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Prevalence of viral antibodies and helminths in field populations of house mice (*Mus domesticus*) in southeastern Australia

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SUMMARY

A 13-month study of wild mice (*Mus domesticus*) in wheatlands in southeastern Australia contrasted changes in the seroprevalence of antibody to 13 viruses and the occurrence of helminths with changes in their population dynamics. Mice were seropositive for mouse hepatitis virus (MHV), rotavirus, minute virus of mice (MVM), mouse adenovirus (MAdV), reovirus (reo 3), and murine cytomegalovirus (MCMV). The seroprevalences of all but rotavirus varied significantly with time and increased with host density. Near the end of the study, host density declined rapidly and the seroprevalence of MVM and reo 3 increased significantly. These two viruses had low seroprevalence when host survival was high and high seroprevalence when host survival was low, indicating they may play a role in regulating mouse populations. In the case of MVM, there was evidence of a viral epizootic during the decline in mouse abundance. The prevalence of four helminths (*Taenia taeniaeformis*, *Syphacia obvelata*, and *Vampirolepis* spp.) differed significantly with time but showed no apparent association with host density. These findings highlight the need for further study on the effect of viruses on the population dynamics of mice.

INTRODUCTION

Populations of the house mouse (*Mus domesticus*) irregularly erupt to form plagues which cause substantial economic and social problems in the cereal-growing regions of Australia [1, 2]. There is no effective method of control and the control of mouse numbers by biological rather than chemical means is more socially and environmentally acceptable [1, 3]. Towards this end, research has focused on the potential of a parasitic nematode to prevent mouse plagues [e.g. 4, 5] and on viruses which occur naturally in mice at 15 locations in southeastern Australia [6]. A survey of the latter revealed antibodies to 8 of 14 viruses. It is not

known what role, if any, these viruses play in regulating mouse numbers in non-plague years, either singly, or in combination with another virus or a macro-parasite, e.g. helminths, blood protozoans.

Smith and colleagues [6] indicated that the seroprevalence of some viruses may be strongly influenced by the density and breeding status of mouse populations at the time of sampling. However, that survey consisted of a single collection from each locality except for some samples taken some 2 years apart. The principal aim of the present study was to monitor changes in the seroprevalence of antibody to different viruses and to associate these changes with changes in the demography and breeding of mouse populations. The study was located in the Mallee wheatlands of northwestern Victoria where mouse plagues occur on average every 7 years [1]. In the $2\frac{1}{2}$ years prior to the study, the density of the mouse population in these wheatlands was low [7].

Apart from the need to develop an effective method of biological control of mouse plagues, there is the issue of whether parasites can regulate populations of mammals. The role of micro- and macro-parasites in the regulation of small mammal populations was the focus of much research by Elton and others in the 1920s and 1930s [8, 9] but the results were equivocal. With a few exceptions [e.g. 10–13] interest in parasites dwindled as the focus switched to other factors, such as social behaviour and resource availability [see 14–16 for reviews]. The reason for the lack of studies of parasites in small mammals is puzzling given the paucity of field data for or against the role that parasites may play in regulating population densities, and the development [17] and application [18] of mathematical models and the existence of well designed laboratory studies [19] which indicate that micro- and macro-parasites have a strong potential to regulate populations of small mammals.

Mice living in the cereal-growing regions of southeastern Australia are good subjects for studies which investigate the possibility that parasites can regulate mammal populations, for two main reasons. Firstly, animals which have a short generation time and undergo marked fluctuations in population density provide the best model for studying the potential of parasites to regulate populations [20]. Secondly, techniques for detecting serum antibody in laboratory mice have been developed recently for most of the well-defined murine viruses [see 21 and references therein] and this technology has been applied routinely to screen sera in wild mouse populations [6].

In the present study, a mouse population was monitored every 3 weeks for demographic changes as part of a long term study and nearby populations were sampled six times over a period of 13 months for viral antibodies and helminths. Two habitats, cropped fields and areas within 50 m of permanent water, were chosen because both mouse and helminth populations display different dynamics in these habitats [12, 22]. Also of interest were the associations between the prevalence of different viral antibodies and different species of helminths. These associations have not been examined before in wild mouse populations.

Scott and Dobson [20] list five prerequisites to determine whether a parasite has the potential to regulate host population abundance. Two relate to the need to simulate natural conditions and two relate to measuring the effect of the parasite on host survival and reproduction as host density increases. The present design

meets these four prerequisites. The fifth, that there be separate uninfected populations or that the populations be monitored before and after the addition of a parasite, could not be met. This presents a caveat on our ability to infer regulation. Nevertheless, based on Scott and Dobson [20], the study will provide a strong indication that an infectious agent could have a role in regulating mouse populations, if three conditions are met: (i) the parasite persists at low host density; (ii) the prevalence of the parasite is density dependent in the host population, and (iii) host survival decreases with increase in the prevalence of the parasite.

METHODS

Study site

The study area was the central Mallee wheatlands of Victoria, Australia. Climate, habitat (wheatlands), farming practices and soil type were similar within this area. Annual rainfall is 340 mm and the region has a Mediterranean climate, with hot summers and predominantly winter rainfall [see 22 for details]. The soils are predominantly sandy loams, and winter cereals are the main crops.

This paper reports on data obtained from two separate studies. Firstly, a capture–mark–release study of mice at the Mallee Research Station (MRS), Walpeup (142.02° E, 35.08° S), which began in 1983 and finished in 1989. Secondly, a study of helminths and viruses, which began in October 1986 and finished in October 1987. All mice for the second study were collected within a 28×5 km area and most were collected within 5 km of Walpeup. The first study provided detailed demographic data which formed the basis for examining the effect of the respective infectious agents on mouse populations.

