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Ecological aspects of the epidemiology of infection with leptospires of the Ballum serogroup in the black rat (*Rattus rattus*) and the brown rat (*Rattus norvegicus*) in New Zealand

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SUMMARY

Epidemiological aspects of infection with leptospires of the Ballum serogroup in black rats (*Rattus rattus*) and brown rats (*Rattus norvegicus*) are described. Rats inhabiting a variety of habitats were investigated and isolates identified as belonging to the Ballum serogroup were obtained from 21 of 61 black rats (34%) and 63 of 243 brown rats (26%). The high level of endemic Ballum serogroup infection in these species reported here has not been described in other countries.

A statistical relationship was shown between the prevalence of infection in brown rat populations and population density but this was not evident for black rats. Epidemiological data indicates that the black rat is a maintenance host for leptospires of the Ballum serogroup in New Zealand. The brown rat does not appear to be an efficient maintenance host for these leptospires, however endemic infection can be maintained in high-density populations inhabiting synanthropic foci.

An hypothesis of 'competitive exclusion' (preferential maintenance of a particular serovar by a host species) is introduced with regard to leptospiral infection in brown rats. It is concluded that the establishment and maintenance of an endemic focus of leptospirosis is dependant on: introduction of a particular serovar; a suitable host; and a suitable host habitat. Within a maintenance population direct transmission appears to be more important than indirect transmission via the environment.

INTRODUCTION

Rats have been shown to be carriers of leptospires throughout the world and are important resevoirs of infection for domestic stock and man. The two species present in New Zealand, the brown rat (*Rattus norvegicus*) and the black rat (*Rattus rattus*) have a cosmopolitan distribution and many different serovars have been isolated from these species in other countries (U.S.D.H.E.W., 1966; U.S.D.H.E.W., 1975). The brown rat is generally regarded as the maintenance host for leptospires of the Icterohaemorrhagiae serogroup and is an occasional carrier of other serovars (Babudieri, 1958; Fennestad & Borg-Petersen, 1972; Michna & Ellis, 1974). The black rat is a less frequent carrier of leptospires (Broom, 1958; Emanuel, Mackerras & Smith, 1964; Torten *et al.* 1970).

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Table 1. *Trap night averages for black rats and brown rats trapped in different habitats*

Habitat	Location	No. trap-nights	Trap-night average (per 100 trap-nights)	
			Black rats	Brown rats
Farmland	Massey	1023	0·9 (0·23)*	0·3
	Otawhao	288	2·1 (0·53)	0
Farm buildings	Massey	537	0·4 (0·1)	1·9
	Linton	616	0·8 (0·2)	1·9
Forest	Woodville	156	2·6 (0·65)	0
	Tiritea	278	4·0 (1·0)	0

* Indices of abundance for black rats, relative to Tiritea.

Previous investigations of rats in New Zealand have resulted in the isolation of leptospires of the Icterohaemorrhagiae and Ballum serogroups (Kirschner & Gray, 1951; Brockie, 1977; Carter & Cordes, 1980) though others have reported negative results (Blakelock & Allen, 1956; Buddle & Hodges, 1977). It appears from these studies that endemic infection with leptospires of the Icterohaemorrhagiae serogroup in brown rats is restricted to a limited number of geographical regions.

This paper describes some epidemiological aspects of leptospiral infection in rats inhabiting both natural and synanthropic foci in the southern half of the North Island. New Zealand provides an unusual situation in which to investigate leptospiral infection in free-living populations because the only indigenous mammals are two species of bats. All other mammalian species have been introduced, mostly in the last 200 years, and have occupied vacant ecological niches. Consequently, only a small number of species inhabit a particular ecosystem, and each of these species is often present in relatively high densities. Few different leptospiral serovars have been isolated from these species and thus the type ecosystem for a particular serovar (maintenance ecosystem for a particular infectious agent in nature) (Schwabe, Reimann & Franti, 1977) can be relatively easily defined.

MATERIALS AND METHODS

Collection of rats

Rats were collected from several habitats including forest, farmland, suburbs, and refuse dumps. The animals were either trapped or shot. Trapping was by snap or cage traps and these were baited for 3 days before activating. Collecting methods were not designed to trap only one species in any one location sampled, however the majority of black rats were collected by trapping and brown rats by shooting.

