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The transmission rate of MCMV in house mice in pens: implications for virally vectored immunocontraception

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Abstract. Pest mammals have severe economic, environmental and social impacts throughout the world. Fertility control could reduce these impacts. Murine cytomegalovirus (MCMV) is being considered as an immunocontraceptive vector to control outbreaks of house mice (*Mus domesticus*) in Australian grain-growing regions. For successful control, a modified MCMV must transmit at a sufficient rate to keep populations of house mice below acceptable economic thresholds. We used disease models developed previously by using observations of free-ranging wild-mouse populations to assess the transmission rate of two laboratory strains of MCMV (N1 and G4) collected in a previous experiment. Mice contained in pens were deliberately infected with the N1 strain only, or with the N1 strain followed by the G4 strain. If we assume density-dependent transmission, which is the more likely mode of transmission, we found the N1 strain of MCMV transmitted at a rate ~1/300 of the rate of field strains, and hence too slowly for successful virally vectored immunocontraception (VVIC). If transmission was frequency-dependent, the rate of transmission was ~1/3 of the rate of field strains, and hence may allow successful VVIC. The G4 strain transmitted at least as slowly as the N1 strain, and possibly much more slowly; however, we could not determine whether this was an inherent property of the G4 strain or whether it was caused by competition with the N1 strain. Given the reliance of successful VVIC on rapid transmission, we recommend that future work in any VVIC system explicitly quantifies the transmission rate of recombinant viruses relative to field strains, both in the presence and absence of competing strains.

Introduction

Pest mammal populations have severe economic, environmental and social impacts throughout the world. The impacts of rodents in particular are both large and pervasive. Rodents reduce the yields of crop; e.g. in Asia it has been estimated that without the impact of rodents the rice yield would feed an additional 200 million people per year (Stenseth *et al*. 2003); they harbour diseases that affect humans in both developed and under-developed countries, e.g. hanta virus (Yanagihara 1990); and they threaten native species, particularly on islands (Towns *et al*. 2006). Pest populations are usually reduced and/or maintained below a particular density to remove their impact, and typically bait-delivered toxins are used to achieve this (Stenseth *et al*. 2003; Towns *et al*. 2006). However, rodenticides can be expensive and labour-intensive to deploy, their use is not always cost-effective and they can affect non-target organisms. In addition, there is increasing public concern about the use of toxins for pest control (Singer 1997; Grandy and Rutberg 2002).

Fertility control has been proposed as an alternative or complementary means to reduce or limit the density of pest mammal populations (Courchamp and Cornell 2000; Delsink *et al*. 2002; Cowan *et al*. 2003). Although fertility control is technically feasible for individual animals, an effective delivery system is one of the major challenges for using this technique with free-ranging wildlife. Since the early 1990s, virally vectored immunocontraception (VVIC) has been studied as one possible delivery system. The idea is to insert a gene for a female reproductive protein into a virus, which will cause infertility of an infected host exposed to the virus (Tyndale-Biscoe 1994). The term immunocontraception was coined because the infertility effect arises as a result of an immune response against self-reproductive antigens. Once released, the virus would move through the population, causing infertility in infected individuals. The possible advantages of this approach over others are that target specificity could be achieved through a combination of the immunocontraceptive effect and the choice of virus, and the self-disseminating nature of the system could greatly reduce the costs of pest control. However, future research will be required to test this and to satisfy the strict regulatory requirements applied to the use of genetically modified organisms (Williams 2002).

Populations of introduced house mice, *Mus domesticus*, in the wheat-growing regions of south-eastern Australia increase rapidly at irregular intervals, causing losses exceeding

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AU$40 million (Singleton 1997). Because this species is not native to Australia and because Australia is considered relatively isolated from areas where house mice are a native species, a research program has been in place to develop VVIC for this species (Singleton *et al*. 2002). This self-disseminating system is one example of several under development for mammals such as rabbits, *Oryctolagus cuniculus*, possums, *Trichosurus vulpecular*, and foxes, *Vulpes vulpes* (Cowan 1996; Robinson *et al*. 1997; Strive *et al*. 2007). The work with house mice is at the forefront of research into self-disseminating systems, partly because of the large body of background knowledge of this intensively studied species, and partly because the high reproductive rate of mice reduces the time required for definitive experiments.