The capture–mark–release study

Mouse populations at the MRS were live-trapped every third week using Longworth traps. Individual mice were measured, examined for breeding condition, ear-tagged and then released at the site of capture. The study design and the demographic and breeding data collected for each mouse followed that reported elsewhere [22]. No mice were removed during the capture–mark–release study and the closest site where mice were removed for the study of infectious agents was $> 0{\cdot}5$ km away.

Abundance index. The abundance of mice was reported as an adjusted index of the number of mice caught per 100 trap nights using a density–frequency transformation [23].

Survival estimates. Survival was estimated per 21 days for each of four weight cohorts of mice: $< 12{\cdot}0$ g, 12·0–13·9 g, 14·0–15·9 g, $\geqslant 16{\cdot}0$ g. Mice $< 12{\cdot}0$ g were considered sub-adults because none was pregnant or had scrotal testes. The survival estimate was based on the minimum number of mice known to be alive and therefore was a measure of minimum survival. Analysis was restricted to the 10 trapping sessions during the period February 1987 to October 1987 because too few mice were recaptured at other times.

Collections for serology and helminthology

Mice were live-trapped in two habitats: (i) standing crop or stubble, and (ii) within 50 m of permanent water. The sites chosen in the present study are representative of two common but diverse habitats in the cereal-growing region. Differences in habitat features, such as water versus semi-aridity and undisturbed versus disturbed habitat, could markedly influence the transmission and persistence of viruses and/or parasites.

The collection of sera and post mortem examinations for helminths began in October 1986 and were conducted on average every 8 weeks (range 6–12) until October 1987. Six collections were made with 25–34 mice sampled from each habitat on each occasion. Only mice > 75 mm in length (most > 80 mm) were screened for antibodies to viruses because about 0·3 ml of serum was required to run all the serological tests. These mice were estimated to be > 8 weeks of age and most were > 12 weeks of age. Where possible, an equal sex ratio was sought in all samples.

Serology

Mice were bled from the sub-orbital venous plexus. The techniques used in the collection and storage of sera and screening for the presence of antiviral antibodies and antibodies to *Mycoplasma pulmonis* are described elsewhere [6]. Sera were screened for evidence of antibodies to *M. pulmonis* and mouse hepatitis virus (MHV), rotavirus (epizootic diarrhoea of infant mice (EDIM) virus), lymphocytic choriomeningitis virus (LCMV), ectromelia virus, mouse adenovirus (two strains: MAdV-FL, MAdV-K87), minute virus of mice (MVM), murine cytomegalovirus (MCMV), reovirus serotype 3 (reo 3), Sendai virus, Theiler's mouse encephalomyelitis virus (TMEV), pneumonia virus of mice (PVM), polyoma virus, and Hantaan virus. Antiviral antibodies were detected by immunofluorescence except antibodies to MCMV which were detected by an enzyme-linked immuno-sorbent assay [6]. These viruses were chosen because, apart from the murine retroviruses, they represent the commonly described viral infections of laboratory mice and because antibodies to eight of these were detected in a recent survey of viruses in wild mice in Australia [6, 21].

The sera from a minimum of 40 mice (20 per habitat) were screened from each of the 6 trapping sessions. At low population density ($n < 100$), a sample of 20–25 mice provided a 95% probability of detecting at least one seropositive mouse in a population with an expected prevalence of 10%. At higher densities ($n > 100$), the expected prevalence is 10–15% [24].

Helminthology

The internal helminths were screened at ×100–500 magnification using a binocular microscope. The techniques used for the collection and identification of internal helminths followed those of Singleton [12], except that mice were not inspected for sub-cutaneous parasites such as *Muspicea borelli*. What was thought initially to be one species of cestode in the small intestine and bile ducts, was later identified as being two species, *Vampirolepis fraterna* and *V. straminea*.

Table 1. *Percentage survival of mice from four weight cohorts trapped at the Mallee Research Station, Walpeup, from February 1987–October 1987*

	Weight cohorts (g)										
	< 12		12–13·9		14–15·9		⩾ 16		Overall		
Month	*n**	Surv†	*n*	Surv	*n*	Surv	*n*	Surv	*n*	Surv	S.E.‡
Feb.	35	2·9	23	13·0	12	25·0	5	20·0	75	15·4	7·64
Mar.	59	1·7	35	0·0	36	13·9	18	11·1	148	6·7	3·15
Apr.	98	28·6	35	25·7	13	61·5	16	56·3	162	43·2	6·69
May	152	18·4	70	24·3	26	53·9	21	28·6	269	31·4	4·99
Jun.	146	21·9	86	36·1	44	27·3	95	30·5	371	29·0	3·39
Jul.	87	42·5	112	50·9	70	62·9	70	51·4	339	52·1	3·57
Aug.	120	31·7	124	29·0	94	42·6	51	31·4	389	33·7	3·33
Sep. 1	81	13·6	116	21·6	99	34·3	52	19·2	348	22·3	2·96
Sep. 2	20	10·0	69	18·8	82	19·5	65	21·5	236	17·6	3·44
Oct.	1	0·0	7	14·3	16	18·8	68	22·1	92	14·0	5·70
Total	799	17·4	677	23·5	492	36·2	461	29·3	2429		
S.E.‡		1·68		2·53		3·47		3·75			

* *n*, sample size.
† Surv, percentage survival.
‡ S.E., ±1 standard error.

