	3	0	0	0
	7	4	4	0	0	0
	20	4	3	0	0	1
BALB/c mice	3	8	8	0	0	0
	7	8	1	4	3	0
	20	8	0	0	2	6

[a] Mice of both species received 2×10^4 pfu of murine cytomegalovirus intraperitoneally, and serum was collected at the times shown.

[b] Values represent number of individuals within a titer.

pathic effects typical of MCMV infection, which was evident by the third day of culture. Discrete plaques stained positively for MCMV antigens by immunostaining. No cytopathic effect was detected in MEF cultured with salivary gland homogenates from *L. lakedownensis* over a 10 day observation period.

DISCUSSION

On mainland Australia, the coexistence of *M. domesticus* with native rodents in habitats as diverse as cropfields, coastal forests, and the arid zones of central Australia is not uncommon (Newsome and Corbett, 1975; Haering and Fox, 1997), but they are reported to be competitively excluded by native species during the later stages of mammalian succession in natural habitats (Lidicker, 1966; Fox and Pople, 1984). However, their coexistence with *L. lakedownensis* on Thevenard Island is unusual since both rodent species are forced to share the same habitat, and have done so for up to 10 yr. Therefore, the possibility of transmission of pathogens of *M. domesticus* to *L. lakedownensis* is increased through their close cohabitation, such as contact with feces, secreta, or aerosols.

Serosurveys conducted on wild rodents other than *M. domesticus* also show evidence of infection with many viruses. Ectromelia virus, pneumonia virus of mice, Sendai virus, LCMV and Theiler's mouse encephalomyelitis virus (TMEV) infect three species of wild British rodents (Kaplan et al., 1980), and infection with TMEV, reovirus type 3, ectromelia, LCMV, mouse adenovirus and mouse hepatitis virus are reported from wild meadow voles (Descôteaux and Mihok, 1986). Interestingly, only antibodies to MCMV were detected in the sera of house mice on Thevenard Island, in contrast to eastern Australia where antibodies were detected to a number of common murine viruses, including MCMV (Smith et al., 1993). However, population densities of this species were low on Thevenard Island compared to those reported for *M. domesticus* from southeastern Australia (Singleton et al., 1993). Some human viruses do not persist in isolated populations when human density is low (Black, 1975). For example, measles virus fails to persist in semi-isolated human populations below a critical population size of about 500,000 people because birth rates are too low to provide sufficient susceptible hosts for the maintenance of the virus in the population (Bartlett, 1960; Black, 1966). In contrast, varicella zoster virus, which like MCMV is a herpes virus with the ability to persist by establishing latent infection, can survive in small human populations on islands (Black, 1966). Since MCMV establishes latent infection in mice from which periodic reactivation may occur (Osborn, 1982), providing continuing opportunities for transmission, it is likely that the virus could persist in small populations such as those found on islands. A small host population may mitigate against the survival of other murine viruses which do not establish persistent infection. A recent serological study of wild *M. domesticus* on Gough Island in the south Atlantic Ocean supports this idea (G. Singleton, pers. comm.). Anecdotal evidence suggests that house mice were introduced accidentally onto Thevenard Island with cargo either for an oil storage facility there or from fishing vessels that anchor off the island. This suggests that the founder population of *M. domesticus* may have been small, and in the absence of further and unlikely introductions to the island following the establishment of quarantine procedures, murine viruses other than MCMV may have declined or been eliminated because of their inability to persist at low host density. The persistence of murine viruses other than MCMV also may be sensitive to population bottlenecks, such as founder events or severe population declines following cyclones. Therefore, it would be of interest to test the hypothesis that small island populations of house mice will only sustain viruses which establish persistent infection.

Our results show that seropositivity to MCMV in *M. domesticus* on Thevenard Island fluctuated over the 29 mo period covered by this study, from 97% to as little as 45% in the population sampled, making an interesting comparison with the stable and high levels of seropositivity in a mainland population of house mice (Singleton et al., 1993). The age of the host is a critical issue in the acquisition of endemic viral infections, the chance of becoming infected, and their seroprevalence with time. Although the ages of individual mice were not determined in this study, consistency was maintained by sampling blood from house mice that were >9 g in body mass. Of these mice, there were no significant correlations between body mass or reproductive condition with seropositivity. However, the simultaneous increase in population abundance, and decrease in seropositivity, seen in June 1995 may reflect an influx of previously uninfected (juvenile) mice into the population following recovery from the population decline caused by tropical cyclone (TC) Bobby in February of that year. High seropositivity detected during other periods may reflect an ageing murid population that is being repeatedly infected with MCMV, or a reactivation of the virus from the latent state. Further population studies are required to understand the relationship between environmental conditions, age-structure, and MCMV seroprevalence on Thevenard Island.