A genetically engineered recombinant strain of murine cytomegalovirus (recMCMV) is being considered as the immunocontraceptive vector to control outbreaks of house mice (Singleton *et al*. 2002; McLeod *et al*. 2007; Redwood *et al*. 2007). Virus–host models have been developed to explore the likely success of VVIC for mice (Arthur *et al*. 2005, 2007). These models suggest it could be successful if (1) the strain of MCMV modified to carry a reproductive antigen can transmit at a similar rate to field strains of the virus, (2) effective control is not compromised by competition between recMCMV and field strains because of cross-immunity to infection and (3) effective control is not compromised by previous infection with field strains of MCMV blocking the development of infertility in mice subsequently infected with recMCMV (Arthur *et al*. 2005, 2007).

MCMV is a large-DNA herpes virus, which is transmitted via close contact (Shellam 1994). It establishes a persistent latent infection, often in the salivary glands, which is not normally lethal to the host (Shellam 1994). MCMV exists naturally in Australia, with wild mice often having a high prevalence (60–100%) of antibodies to the virus (Smith *et al*. 1993; Moro *et al*. 1999; Singleton *et al*. 2000). Multiple strains have been isolated from individual mice, indicating infection from multiple strains occurs. This could be a useful property for successful VVIC with recMCMV, because it indicates cross-immunity to MCMV may not preclude infection with another strain. However, the mechanisms of multiple infections are not known (Booth *et al*. 1993); neither is it known whether infection with one strain reduces either the likelihood or rate of infection by other strains. This is important, because modelling studies indicate that competition between competing strains would reduce the effectiveness of VVIC (Arthur *et al*. 2007).

A sufficient transmission rate of the recombinant virus is needed to keep populations of house mice below acceptable economic and social density thresholds (Arthur *et al*. 2005, 2007); however, it is currently uncertain whether the isolation or modification of MCMV affects its transmission rate. The candidate strains currently under development for VVIC originally were isolated from the salivary glands of wild mice (Booth *et al*. 1993). The epidemiology of two of these strains in enclosed populations of house mice has been investigated (Farroway *et al*. 2005), namely the G4 strain and the N1 strain (Booth *et al*. 1993). That experiment was designed to determine (1) whether an isolated strain (N1) would transmit in enclosed populations of house mice and (2) whether earlier infection with the N1 strain would prevent infection with the G4 strain. In all, 25% of founding mice in six enclosures were infected with the N1 strain, and then 6 weeks later, in four of the enclosures as many of the mice inoculated originally with the N1 strain as could be caught were inoculated with the G4 strain (Farroway *et al*. 2005). During the study, data were collected on changes in seroprevalence and on the presence of viral DNA in salivary glands of mice.

At the end of the experiment, 80% of the original mice not initially infected with the N1 strain and ~30% of all mice born during the experiment had become infected by the N1 strain through natural transmission, leading the authors to conclude that 'We have shown that the transmission of an MCMV strain (N1) occurs rapidly through wild mice populations held in outdoor enclosures that mimic conditions experienced by free-living mice in a mouse-plague-prone region of rural Australia' (Farroway *et al*. 2005: p. 706). A small number of mice also became infected with the G4 strain, showing that earlier infection with the N1 strain did not preclude infection with the G4 strain.