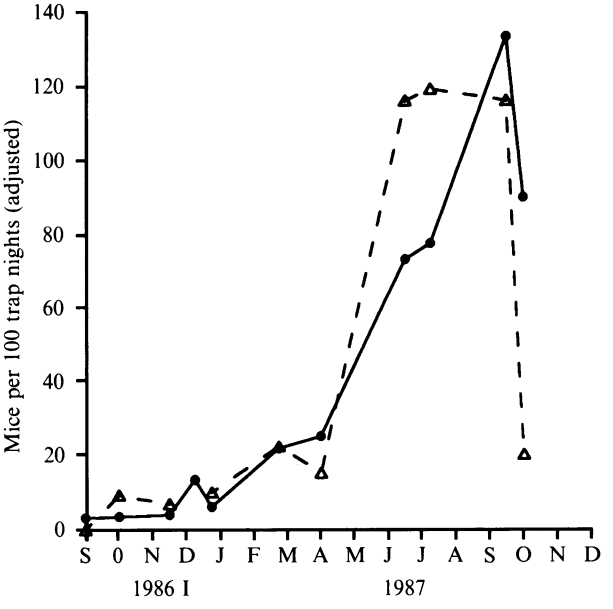

Fig. 1. Abundance indices of mice trapped in crops (△) and in areas near permanent water (●).

Consequently, the combined prevalence of the two species was presented as *Vampirolepis* spp.

Statistical analyses

The use of the terms prevalence and intensity of infection follow Margolis and colleagues [25]. Survival was analysed by considering numbers of survivors in each

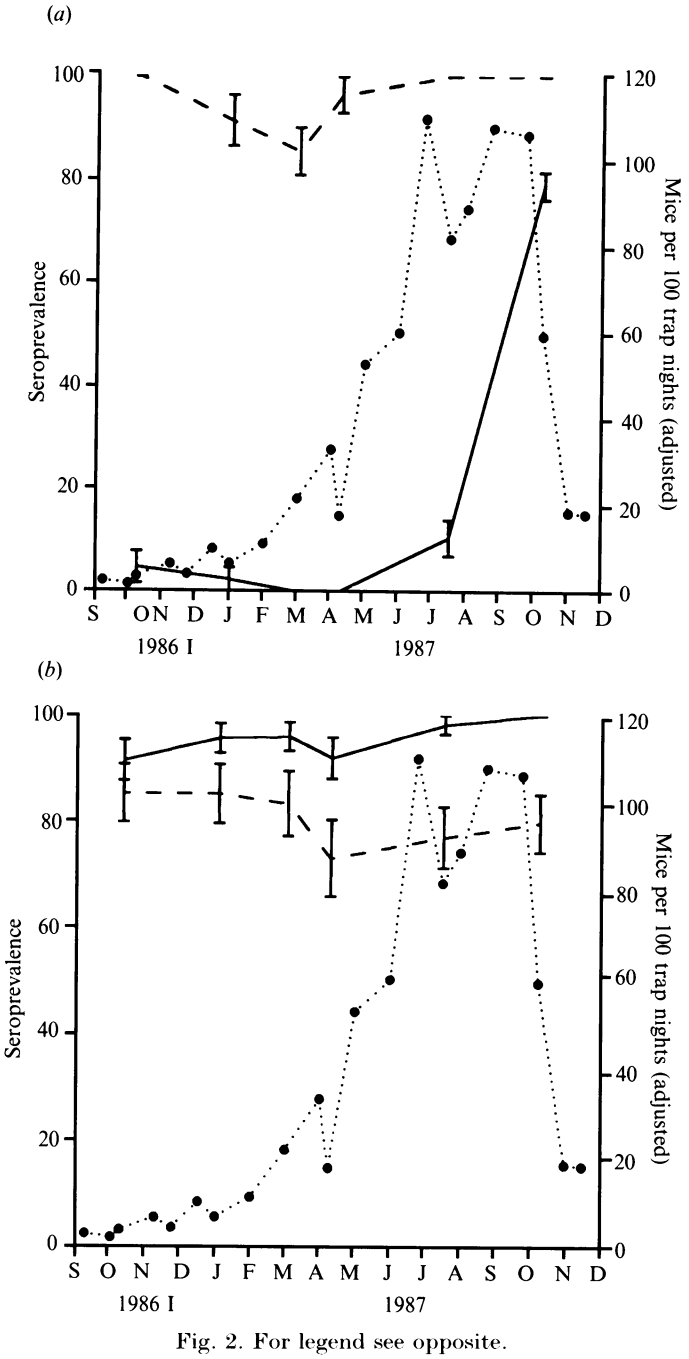

Fig. 2. For legend see opposite.

category as a binomial variate and fitting a generalized linear model with binomial errors and logit link [26]. Sex, age, cohort ($n = 4$), census ($n = 10$) and their interactions were included in the initial model, but sex, and interactions involving sex, were excluded from the final model as they were having no effect. As numbers of mice in each category were generally large (Table 1), the deviance ratios in the analyses of deviance obtained from fitting the generalized linear model were regarded as F-ratios for testing significance.