Snap traps killed the rats and blood samples were not therefore available for serology. Both serological and bacteriological examinations were carried out on live rats captured in cage traps. The most successful method of collecting brown rats in locations where there were high populaton densities was by night-shooting using 0·22 calibre birdshot. Blood samples were collected immediately by opening the thorax. Black rats were never seen by spot-lighting and it appeared that they were absent from habitats that supported high densities of brown rats.

Table 2. *Subjective estimates of the relative abundance of brown rats inhabiting different refuse dumps*

		Density estimates			
Location	Size	Subjective	No. shot/ 1½ hrs	360° spot light count/ 2 min	Estimate of relative abundance
Feilding	Large	XXXXXX (1·0)*	30 (1·0)	45 (1·0)	1·0
Longburn	Small	XXX (0·5)	12 (0·4)	8 (0·18)	0·36
Kiwitea	Small	XX (0·33)	6 (0·2)	3 (0·07)	0·21
Palmerston	Large	X (0·17)	6 (0·2)	2 (0·04)	0·14

* Indices of abundance relative to Feilding.

All rats were weighed, sexed and classified as either sexually immature or mature. Sexual maturity is reached in black rats at approximately 80 g (Bentley & Taylor, 1965; Innes, 1977) and from 115 to 240 g in brown rats (Leslie, Perry & Watson, 1946; Calhoun, 1962). The lower limit was used in this study.

Trapping success was expressed in terms of trap-night averages (TNAs) and these values were used as indices of relative abundance of a species in different habitats (Table 1). A small number of black rats were caught in possum traps and they were excluded from calculations of TNAs. Indices of relative abundance for brown rats inhabiting different refuse dumps were made from subjective density estimates (Table 2). Each variable was given equal weighting.

Serological and bacteriological examinations

Sera were tested against live *L. ballum*, *copenhageni*, *pomona*, *hardjo* and *tarassovi* antigens (representing all serogroups from which leptospires have been isolated in New Zealand), using a modified microscopic agglutination test (MAT) (Cole, Sulzer & Pursell, 1973). The initial serum dilution used was 1 : 12. A random sample of 50 sera was also tested against *L. australis*, *autumnalis*, *bataviae*, *biflexa*, *canicola*, *grippotyphosa* and *pyrogenes* antisera. As initial serology from brown rats inhabiting refuse dumps revealed only low and infrequent titres, serological examinations were not carried out on a proportion (26 %) of brown rats taken from these locations during later surveys.

Kidneys were cultured within 12 h of death. After aseptic removal from the carcass each kidney was put in a gamma-sterilized plastic bag containing 50 ml of Stuarts base medium (SBM) (Stuart, 1946) and homogenized in a Colworth Stomacher 400 (Seward & Co. Ltd, London). The Stomacher could not be used for small amounts of tissue and therefore kidneys from immature rats were homogenized by forcing through a 5 ml, sterile disposable syringe fitted with a 14 gauge needle. One ml of homogenate was inoculated into 9 ml of SBM and two serial ten-fold dilutions in SBM were made from this primary dilution. Approximately 0·3 ml of each dilution was then inoculated into 5 ml of semisolid EMJH medium (Difco) containing 200 μg/ml of 5-fluorouracil (5FU). A parallel series of cultures from each kidney was made in media containing 400 μg/ml of 5FU.

Cultures were incubated at 30 °C and examined by dark-field microscopy at 2 week intervals for 3 months. Isolates were subcultured in liquid EMJH medium

Table 3. *The bacteriological prevalence of serogroup Ballum infection in black rats and brown rats from different habitats in the southern half of the North Island of New Zealand*

		Black rats			Brown rats		
Habitat	Location	No.	No. positive	Prevalence (%)	No.	No. positive	Prevalence (%)
Farmland	Massey	9	4	44	3	0	0
	Otawhao	16	8	50	0	—	—
Farm buildings	Massey	2	0	0	10	0	0
	Linton	5	2	40	12	0	0
Forest	Woodville	15	5	33	0	—	—
	Tiritea	11	2	18	0	—	—
Suburbs	Palmerston North	3	0	0	5	0	0
Refuse dump	Feilding	0	—	—	123	53	43
	Longburn	0	—	—	55	8	14
	Kiwitea	0	—	—	6	1	17
	Palmerston North	0	—	—	29	1	3
Total		61	21	34	243	63	26

until of sufficient density for serogrouping by cross-agglutination against reference antisera in the MAT. For definitive serovar identification by cross-agglutination absorption, antisera against two isolates were prepared in rabbits (Kmety, 1974) and both were sent to the WHO Leptospirosis Reference Laboratory, C.D.C., Atlanta, Georgia.