At the time of the study by Farroway *et al*. (2005), virus–host models did not exist for MCMV in house mice and the study was not designed to measure transmission rates explicitly. In the present paper, we use the data collected by Farroway *et al*. (2002, 2005) to estimate the transmission rate of the N1 strain by using the model developed by Arthur *et al*. (2005). We also estimate the transmission rate of the G4 strain, although we recognise that the inferences we can make about transmission of the G4 strain are constrained by the fact that in the original experiment G4 was present only in enclosures where the N1 strain was also present. Hence, we have no way of determining whether or by how much the estimated transmission rate of G4 was affected by the presence of N1. Finally, we assess how the estimated transmission rates compare with the rates estimated from field data and discuss what implications this has for the likely effectiveness of VVIC.

Methods

Data were obtained from an experiment described in detail by Farroway *et al*. (2002). Briefly, eight populations of specific-pathogen-free mice (i.e. mice with no earlier exposure to MCMV) were established in pens (each 225 m^2) at Walpeup (35.08°S, 142.02°E), Victoria, Australia. Fourteen female and eight male mice were placed in each enclosure, for a total of 22 mice at a population density equivalent to 978 ha^{-1}. Two of the enclosures were kept as negative-control enclosures where MCMV was not released and mice were trapped and handled as in the other enclosures to test for unintentional researcher transmission of MCMV. At the end of the experiment, mice in these two enclosures were free of MCMV by all detection methods, indicating unintentional researcher transmission of MCMV did not occur (Farroway *et al*. 2005) and, hence, that the results observed in the other enclosures were due to natural transmission of the released MCMVs.

At the start of the experiment, three male and three female mice in each of six enclosures were inoculated with the N1 strain of MCMV. At Week 6, in four of the six enclosures with the N1 strain, as many of the originally inoculated mice as could be caught (4, 5, 6 and 5) were also inoculated with the G4 strain of

MCMV. The enclosure study was terminated at 12 weeks. An individual was considered seropositive for MCMV infection on the basis of an enzyme-linked immunosorbent assay (ELISA) of a 1 : 100 dilution of sera. This ELISA was not strain-specific. This dilution was chosen because of the large number of false positives in virus-free pens obtained at a 1 : 20 dilution. Two cohorts of juvenile mice were born into each pen during the experiment. At the end of the experiment mice were also screened for MCMV by using quantitative PCR on extracts from mouse salivary glands (Farroway *et al*. 2005).

The two virus strains used in the experiment were previously isolated from wild mice from Nannup (N1) and Geraldton (G4) in Western Australia (Booth *et al*. 1993). These strains were chosen because it was thought that a well established salivary-gland strain would be more likely to persist and transmit among wild mice (Farroway *et al*. 2002).

Disease model

We used density-dependent and frequency-dependent transmission models described by Arthur *et al*. (2005), with some modifications. Vertical transmission was ignored as in Arthur *et al*. (2007). We assumed no mice died during the experiment to simplify the modelling, which is close to what was observed (Farroway *et al*. 2002) – the death rate across all six enclosures was 0.004 week^{-1} (on the basis of the number of founders known to be alive at the end of the experiment). There was also no strong evidence that the presence of MCMV affected the survival of juveniles born in the pens, on the basis of comparisons between enclosures with and without MCMV (Farroway *et al*. 2002). We modelled the appearance of juveniles as single-time-step events that were deemed to have occurred at 8 and 11 weeks after the start of the experiment, on the basis of past life history of mice in these pens (Chambers *et al*. 1997). The number of juveniles in each cohort was determined at the end of the experiment when all mice were removed from each pen (Farroway *et al*. 2002). Transmission was modelled as

$$\frac{dS}{dt} = -\beta SI/(1-\varepsilon+\varepsilon N),$$

$$\frac{dE}{dt} = \beta SI/(1-\varepsilon+\varepsilon N) - \sigma E,$$

$$\frac{dI}{dt} = \sigma E,$$

where S = density of susceptible mice ha^{-1}, E = density of infected but not infectious mice (i.e. 'exposed' mice), I = density of infectious mice, β = disease transmission rate, σ = rate of moving from infected to infectious class, i.e. $1/\sigma$ is the latent period, n = the total population density and, hence, when $\varepsilon = 0$, transmission is density-dependent and when $\varepsilon = 1$, transmission is frequency-dependent. Mice were considered to remain infectious for the remainder of the experiment as in Arthur *et al*. (2005).