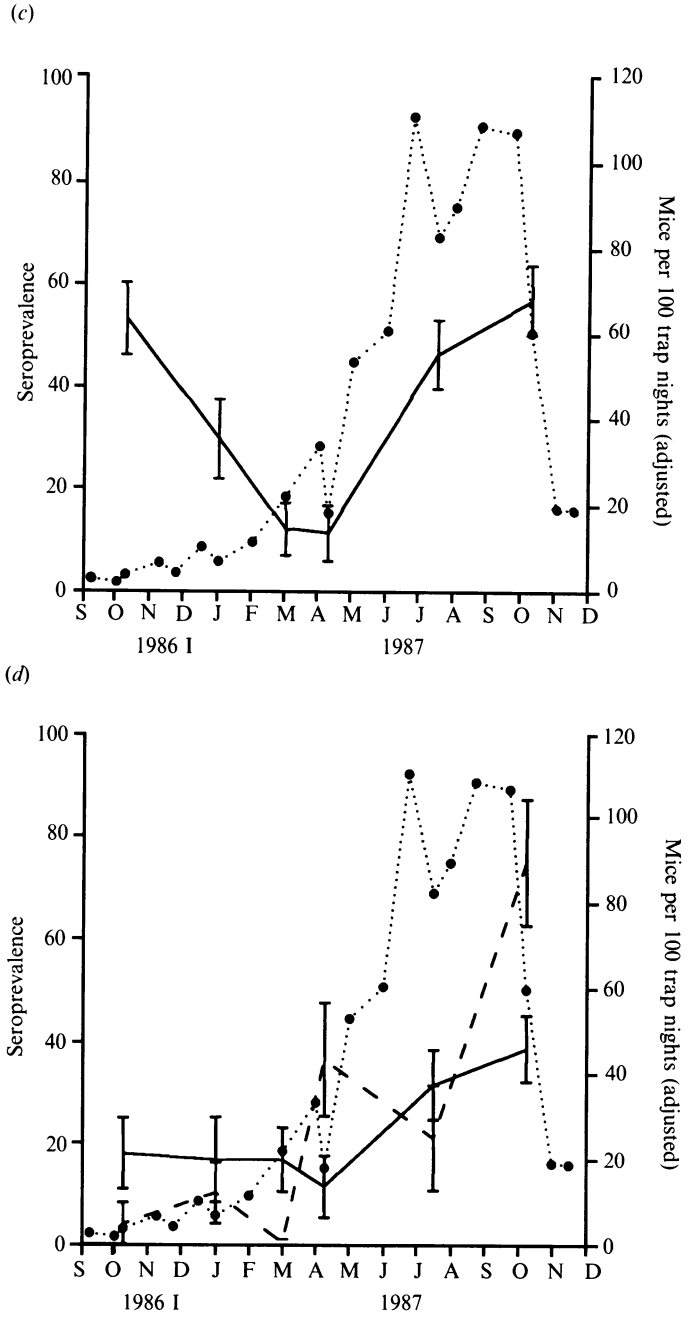

Fig. 2. Seroprevalence (%) (± 1 standard error) of antibodies to six viruses. Dotted line represents abundance indices of mice (habitats combined). (*a*) MHV (broken line) and MVM (solid line); (*b*) rotavirus (broken line) and MCMV (solid line); (*c*) MAdV-K87 (solid line); (*d*) reo 3, females (broken line) and males (solid line).

Presence of each of the antiviral antibodies and the helminths was recorded as a binary response (present/absent) for each mouse. These were then analysed by fitting generalized linear models with binomial error and logit link [26] separately for each antibody or helminth. The effects of census ($n = 6$), habitat (water or crop), sex and their interactions were tested. The data were not orthogonal

because there were unequal numbers of mice in each census/habitat/sex category. Consequently, main effects were adjusted for other main effects and interactions were adjusted for all main effects and other interactions of the same or lower order.

The importance of mouse weight and length as a covariate was tested in each case, and where significant they were included in the model. Due to the binary nature of the data, the significance of each main effect or interaction was determined from the analysis of deviance by regarding its deviance as a chi-squared variate on the same degrees of freedom. The effects of census, habitat and sex on intensity of infection of the nematode *Syphacia obvelata*, and on the weights and lengths of the mice, were obtained from analyses of variance. Intensity and weight were log transformed prior to analysis.

RESULTS

The capture–mark–release study

Demography and reproduction. The density of mice in both habitats was low at the commencement of the study in October 1986 with an abundance index of < 10. The index increased rapidly from February 1987 through to July 1987 to a peak of > 100. Mouse abundance declined rapidly in September 1987 in cropped fields and in the surrounds of permanent water. Mouse abundance, however, remained high near permanent water (Fig. 1).

Mice were in reproductive condition (pregnant or lactating) from October 1986–May 1987. During this period there were four collections of mice for parasites. The respective percentages of the adult female population breeding at these times were: sample 1 (October 1986), 89% ($n = 18$); sample 2 (January 1987), 42% ($n = 59$); sample 3 (March 1987), 63% ($n = 38$); sample 4 (April 1987), 33% ($n = 30$). Breeding recommenced in mid-October 1987 (sample 6; $n = 20$) but only one adult female was pregnant.

Survival. From February–October 1987 there were significant differences in the survival of mice at the MRS with regard to time ($F = 11{\cdot}80$, $P < 0{\cdot}001$, D.F. = 9, 39) and cohort ($F = 7{\cdot}80$, $P < 0{\cdot}001$, D.F. = 3, 39). Overall, survival peaked at 52% in July, however it dropped below 20% in February, March, late September and October (Table 1). Of the cohorts, survival of mice was lowest (17%) in the $< 12{\cdot}0$ g cohort (= subadults), and highest (36%) in the 14·0–15·9 g cohort (Table 1).

The association between host abundance (at time $t-1$) and survival was then modelled by splitting the 9 degrees of freedom for time into 1 degree of freedom for abundance and 8 degrees of freedom for remaining time effects. There was a significant association between density and survival ($F = 26{\cdot}98$, $P < 0{\cdot}001$, D.F. = 1, 39); survival was positively correlated with density. Even with survival adjusted for density effects, differences with regard to time of sampling remained significant ($F = 9{\cdot}91$, $P < 0{\cdot}001$, D.F. = 8, 39).