RESULTS

Black rats were trapped in farmland, forest and suburban habitats, thus reflecting the widespread distribution of this rodent in New Zealand compared with other countries where it is generally confined to cities and ports. Brown rats were trapped only in synanthropic habitats that provided a food source associated with the activity of man. Different habitats supported markedly different population densities of rats (Tables 1 and 2) and farm buildings were the only habitat where both black and brown rats were found.

A total of 61 black rats and 243 brown rats were collected. Sera were obtained from 30 black rats and 168 brown rats and serological testing revealed very few titres in both species. Antibodies to serovar *ballum* were found in eight black rats (27%) and ranged from 1:24 to 1:192. Only six brown rats (4%) had titres to this antigen, ranging from 1:12 to 1:48. Low titres (1:24 or less) against serovars *pyrogenes* (2), *copenhageni* (1), *tarassovi* (1) and *canicola* (1) were detected in five black rats. Similar titres against serovars *pyrogenes* (3) and *tarassovi* (2) were detected in five brown rats.

Leptospires were isolated from 21 black rats (34%) and 63 brown rats (26%). All isolates belonged to the Ballum serogroup and one isolate from each species was typed by cross-agglutination absorption as serovar *ballum*.

In many cases isolates were made from seronegative rats, with 67% of

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culturally-positive black rats and 90% of culturally-positive brown rats being in this category. Only one black rat was seropositive but negative on culture. There was no significant difference ($P > 0{\cdot}05$) in the prevalence of infection by sex in either species, however there were marked differences in age-specific prevalences of infection. All isolations from black rats were from sexually-mature animals, and only two of 23 sexually-immature brown rats were positive (8·7%). These differences were statistically significant for both species ($P < 0{\cdot}05$).

The prevalence of infection in brown rats from different locations showed considerable variation (Table 3). Prevalences ranged from 3 to 43% for brown rats inhabiting refuse dumps, and these differences were highly significant ($P < 0{\cdot}001$). No isolations were made from brown rats inhabiting other environments. In comparison, differences in the prevalence of infection in black rat populations were much less pronounced (Table 3), and isolates were recovered from black rats from all habitats except suburban Palmerston North.

A consideration of the relative indices of abundance (Tables 1 and 2) and the prevalence of infection in different populations provides some important epidemiological information on infection with leptospires of the Ballum serogroup in rats. There was a strong correlation between indices of relative abundance and the prevalence of infection in brown rats inhabiting different refuse dumps ($r = 0{\cdot}96$, $P < 0{\cdot}01$) and analysis using the GLIM package for generalized linear models (Nelder & Wedderburn, 1972) demonstrated that the regression of prevalence of infection on relative abundance ($y = 41{\cdot}2x + 1{\cdot}96$, $t = 4{\cdot}83$) was statistically significant ($P < 0{\cdot}05$). The relative abundance of brown rats inhabiting farmland and farm buildings could not be compared with refuse dumps because of the different method of capture, however it was estimated that they were present in considerably lower numbers than even the lowest density refuse dump population. No isolations were made from these populations.

In comparison with brown rats, no statistically significant relationships could be shown between relative indices of abundance of black rats in different habitats and the prevalence of infection. Thus the prevalence of infection with leptospires of the Ballum serogroup in different populations of black rats did not appear to be associated with population density.

The Ballum serogroup isolates grew vigorously in artificial media and of the 84 isolates obtained, 60 grew in all six culture tubes inoculated with each kidney homogenate. The contamination rate of media containing 200 μg FU/ml was 8% and that of media containing 400 μg 5FU/ml was 6%.

DISCUSSION

The black rat is widely dispersed throughout farmland, forest and suburban ecosystems in New Zealand, whereas the brown rat has a patchy distribution related to the activity of man (Wodzicki, 1950; Best 1968). When favourable conditions for the brown rat exist, this species prevails over the black rat (Daniel, 1969) however the two species can live in the same habitat if the population density of brown rats is low (Wodzicki, 1950). These ecological differences were confirmed in the present study and it is apparent that there is minimal opportunity for transfer of leptospires between the two species.