Estimating the transmission rates of strains N1 and G4 by using quantitative PCR data

Strain-specific quantitative PCR (qPCR) data were obtained for the final trapping session only (Table 1), providing one data point for each of the six pens. Different proportions of each cohort were assessed by qPCR in each enclosure (Farroway *et al*. 2005). Our simulation model did not account for different cohorts separately, so we had to modify the final prevalence value to account for this. For example, in Pen 7 all founders were sampled

Table 1. Enclosure results used to fit models for MCMV transmission

Pens 4 and 6 received strain N1 only. Pens 2, 3, 7 and 8 received strains N1 and G4. Pens 1 and 5 were negative-control pens where no virus was released and are not shown here

Pen	Cohort (total[A])	Seropositive/sample (%)				PCR-positive/sample at Week 12 (%)	
		Week 5	Week 7	Week 9	Week 12	N1	G4
4	Founders	3/7 (43)	3/8 (38)	9/17 (53)	14/20 (70)	16/20 (80)	–
	Cohort 1 (72)	–	–	1/26 (4)	4/45 (9)	23/42 (55)	–
	Cohort 2 (32)	–	–	–	1/25 (4)	5/23 (22)	–
6	Founders	2/9 (22)	1/9 (11)	6/21 (29)	16/20 (80)	18/21 (86)	–
	Cohort 1 (79)	–	–	1/10 (10)	2/45 (2)	21/45 (47)	–
	Cohort 2 (12)	–	–	–	0/13 (0)	2/11 (18)	–
2	Founders	1/6 (17)	4/12 (33)	4/15 (27)	20/22 (91)	22/22 (100)	4/22 (18)
	Cohort 1 (72)	–	–	0/4 (0)	2/43 (5)	14/41 (34)	3/41 (7)
	Cohort 2 (30)	–	–	–	2/23 (9)	7/22 (32)	0/22 (0)
3	Founders	3/13 (23)	3/12 (25)	9/18 (50)	18/22 (82)	20/22 (91)	2/22 (9)
	Cohort 1 (64)	–	–	3/7 (43)	8/42 (19)	17/41 (41)	2/41 (5)
	Cohort 2 (7)	–	–	–	2/9 (22)	1/9 (11)	0/9 (0)
7	Founders	2/15 (13)	2/7 (29)	9/19 (47)	21/22 (95)	18/22 (82)	3/22 (14)
	Cohort 1 (40)	–	–	4/6 (67)	1/27 (4)	10/27 (37)	0/27 (0)
	Cohort 2 (20)	–	–	–	6/20 (30)	3/19 (16)	0/19 (0)
8	Founders	4/9 (44)	2/11 (18)	8/21 (38)	15/21 (71)	18/20 (90)	0/20 (0)
	Cohort 1 (46)	–	–	4/4 (100)	6/32 (19)	16/32 (50)	0/32 (0)
	Cohort 2 (17)	–	–	–	0/15 (0)	3/13 (23)	0/13 (0)

[A]The total number of juveniles found at Week 12 is given for each cohort (in parentheses).

and 18 of 22 were positive, 27 of 40 Cohort 1 animals were sampled and 10 were positive, 19 of 20 Cohort 2 animals were sampled and three were positive. From this we calculated the total number of positive mice that would be in the pen as

$$\frac{18}{22} * 22 + \frac{10}{27} * 40 + \frac{3}{19} * 20 = 36.$$

This gives a prevalence of 36/82 and, hence, from a random sample of 68 (= 22 + 27 + 19) animals you would expect 30 to be positive. Hence, we used 30/68 as our final prevalence value for Pen 7 to fit the models. PCR-positive mice were assumed to be in the *I* class.