Serology

Overall. Mouse populations at each habitat were seropositive for six viruses: MHV, rotavirus, MCMV, MAdV-K87, reo 3 and MVM. Of these, only antibodies to MVM were not detected at each trapping session. No antibodies to MVM were detected

Table 2. *Differences in seroprevalence between mice captured in crops and in areas near permanent water*

Virus	Habitat	Seroprevalence*	χ^2_1	*P*-value
Rotavirus	Water	85·3	4·52	< 0·05
	Crop	75·0		
MVM†	Water	26·5 (2·24)	7·55	< 0·01
	Crop	19·8 (0·85)		
MAdV‡	Water	45·5 (3·83)	11·88	< 0·001
	Crop	25·9 (3·77)		
reo 3‡	Water	20·4 (3·24)	8·10	< 0·01
	Crop	35·0 (3·82)		
MCMV‡	Water	97·7 (1·16)	6·92	< 0·01
	Crop	90·6 (2·62)		

* One standard error is shown in parentheses.
† Seroprevalences are adjusted to the mean weight.
‡ Seroprevalences are adjusted to the mean length.

in March and April 1987 (Fig. 2*a*, 2*b*, 2*c*, 2*d*). No antibodies to LCMV, TMEV, MAdV-FL, PVM, or polyoma, ectromelia, Sendai, Hantaan viruses or *M. pulmonis* were detected at any of the six trapping sessions.

Effects of weight and length. There were significant differences in the length ($F = 8·00$, $P < 0·001$, D.F. = 5, 280) and log weight ($F = 8·35$, $P < 0·001$, D.F. = 5, 280) of mice between the six sampling sessions (hereafter referred to as time). There was also a significant time by sex interaction for length ($F = 4·19$, $P < 0·01$, D.F. = 5, 280) and log weight ($F = 4·74$, $P < 0·001$, D.F. = 5, 280). This effect was due primarily to females being shorter and lighter than males in October 1987; the opposite occurred previously whereby females were longer and heavier than males. Because of these differences the viral and helminthological data were tested first to determine whether weight and/or length significantly covaried with the prevalence or intensity of infection. If there was a significant association then the data set was adjusted for weight or length.

Prevalence of antibodies to MVM and MCMV showed significant covariance with both length and weight ($P < 0·05$ for MVM and reo 3; $P < 0·001$ for MCMV). Prevalence of antibodies to reo 3 showed significant covariance with length only ($\chi^2_1 = 4·81$, $P < 0·05$). Length was used as a covariate for MCMV and reo 3 and weight as a covariate for MVM in the analyses reported below. The prevalence of antibodies to MAdV significantly covaried with weight ($\chi^2_1 = 4·56$, $P < 0·05$). Weight was used as a covariate for this virus in the analyses reported below.

Temporal differences. The prevalence of antibodies differed significantly with time for MHV ($\chi^2_5 = 20·71$, $P < 0·001$), MVM ($\chi^2_5 = 186·86$, $P < 0·001$), MAdV ($\chi^2_5 = 42·51$, $P < 0·001$), reo 3 ($\chi^2_5 = 25·52$, $P < 0·001$) and MCMV ($\chi^2_5 = 31·30$, $P < 0·001$). In all cases, prevalences increased with the increase in mouse density in July 1987 (Fig. 2*a–d*). The response of MVM to changes in host density was the most pronounced.

Sex differences. The prevalence of antibodies only differed significantly between

Table 3. *Prevalence (%) of viral antibodies in mouse populations from two habitats for (i) the main trapping localities, and (ii) all localities combined, October 1986–October 1987*

Location, habitat and time	*n**	MHV	reo 3	MAdV-K87	MVM	Rota-virus	MCMV	(*n*)†
Ouyen								
Near water								
Oct.	5	100	0	60	0	100	100	
Jan.	10	100	10	50	0	90	100	
Mar.	5	100	0	0	0	100	100	
Apr.	10	100	20	0	0	44	82	
Jul.	10	100	50	60	70	90	100	
Oct.	5	100	80	80	80	80	100	
Cropped areas								
Oct.	5	100	40	40	0	100	80	
Jan.	9	78	33	22	0	89	88	
Jul.	12	100	50	25	0	92	92	
Walpeup								
Near water								
Oct.	6	100	33	50	0	100	67	
Jan.	7	100	14	29	0	86	100	
Mar.	9	100	11	11	0	89	100	
Apr.	4	75	0	0	0	100	100	
Jul.	13	100	0	69	0	77	100	
Oct.	22	100	32	73	95	86	100	
Cropped areas								
Oct.	11	100	0	45	0	64	82	
Jan.	11	100	9	9	0	73	100	
Mar.	20	90	20	10	0	80	87	
Apr.	1	100	100	NT‡	0	100	100	
Jul.	5	100	80	40	0	40	100	
Oct.	24	100	65	42	96	75	100	
3–5 km south of Walpeup								
Near water								
Oct.	6	100	50	83	33	67	100	
Jan.	1	100	0	0	100	100	100	
Apr.	6	100	17	33	0	100	100	
Jul.	2	100	50	0	0	100	100	
Oct.	4	100	25	50	100	75	100	
Cropped areas								
Oct.	4	100	0	25	0	100	75	
Apr.	19	100	37	5	0	74	62	
All water (n = maximum)								
Oct.	20	100	21	65	11	90	91	(23)
Jan.	21	95	10	33	5	90	96	(25)
Mar.	20	79	5	11	0	90	96	(25)
Apr.	20	95	16	10	0	74	92	(25)
Jul.	27	100	22	63	26	85	100	(16)
Oct.	31	100	39	71	94	84	100	(31)
All crop (n = maximum)								
Oct.	20	100	10	50	0	80	82	(28)
Jan.	20	90	20	16	0	80	88	(24)
Mar.	20	90	21	14	0	77	93	(28)
Apr.	20	100	35	11	0	72	77	(26)
Jul.	26	100	44	24	0	68	96	(34)
Oct.	24	100	65	42	96	75	100	(26)

* n, sample size.
† (n), sample size for MCMV sera; n = (n) for the main trapping localities.
‡ NT, not trapped.

sexes for MCMV ($\chi^2_1 = 5{\cdot}54$, $P < 0{\cdot}05$); adjusted for an overall mean length of 81·1 mm, the prevalence in males was 96·2% (±1·40 S.E.) and in females was 90·7% (±3·10 S.E.). The prevalence of antibody to reo 3 differed significantly by sex and time ($\chi^2_5 = 16{\cdot}06$, $P < 0{\cdot}01$). The prevalence in females was about half that in males in 3 of the 6 samples but in samples 4 (April 1987) and 6 (October 1987) the opposite occurred; the prevalence in females was 39% and 77%, and in males 14% and 42%, respectively (Fig. 2*d*).