Table 4. *Prevalence of Ballum and Icterohaemorrhagiae serogroup infection in brown rats in other countries*

Author	Country	No. sampled	Ballum		Icterohaemorrhagiae	
			No.	Prevalence (%)	No.	Prevalence (%)
Broom, 1958	U.K.	850	0	—	370	43
Brown & Gorman, 1960	U.S.A.	30	0	—	0	—
Alexander *et al.* 1963	Puerto Rico	104	2	2	21	20
Minette, 1964	Hawaii	310	5	2	266	86
Schnurrenberger *et al.* 1970	U.S.A.	432	11	3	201	47
Fennestad & Borg-Petersen, 1972	Denmark	82	0	—	17	21
Michna & Campbell, 1970	U.K.	134	1	1	20	15

Table 5. *Prevalence of Ballum and Icterohaemorrhagiae serogroup infection in brown rats in New Zealand*

Author	No. sampled	Ballum		Icterohaemorrhagiae	
		No.	Prevalence (%)	No.	Prevalence (%)
Kirschner & Gray, 1951	52	0	—	2	4
Brockie, 1977	25*	0	—	5	20
	51†	8	16	0	—
Hathaway, 1978	243	63	26	0	—
	123‡	53	43	0	—
Carter & Cordes, 1980	132	12	9	33	25

* Sample from Eastern Waikato. † Sample from Southern Taranaki. ‡ Sample from Feilding refuse dump.

The prevalance of *ballum* titres in black and brown rats was very low and the ratios of serological to bacteriological prevalence were 24:57 and 3:28 respectively. It is evident that serology is an inadequate method for the detection of Ballum serogroup infection in rats and this was especially pronounced for brown rats. Evidence of the inability of leptospires of this serogroup to stimulate high or sustained titres has also been reported in other free-living species (Shotts *et al.* 1975). Titres against antigens other than *ballum* were low and sporadic, and were of unknown significance.

Leptospires of the Ballum serogroup were isolated from both species of rats inhabiting synanthropic foci, confirming the work of others (Brockie, 1977; Carter & Cordes, 1980). Endemic infection was also found in black rats inhabiting natural foci and it is concluded that the black rat is a maintenance host for the leptospires of the Ballum serogroup in New Zealand. The black rat has not been established as a maintenance host for particular serovars in other countries.

Surveys of brown rats in other countries have revealed only low prevalences of Ballum serogroup infection (Table 4). In contrast, high prevalences of Icterohaemorrhagiae serogroup infection have been found and the brown rat is recognized as a maintenance host for these leptospires. Comparative data (Tables 4 and 5) indicates that when there is a high level of endemic Icterohaemorrhagiae serogroup

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infection in brown rats, the prevalence of Ballum serogroup infection is either low or zero. The absence of Icterohaemorrhagiae and other serogroup infections in the rat populations studied in the present investigation appears to facilitate a high prevalence of Ballum serogroup infection in high-density populations. This situation has not been reported in other countries. The patchy distribution of the brown rat in New Zealand probably limits the spread of Icterohaemorrhagiae serogroup infection but if these organisms were introduced to brown rat populations in the southern half of the North Island they might considerably reduce the prevalence of Ballum serogroup infection in high density populations. This hypothesis of 'competitive exclusion' may also relate to leptospirosis in other free-living species in different ecosystems.

The prevalence of infection in black rats appeared to be independant of population density and independant of the different environmental conditions that prevailed in different habitats. In comparison, the prevalence of infection in brown rats was closely associated with population density, with prevalences on refuse dumps with similar environmental conditions but different management practices ranging from 3 to 43%. Infection was absent from low-density populations, and these results suggest that a high frequency of contact is necessary to ensure transmission and hence maintain an endemic focus of Ballum serogroup infection in brown rats. Such contact, both sexual and non-sexual, is maximal in crowded environments (Calhoun, 1962).

The fact that brown rats appear to maintain endemic foci of Ballum serogroup infection only under conditions of high population density suggests that this species is an inefficient maintenance host for these leptospires, however there is no information available on the characteristics or duration of leptospiruria in brown rats infected with leptospires of the Ballum serogroup. Other observations supporting this hypothesis are that leptospires of the Icterohaemorrhagiae serogroup, when present, seem to be maintained preferentially, and surveys in other countries where brown rats share habitats with other rodents with endemic Ballum serogroup infection have shown that they are only rarely infected (Brown & Gorman, 1960; Clark, 1961; Schnurrenberger, Hanson & Martin, 1970). The source of Ballum serogroup leptospires for high density populations of brown rats is speculative but may be from house mice (*Mus musculus*) which are common carriers of these organisms in New Zealand (Hathaway, 1978).