The main parameter of interest was the transmission rate (β) of the two strains. To fit models to the data, density of the population was expressed as mice ha^{-1}, on the basis of the number of mice in a pen and a pen size of 225 m^2. Inoculated mice were placed in the *E* class. We assumed mice became infectious 2 weeks after the initial infection (Arthur *et al*. 2005), i.e. $\sigma = 0.5$ $week^{-1}$. We assumed all parameters were the same whether the mice were inoculated with MCMV, or obtained it via natural transmission. Models were run in daily time steps. The models were fitted to the prevalence estimates obtained at the end of the study by maximum likelihood estimation assuming binomial observation error (Hilborn and Mangel 1997). For strain N1, starting prevalence (in the *E* class) was based on the six inoculated mice. For each model type, we asked whether or not the transmission rate was affected by the presence of the G4 strain. For strain G4, the design of the original study (Farroway *et al*. 2002, 2005) meant that we could estimate the transmission rate of G4 only in the presence of N1. We used Akaike information criteria (AIC) to assess support for the models (Burnham and Anderson 1998). The 95% confidence interval for β was based on the likelihood profile (Hilborn and Mangel 1997).

Model fitting – seroprevalence data

Seroprevalence provides an alternative means of monitoring MCMV dynamics and was used to fit models to field data in a previous paper (Arthur *et al*. 2005). Seroprevalence data were obtained at four times throughout the pen experiment (Table 1). We made several assumptions to fit models to these data. We ignored the effect of changes in density due to recruits into the population and considered changes in seroprevalence only among the founders. This was done for two main reasons. (1) There was some indication that seroprevalence may not reflect true infection with MCMV in juveniles because of maternal antibodies. Of 16 Cohort 1 animals that were showing antibodies to MCMV in Week 9, 11 (69%) had lost this antibody status by Week 12. In contrast, almost all founders that became seropositive for MCMV remained so until the end of the experiment. (2) New recruits entered late in the experiment, so the effect of increased population density had little time to be observed, given that it takes on average 3 weeks for new infections to become observable by using seroprevalence data (see below). We assumed mice seroconverted at the same rate they moved from the *E* to the *I* class to simplify the modelling, i.e. 0.5 $week^{-1}$, and hence *I*-class mice were considered seropositive. Finally, we assumed all parameters were the same whether mice were inoculated with MCMV, or obtained it via natural transmission.

We fitted the models to the seroprevalence data by using maximum likelihood estimation assuming binomial observation error (Hilborn and Mangel 1997). AIC was used to assess support for *a priori* specified models (Burnham and Anderson 1998). We assessed whether there was evidence that β was the same in all six enclosures or varied on the basis of the treatment (two strains *v.* one strain). Assuming no change in density meant that we could not assess the relative support for frequency-dependent *v.* density-dependent transmission with the seroprevalence data. Confidence intervals were estimated by the same methods as described above. All modelling and analyses were carried out by using Program R 1.7.0 (R Development Core Team 2007).

Results

The raw data from the experiment are shown in Table 1. Seroprevalence increased throughout the experiment, indicating transmission of MCMV among mice. At the end of the experiment, in general, more mice were positive for MCMV infection on the basis of qPCR of salivary glands than on the basis of serology. This is not unexpected because PCR is a more sensitive technique for detecting infection. These data were used to fit the quantitative models of viral transmission.