Habitat differences. The seroprevalence of rotavirus, MVM, MAdV and MCMV was significantly higher in mice captured near permanent water. In contrast, the seroprevalence of reo 3 was significantly higher in mice captured in crops (Table 2).

Micro-geographic differentiation. During the 13 months, changes in seroprevalence of all viruses followed similar trends in mice in the three main trapping localities (Table 3). Notable exceptions were higher seroprevalences of reo 3 and MVM in mice caught near water at Ouyen in July 1987, compared to the seroprevalence of all mice caught near water for that month. Indeed, only 7 of 53 mice sampled from crop and water habitats were seropositive to MVM in July and all were from a sample of 10 mice caught at Ouyen.

Parasitology

One helminth (*Syphacia obvelata*), two cestodes (*Vampirolepis straminea*, *V. fraterna*) and one metacestode (*Taenia taeniaeformis*) were recorded in mice in all but one of the six samples; *T. taeniaeformis* was not recorded in April 1987 (Table 4). *T. taeniaeformis* occurred too infrequently throughout the study for statistical analyses. Prevalence of helminths differed significantly with time for *Vampirolepis* spp. ($\chi^2_5 = 16{\cdot}50$, $P < 0{\cdot}01$) and *S. obvelata* ($\chi^2_5 = 43{\cdot}14$, $P < 0{\cdot}001$). The difference for *S. obvelata* was not associated with increases in host density; prevalence was lowest in October 1986 and October 1987 (Table 4).

Vampirolepis spp. were more prevalent among mice caught near water ($\chi^2_1 = 11{\cdot}92$, $P < 0{\cdot}001$) but there was also a significant sex by habitat effect ($\chi^2_1 = 8{\cdot}39$, $P < 0{\cdot}01$). This was due to a significant habitat effect among males but not females; prevalence of infected male mice was 19·1% (±4·05 S.E.) among those caught near water and 9·9% (±0·99) among those caught in crop/stubble. Prevalence of infection in females was 10·5% (±4·73) and 10·6% (±4·41), respectively.

Variation in intensity of infection was analysed only for *S. obvelata*. There was a significant time effect on log of intensity of infection ($F_{5,273} = 6{\cdot}93$, $P < 0{\cdot}001$). Again, this effect was not associated with increases in host density: the intensities of infection in January 1987 (116·1 worms) and July 1987 (142·7 worms) were double those of the other four samples.

Associations between viruses

All 242 mice screened had antibodies to at least one virus; 53% were seropositive for at least four viruses in Oct 1986; 37% in Jan 1987; 18% in March 1987; 20% in April 1987; 59% in July 1987; and 93% in Oct 1987 (Table 5).

Table 4. *Prevalence (%) and mean intensity of helminths in mice captured in crops and in areas near permanent water, October 1986–October 1987*

Time	Habitat	*n**	*Vampirolepis* spp. Prev	*T. taeniaeformis* Prev	*T. taeniaeformis* Int	*S. obvelata* Prev	*S. obvelata* Int
Oct.	Water	24	0	17·0	2·0	71·0	71·4
	Crop	28	3·6	7·1	8·5	75·0	70·0
Jan.	Water	18	11·1	5·6	1·0	100	96·2
	Crop	21	0	0	0	100	133·6
Mar.	Water	24	3·9	8·3	2·5	92·3	65·0
	Crop	23	4·0	0	0	92·0	29·8
Apr.	Water	24	4·0	4·0	1·0	100	84·2
	Crop	25	0	0	0	96·0	49·5
Jul.	Water	23	17·4	13·0	1·0	95·7	199·8
	Crop	20	10·0	5·0	1·0	100	75·1
Oct.	Water	26	38·0†	0	0	100	49·3
	Crop	26	7·7	3·9	1·0	84·6	62·7

* *n*, sample size.
† Mixed species: *V. fraterna* & *V. microstoma*.

Table 5. *Distributions of the numbers of different viral antibodies and helminths per mouse over the six sampling periods*

Sampling period	*n*†	Number of viruses* (serology) 1	2	3	4	5	6
1986							
Oct.	38	1	3	14	17	2	1
1987							
Jan.	38	2	7	15	13	1	0
Mar.	33	0	12	15	6	0	0
Apr.	30	1	11	12	6	0	0
Jul.	49	0	6	14	22	5	2
Oct.	54	0	0	4	12	28	10
Totals	242	4	39	74	76	36	13

	n	Number of helminth species 0	1	2	3
1986					
Oct.	52	12	32	7	1
1987					
Jan.	39	0	36	3	0
Mar.	47	0	43	4	0
Apr.	49	0	48	1	0
Jul.	42	1	32	8	1
Oct.	52	3	37	12	0
Totals	281	16	228	35	2

* Detected as antibody.
† *n*, sample size.