Climatic factors are recognized as important variables which influence population size and therefore the persistence of a disease (Levine, 1963; Dobson and Grenfell, 1995). Declines in the number of mice between January and April on Thevenard Island in both 1995 and 1996 were probably related to two TC's. TC Bobby (February 1995) was associated with the more extreme weather, resulting in excess of 400 mm of rain falling on the island within 3 days and gale force or stronger winds for 24 hr. Although TC Olivia (April 1996) caused significantly less rainfall (approximately 50 mm), it produced gale force or stronger winds for approximately 6 hr. Interestingly, there was no statistically significant correlation in our data between either rainfall (data not shown) or population density (Fig. 2a), and fluctuations in seropositivity to MCMV.

The lack of detectable seroconversion to MCMV in *L. lakedownensis* on Thevenard Island suggests that the virus is unable to infect and replicate in this species under normal environmental circumstances (notwithstanding the detection of a single plaque in only one homogenate as discussed above). The failure to isolate MCMV from the salivary glands, liver and spleen of experimentally inoculated short-tailed mice indicates that the virus is unable to replicate in these tissues and therefore may not be able to infect this host. All *L. lakedownensis* individuals maintained a healthy condition post-inoculation with MCMV unlike the BALB/c mice which developed clinical signs of MCMV infection. Nonetheless, the production of antibodies to MCMV by experimentally inoculated short-tailed mice demonstrates that this species is potentially capable of responding immunologically to MCMV antigens. Taken together, these data suggest that MCMV infection was restricted to its natural house mouse host on Thevenard Island.

In eastern Australia, wild populations of *M. domesticus* periodically reach plague proportions as a result of favourable climatic conditions, the availability of food, and the lack of competition from other granivores (Singleton and Redhead, 1990). At these times, maximum population densities may reach 3,000 mice/ha (Redhead and Singleton, 1988). In contrast, the population density of house mice on Thevenard Island, calculated from the number of individual mice known to be alive (D. Moro, unpubl. data), was low (0.7–13 mice/ha), and compares with densities of approximately 40 mice/ha in irrigated lands in southeastern Australia in non-

plague years (Redhead and Singleton, 1988). However, it is important to note that the generally low abundance on Thevenard Island was sufficient to support a seroprevalence to MCMV of at least 45% at all times.

Caution must be used when extrapolating results obtained from an ELISA that has been developed for *M. domesticus* sera to another species because of the risks of making statistical errors (Dobson and Hudson, 1995). The *L. lakedownensis* sera were nonreactive and generally produced lower background readings than the negative controls of normal mouse serum. Only limited seroconversion was detected from day 20 in short-tailed mice which were experimentally inoculated with MCMV, and we are confident that false negative results are unlikely. Although no viral replication was detected in *L. lakedownensis,* the possibility exists that replication below the level of detection could have occurred in the tissue tested or in organs not cultured for the virus.

This study has shown that on Thevenard Island, where two species of murids coexist, only antibodies to MCMV were detected in the sera of *M. domesticus.* There was no evidence of past infection with MCMV or other murine viruses, or with *M. pulmonis,* in the *L. lakedownensis* population. This, together with other evidence presented here, suggests that short-tailed mice are at no risk of infection with the introduced murine herpesvirus, MCMV. However, we also are aware of the possibility that bacteria and parasites may cross-infect *M. domesticus* and *L. lakedownensis.* With respect to bacterial infection, polyarthritis with swollen limb joints associated with *Streptobacillus moniliformis* infection is described in free-living *M. domesticus* in Australia (Taylor et al., 1994), and the fatal infection of the native Australian murid *Notomys alexis* by this organism following the bite of wild rats has been reported (Hopkinson and Lloyd, 1981). Interestingly, swollen limbs suggestive of this infection were occasionally observed in house mice trapped on Thevenard Island, but no such lesions were observed in short-tailed mice (D. Moro, unpubl. obs.). In conclusion, the approach employed in the present study in which evidence was sought for cross infection of *L. lakedownensis* with viruses from *M. domesticus,* could be applied to the study of other endangered species that occur sympatrically with exotics.

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