Estimating transmission rates

Quantitative PCR data

There was no strong evidence that the transmission rate of the N1 strain was different depending on whether the G4 strain was present or not (Table 2). The support for the density-dependent transmission model for the N1 strain was approximately twice that for the frequency-dependent transmission model (Table 2). Most of the mice originally inoculated with G4 were not qPCR positive for G4 by the end of the experiment (Week 12 in Table 1). In contrast, almost all mice inoculated with N1 were positive for this strain at the end of the experiment. This suggests most mice were not successfully infected with G4 when inoculated. We could not fit our disease models to the G4 data if we assumed that the initial prevalence of G4 was determined by the number of mice injected with G4 at Week 6, because the final prevalence was lower than predicted even with no transmission. In Enclosure 8, no mice were qPCR positive for the G4 strain (Table 1), despite five of the founder mice being inoculated. Hence, we estimated a maximum possible transmission rate for the G4 strain assuming that only one mouse in each of Enclosures 2, 3 and 7 was inoculated. The support for the density-dependent transmission model for the G4 strain was approximately twice that for the frequency-dependent transmission model (Table 2). The maximum possible transmission rate of the G4 strain was similar to that estimated for the N1 strain (Table 2).

Seroprevalence data

There was no strong evidence that transmission of MCMV among founders was different depending on whether one or two strains were present (Table 2). The estimated transmission rate among founders was similar to the rate estimated with the qPCR data for the density-dependent transmission model, and ~1/4–1/3 of the rate estimated with the frequency-dependent model.

Table 2. Model fitting and transmission estimates for strains N1 and G4, by using either quantitative PCR or seroprevalence data

K, number of estimated parameters; AIC, Akaike information criteria; ΔAIC, the difference between the model and the 'best' model; ω_i, Akaike weight or likelihood of the model, given the data and the models in the set. All same, all six pens have the same estimate of the transmission coefficient (β); treatment, the two pens with only the N1 strain have a different estimate of β compared with the four pens with both the N1 and the G4 strain

Model	Grouping	K	AIC	ΔAIC	ω_i	Estimate of β week^{-1} (95% confidence interval)
Models fitted using qPCR data						
N1 strain (most supported model fit the data, $\chi^2_5 = 1.66$, $P = 0.89$)						
β*SI* (density-dependent)						
	All same	1	33.7	0.0	0.45	0.00037 (0.00034–0.00045)
	Treatment	2	35.2	1.5	0.21	N1 only: 0.00040 (0.0002–0.00060)
						N1 in the presence of G4: 0.00038 (0.0002–0.00056)
β*SI*/*N* (frequency-dependent)						
	All same	1	35.8	2.1	0.16	1.06 (0.88–1.29)
	Treatment	2	35.6	1.9	0.18	N1 only: 1.26 (0.88–1.64)
						N1 in the presence of G4: 0.95 (0.73–1.17)
G4 strain (most supported model fit the data, $\chi^2_2 = 1.15$, $P = 0.56$)						
β*SI* (density-dependent)						
	Treatment	1	12.62	0.0	0.70	G4 in the presence of N1: 0.00034 (0.0002–0.0005)
β*SI*/*N* (frequency-dependent)						
	Treatment	1	14.36	1.8	0.30	G4 in the presence of N1: 0.95 (0.55–1.38)
Models fitted using seroprevalence data						
Different strains not distinguishable with these data (most supported model fit the data, $\chi^2_{23} = 15.43$, $P = 0.88$)						
β*SI* (density-dependent)						
	All same	1	105.5	0.0	0.60	0.00031 (0.00025–0.00037)
	Treatment	2	106.3	0.8	0.40	N1 only: 0.00026 (0.00000–0.00054)
						Both strains: 0.00033 (0.00015–0.00051)
β*SI*/*N* (frequency-dependent)						
	All same	1	As for density-dependent model			0.30 (0.24–0.36)
	Treatment	2				N1 only: 0.25 (0.05–0.35)
						Both strains: 0.31 (0.25–0.39)

Because the density of founders did not vary during the experiment, both frequency-dependent and density-dependent models provide the same fit to these data, i.e. the transmission-rate estimate for the density-dependent model is the frequency-dependent estimate divided by the density of founders (e.g. 0.3/978 = 0.00031).