Table 6. *Summary of some key parameters that influence the epizootiology of the six viruses for which antibodies were detected in mice at Walpeup [after 21, 39]*

Virus	Transmission and shedding route	Site of infection	Immune- and age-dependent aspects	Effect on laboratory mice*
Rotavirus (EDIM)	Faecal–oral; not vertical; airborne	Intestine	Disease only in neonates; acute, self-limiting; adults susceptible to subclinical infection	Mortality only among neonates
MHV	Faecal–oral or airborne; mechanical transmission by arthropods; vertical unlikely	Intestine or polytropic (lymphoid organs, liver, brain are major targets)	Enterotropic strains easily transmitted; acute, self-limiting; mortality in young mice	Disease or mortality only in mice susceptible by virtue of age, genotype or immune incompetence; transient depression of immune function
reo 3	Faecal–oral; vertical unlikely	Polytropic	Acute, self-limiting; infant mice more susceptible than weanling mice	Alopecia
MCMV	Close contact; saliva	Salivary gland	Latency common; persistent infection; young mice more susceptible than old mice	Immuno-suppression
MAdV-K87	Faecal–oral	Intestine	Acute, self-limiting; neonates $> 100\times$ more susceptible to infection than weanlings	None
MVM	Faecal–oral; urine; very stable	Polytropic; kidney and intestine are major targets	Acute, self-limiting; disease and mortality only in neonates of certain genotypes	None in adults; disease in some neonates; occasional runting

* All listed infections are subclinical in naturally infected adult, immunocompetent laboratory mice.

Analyses of the associations between the respective viruses were restricted because the seroprevalences of MHV, MCMV and MVM were generally too high (> 90%) or too low (< 10%). Paired associations amongst the other three viruses indicated that mice with antibodies to reo 3 were more likely to be seropositive than seronegative to rotavirus ($\chi^2_1 = 5{\cdot}59$, $P < 0{\cdot}05$, $n = 257$). This association between the 2 viruses was consistent over the 6 samples; only 16 mice were seropositive to reo 3 in the first 3 sample sessions but 15 of these mice also had antibodies to rotavirus.

Association between viruses and helminths

The overall correlation between the number of viruses (from serology) and the number of helminth species recorded in each mouse was not significant ($r = 0{\cdot}09$, $n = 214$). Analyses of associations between specific viruses and helminths were limited to three viruses (rotavirus, reo 3 and MAdV-K87) and one helminth (*Vampirolepis* spp.). Prevalences of the other viruses and helminths were too high or too low throughout the study for determining associations. There was a tendency for mice to have either antibody to MAdV-K87 and be infected with *Vampirolepis* spp. or to have both absent ($\chi^2_1 = 4{\cdot}3$, $P < 0{\cdot}05$, $n = 235$).

DISCUSSION

Mice from the Mallee wheatlands of southeastern Australia were seropositive for six viruses; MHV, rotavirus, MVM, MAdV-K87, reo 3, and MCMV. Antibodies to these viruses occur commonly in mouse populations in eastern and southern Australia [6] but nothing was known about how their seroprevalence varied with time. In the current study, the seroprevalence of MHV, MVM, MAdV, reo 3 and MCMV all varied significantly with time. Generally, seroprevalence decreased or remained low from January to April 1987 and increased in July 1987. This increase occurred during a period of rapid increase in the population density of mice from April to July 1987. No antibodies to LCMV, TMEV, MAdV-FL, or PVM, ectromelia, Sendai, polyoma and Hantaan viruses were detected. In Australia, antibodies to LCMV and Sendai virus have been reported in wild mice, but at low prevalence and at a few localities only [6]. The sample sizes in the present study, indicate that at each time of sampling none of these eight viruses was present with a seroprevalence of > 10% [24]. That none of the viruses was detected in any of the 12 samples (6 times × 2 habitats), suggests that it was unlikely that they were present at all. The prevalence of the four helminths (*T. taeniaeformis*, *S. obvelata*, and *Vampirolepis* spp.) also showed significant differences with time but there was no apparent association with increase in host density. There was no serological evidence of *M. pulmonis* infection.

These results indicate that the seroprevalence of at least some of the infectious agents present in wild mouse populations may be density-dependent, an important prerequisite if a parasite is to be capable of regulating population density [27]. However, our results are, at best, preliminary for two reasons. First, there were only six sampling periods over 13 months. Second, seroprevalence tests are not ideal for epizootiological studies because they provide a history of infection that is not precise. In the current study, we could not determine whether a mouse was

recently infected and shedding virus, the likely time of maximal replication in the host, or whether the mouse had recovered from any potential clinical or functional effects of virus infection.

The first problem can be overcome if the seroprevalence of viruses was measured more often during the major phases of host density; low, increasing, high, declining and low (immediately post-outbreak) again. This would provide a better measure of how changes in mouse density influence the prevalence of murine viruses and vice versa. The second problem is more difficult to surmount. If the class of the immunoglobulin (IgM or IgG) was determined, then the presence of IgM would usually indicate a recent infection. Further progress could be made by sampling systematically across age classes. However, until there is an affordable technique that can be applied routinely to hundreds of animals for detecting virus replication, preferably without sacrificing the animal, the problem will remain.

The most marked increase in seroprevalence occurred with MVM. This rose from zero in most habitats in July 1987 to $> 90\%$ in October 1987. The occurrence of an epizootic of MVM is not surprising given that the virus can be transmitted via faeces, urine, and the oronasal route (Table 6). Moreover, MVM can withstand desiccation, increasing the likelihood of susceptible mice coming in contact with shed virus [28]. In the present study, the seroprevalence of MVM increased rapidly when host density was high, indicating that there may be a threshold host density above which MVM is highly contagious. The unanswered questions are where and how did the virus persist prior to the epizootic and why did it take so long for the epizootic to occur? The results indicate that MVM persisted in small pockets of the host population. The rate of spread of the virus, however, was probably not restricted by social barriers because the seroprevalence of the virus was low during the increase phase in host density, a period when dispersal by mice would be high [22, 29].