Ecological aspects of the epidemiology of Ballum serogroup infection in black rats and brown rats are summarized in Fig. 1. The establishment and maintenance of an endemic focus of leptospires depends on continued transmission of the organism, and this is dependant on the introduction of a particular serovar, a suitable host and a suitable host habitat. A favourable environment for the survival of the serovar outside the host appears to be relatively unimportant for the maintenance of a nidus of infection under these conditions. This is in contrast to the opinions of several workers who have regarded a favourable environment for the survival of leptospires to be a very important factor in the maintenance of an endemic focus of infection in a free-living maintenance host population (Ferris *et al.* 1961; Gordon-Smith *et al.* 1961; Turner, 1967; Twigg *et al.* 1969). It is believed that a favourable environment is much more important for the transmission of the infectious agent to domestic animals and man. In most cases, the serovars that

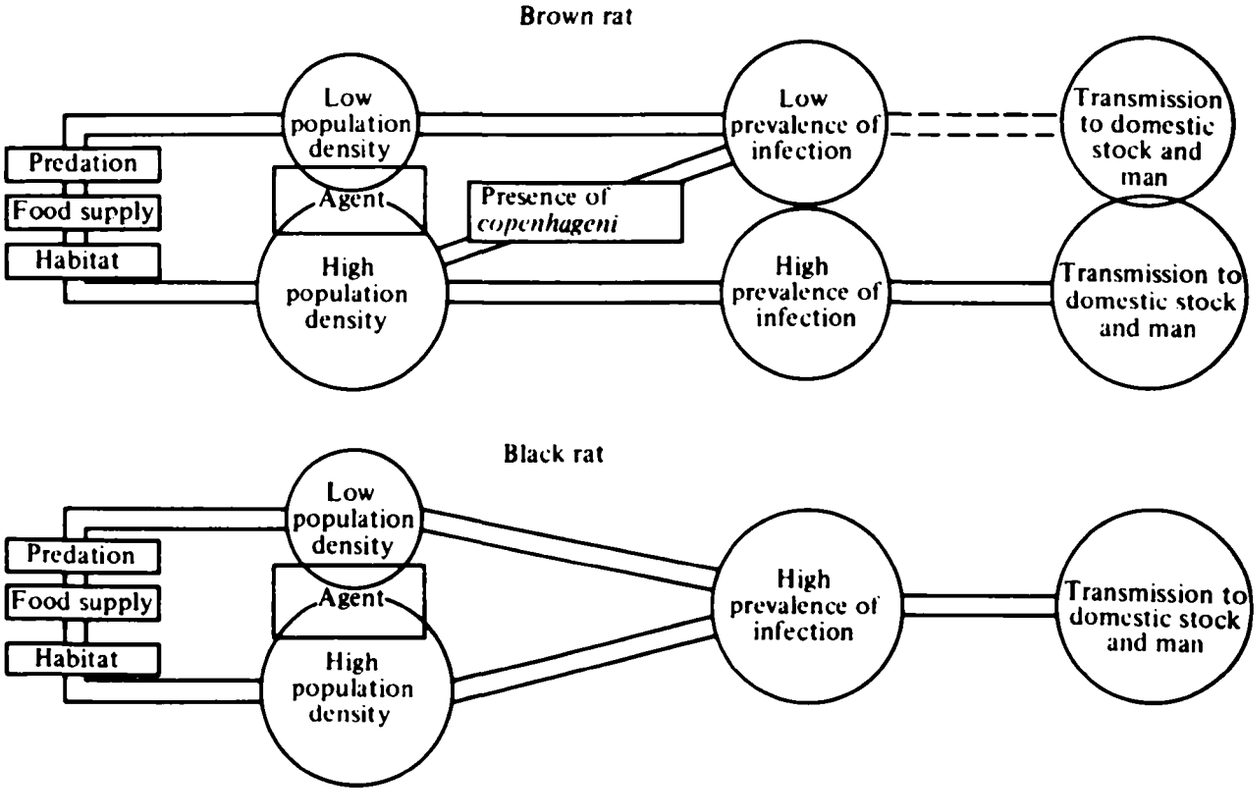

Fig. 1. Ecological aspects of the epidemiology of infection with leptospires of the Ballum serogroup in brown rats and black rats.

are maintained in free-living rodent populations cause only sporadic infections in domestic animals and man in the same habitat, due to the limitations of continual environmental transmission, however an increase in the population density of infected rodents in an ecosystem may result in an increase in the number of sporadic infections in domestic animals and man sharing that ecosystem.

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