Discussion

Our disease models provided adequate fits to both the quantitative PCR data and the seroprevalence data. We could not detect any strong effect on the transmission of the N1 strain once the G4 strain was added to pens – N1 may have transmitted slightly slower in the presence of G4 than it did in isolation (Table 2). The G4 strain also transmitted in the presence of the N1 strain and possibly at about the same rate as the N1 strain. However, this was the maximum possible transmission rate of the G4 strain and was based on an assumption that only one of the mice in each pen was successfully inoculated with G4. The observed data would suggest a lower transmission rate if more than one mouse in each pen was successfully inoculated with G4. Unfortunately, no experiments were conducted in which G4 was added in the absence of the N1 strain, so we cannot determine whether the lack of success with inoculation was due to the presence of N1 or an inherent property of the G4 strain used in the experiment, although laboratory studies suggest G4 should infect almost all mice inoculated with it (Lyn Hinds, pers. comm.).

By using field data of changes in MCMV seroprevalence during an outbreak of house mice and the same types of models as used here, Arthur *et al*. (2007) estimated that the transmission rate of MCMV was 0.1 week^{-1} if transmission was density-dependent or 3.0 week^{-1} if transmission was frequency-dependent. On the basis of these transmission rates they determined that immunocontraceptive MCMV could prevent mouse populations from growing rapidly to damaging levels if >70% of mice infected with the virus became infertile. Successful control was still possible if the transmission rate of the engineered virus was ~30% of the field-estimated value, provided there was no competition between the engineered strain and wild strains and provided earlier infection with wild strains did not block development of the infertility effect on subsequent infection with the engineered strain (Arthur *et al*. 2007).

The estimated transmission rate in the field is ~300 times faster than the rate estimated in the pens for density-dependent transmission and ~3 times faster than the rate estimated in the pens for frequency-dependent transmission. This clearly suggests that if density-dependent transmission were the main mode of transmission of MCMV, the transmission of the N1 strain would be far too slow to prevent damaging mouse-population outbreaks. However, if frequency-dependent transmission were the main mode of transmission, then transmission of the N1 strain would be at least closer to the rate required for successful control.

Neither the models fitted to field data (Arthur *et al*. 2007), nor the models fitted to pen data were able to distinguish

clearly between density-dependent and frequency-dependent transmission; the density-dependent model received approximately twice the support of the frequency-dependent model in the present study, when models were fitted to the PCR data. Fitting models to the seroprevalence data could not distinguish between density-dependent and frequency-dependent transmission because the density of mice in the pens was assumed to remain constant through the experiment and only founders were considered. This assumption had a limited effect on the magnitude of the estimated transmission rate under the density-dependent model, and caused a reduction in the estimated transmission rate under the frequency-dependent model of ~70%, compared with the rate estimated by using PCR data. One explanation for the reduction may be that the pulse of uninfected juveniles into the pens (which was included in the PCR fitting) would temporarily lower the prevalence of infection and hence require a higher estimated transmission rate to achieve the same final prevalence of infection as when the pulse of juveniles is ignored. The assumption creates uncertainty about the magnitude of the frequency-dependent transmission rate if that is the correct mode of transmission; however, the result does not help determine which mode of transmission is more likely.

There is some evidence from other studies that density-dependent transmission of MCMV is more likely. Frequency-dependent transmission occurs when contact rates do not vary with population density. Sexual transmission is one of the most common forms of frequency-dependent transmission, although only when sexual contacts do not scale with density, such as in monogamous mating systems. House mice probably have a polygamous mating system where the number of sexual contacts are more likely to scale with density (Singleton 1983; Waterman 2007). A lethal allele introduced into an island population of mice spread across the island rapidly, consistent with a polygamous mating system and an open deme model of social structure (Anderson *et al*. 1964). This suggests that even if transmission of MCMV is largely via sexual transmission, density-dependent transmission is more likely. Further evidence has suggested that sexual transmission may not be the main mechanism of transmission (Jacob and Sutherland 2004). These researchers found that old females that had never experienced a pregnancy still had 86% seroprevalence for MCMV whereas young females that had experienced two or three pregnancies had 60–67% seroprevalence for MCMV.