What then were the effects of the epizootic of MVM on the mouse population? An indication that a virus may be influencing host dynamics is low seroprevalence when host survival is high and high seroprevalence when host survival is low [17]. The seroprevalences of MVM and reo 3 clearly followed this pattern. In the case of MVM, there was strong evidence of a viral epizootic during the rapid spring decline in mouse abundance in 1987. Whether these two viruses are having a significant impact on host dynamics either through their own virulence or through increasing the susceptibility of mice to other parasites, cannot be determined from the present study. However, the results indicate the merit of conducting further research on the effects of MVM and reo 3 on the dynamics of field populations of mice.

Two other studies of seroprevalence of viruses in small mammals have followed demographic changes in the host population for at least a year. Kaplan and colleagues [11] reported an epizootic of PVM in wood mice, *Apodemus sylvaticus*, in the UK, and Descôteaux and Mihok [13] reported an epizootic of TMEV and possibly reo 3 in meadow voles, *Microtus pennsylvaticus*, in Canada. Again, neither of these studies were able to document more than an association between increased seroprevalence and decreased host survival. Interestingly, antibodies to PVM and TMEV were not recorded in the current study nor in a survey of viruses in mice from 13 other sites in Australia [6].

A sudden decrease in survival of one cohort relative to others could also indicate the pathogenic effects of a virus, this time via an epizootic. This did not happen in the present study, despite the apparent epizootic of MVM. Instead, the survival analyses indicated that time of year and age structure of the host population were the major factors influencing host survival.

Seasonal effects and density effects are often difficult to disentangle without a long term study ($\geqslant 2$ years). Increases in seroprevalence of most of the viruses occurred in July and October 1987. Winter (June–August) is characteristically the time of year when food quantity and quality is at its lowest for mice [30, 31]. Protein malnutrition has been shown to potentiate Sendai [32] and MCMV [33] disease in laboratory mice and is likely to potentiate the effect of the other viruses recorded in the present study. Added to this is the social stress among male mice that occurs with commencement of breeding in early Spring (September and October). The relative effects of malnutrition and social stress on the prevalence and persistence of a virus and the progress of disease in wild mice is not known.

Thus far we have considered primarily the possible effects of individual viruses. This may be misleading given the high degree of co-occurrence of antibodies to other viruses in the current study. Little is known of the concomitant effects of these viruses nor of how their combined effect is influenced by extrinsic and intrinsic factors such as food supply and stress which act on host populations [6]. There is some information on the subject as it pertains to laboratory rodents. Infection of the laboratory rat with respiratory viruses such as sialodacryoadenitis virus (coronavirus) [34] or Sendai virus (paramyxovirus) [35] greatly exacerbates respiratory mycoplasmosis, although the mechanism is not known. Polytropic or respiratory strains of mouse hepatitis virus have been shown to interfere with the host response to pneumonia virus of mice (PVM, paramyxovirus) under both natural and experimental conditions [36]. PVM replication in the respiratory tract was diminished, and the PVM-specific antibody response was reduced and delayed in dually infected mice [36]. Prior MHV infection also compromised the susceptibility of inbred mice to Sendai virus infection [36]. The likely mechanism for MHV-associated alteration of respiratory virus pathogenesis is its potent induction of an interferon response [37, 38].

Associations in seroprevalence were found in the present study between rotavirus and reo 3 and between MAdV-K87 and presence of *Vampirolepis* spp. Unfortunately, the consistently high seroprevalences of MCMV and MHV, low seroprevalences of MVM, and high prevalence of *S. obvelata*, restricted our analyses of associations between the infectious agents. Further studies of these associations in populations of wild mice and of the consequences of such associations on the dynamics of host populations would be rewarding.

There were habitat differences in seroprevalence of five viruses and in the prevalence of the *Vampirolepis* spp. An increase in prevalence of *Vampirolepis* spp. in mice living near permanent water has been reported previously [12] and is consistent with the involvement of invertebrates in the transmission of the parasite. Of the viruses, antibodies to rotavirus, MVM, MAdV and MCMV were more prevalent in mice living near permanent water and antibody to reo 3 was more prevalent in mice living in crops or stubble. There were no significant habitat differences in host abundance or rates of population increase of the host, especially

when the densities of mouse populations were low during the first 10 months of the study. Features of the life history of a virus such as its mode of transmission and degree of persistence in the environment could help explain these habitat differences. For example, some viruses may survive longer in more humid environments than in the fields of the semi-arid wheatlands. An overview of the features of each virus that could influence its epizootiology is presented in Table 6. This overview provides no obvious explanation for the habitat differences recorded in the present study. Perhaps this reflects the fact that the epidemiological information for each virus was drawn primarily from laboratory studies of mice and rats [21, 39].

We conclude by reconsidering the three conditions stated in the introduction. Firstly, infections with six viruses and four helminths were identified. All occurred at low mouse density and most persisted during the rapid increase and then precipitous decline in host abundance. Second, the seroprevalence of five of the six viruses was density dependent. Third, the seroprevalence of antibody to MVM and reo 3 was low during high survival, and high during low survival, of mouse populations. Together, these results indicate an association between the prevalence of these two viruses and the density of the host population. Although future studies should focus on MVM and reo 3, our results also emphasized that the additive or synergistic effects of other viruses during periods of malnutrition or social stress of the host population must be considered.

The results of the present study also need to be viewed in the context that the mouse populations were not in plague densities. Although mouse densities were the second highest recorded during an 8-year study at Walpeup [7], they were at least an order of magnitude lower than those recorded in mouse plagues in this region [1, 2, 22]. Therefore, it will be of immense interest to see how changes in seroprevalence recorded in the present study, compare with those that occur during a plague.

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