If density-dependent transmission is the main mode of transmission of MCMV, there are many possible explanations for the large difference between the field-estimated rate and the pen-estimated rate. The N1 strain was isolated in a cell culture (Booth *et al*. 1993) and had been passaged many times since its isolation from wild mice, and this could select for viruses that have reduced natural transmission and would explain the low transmission rate. Reduced virulence of strain N1, with lower levels of viral replication than for other strains, has been reported, consistent with this interpretation (Lyons *et al*. 1996).

Alternatively, it is possible the pen environment explains the differences in density-dependent transmission estimated for the N1 strain compared with field strains. One issue is the estimate of population density in the two environments. Data from the field studies analysed by Arthur *et al*. (2007) were taken from the farm scale and included a range of micro-habitats, probably supporting different densities of mice (Singleton *et al*. 2000). To compare density-dependent transmission rates under the two circumstances (pen *v.* field) we are assuming that the densities of the mouse populations are comparable (Begon *et al*. 2002). We have no way of testing this with the data we have; however, the conditions in the pens were designed to mimic conditions in the field (Farroway *et al*. 2002).

Another major difference between the pen trial and the field trial is that mice used in the pen trial were pathogen-free, with the exception of the inoculated MCMVs (Farroway *et al*. 2002, 2005), whereas mice in the field can have several different infections (Smith *et al*. 1993). It is possible that infection with other diseases makes mice more susceptible to infection with MCMV. For example, some viruses such as mouse hepatitis virus (MHV), which is highly prevalent in Australian field populations (Smith *et al*. 1993), are immunosuppressive (Smith *et al*. 1987, 1991). Increased stress may also lead to higher susceptibility to infection (Peterson *et al*. 1991), although it seems unlikely that mice should be highly stressed during the early stages of an outbreak when conditions are highly favourable for them and when high field-transmission rates of MCMV were recorded (Arthur *et al*. 2005).

Our quantitative analysis of the data collected by Farroway *et al*. (2005) suggests that their initial conclusion that N1 transmitted rapidly in pens may have been incorrect, particularly if transmission is the more likely density-dependent mode. Given the reliance of successful VVIC on sufficient transmission of the engineered virus (Arthur *et al*. 2007), it is essential future work properly quantifies the transmission rate of engineered strains of MCMV – an issue also raised by Redwood *et al*. (2007). The present study suggests that there may be problems with the low transmission rate of isolated strains. However, it also highlights that it is not clear how the quantitative estimates of transmission rates in pens truly compare with those estimated from field data. To overcome these problems future studies need to be designed so that the relative transmission rates of isolated strains can be compared directly with transmission rates of field strains of MCMV. To do this, both isolated and field strains should be run in the same experimental setting, be that in the laboratory or in outdoor enclosures. On the basis of modelling studies, it is the relative transmission rate that is important, regardless of whether the mode of transmission is frequency- or density-dependent. Future studies should also be designed to determine the mode of transmission of MCMV in house mice.

We are still a long way from VVIC being used for pest-animal control in any system (McLeod *et al*. 2007) and modelling results on whether it would provide a useful strategy are mixed. For example, a strategy involving concurrent baiting and VVIC was the most efficient way of reducing an island population of feral cats, *Felis catus*, and could lead to eradication (Courchamp and Cornell 2000). In contrast, the most recent models for rabbits suggest that lethal control with poison bait is far more effective at reducing rabbit populations than VVIC control with modified myxoma virus would be (McLeod and Twigg 2006).

Whether sufficient transmission of a self-disseminating immunocontraceptive vector for successful fertility control of any pest animal can be achieved is still unknown. Our work with

house mice highlights the importance of transmission and some of the problems associated with it. In any system where self-disseminating immunocontraception is being considered, experiments will need to be designed to quantify transmission of the vector explicitly to determine whether successful control is feasible. Only if satisfactory progress is achieved in these experiments should further development of this technology be considered, notwithstanding the social and ethical issues, which need to be addressed also.

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