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LEPTOSPIROSIS IN FREE-LIVING SPECIES IN NEW ZEALAND

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Abstract: A total of 1296 free-living mammals and birds of 12 species was examined for serologic and bacteriologic evidence of leptospiral infection. Endemic infection with serovar *ballum* was found in several introduced species of mammals. Endemic *ballum* infection is not recognised in the same species in Great Britain, their country of origin.

Possums *(Trichosurus vulpecula)* were found to have a high prevalence of infection with *balcanica,* a serovar that has been isolated from possums in Australia and from cattle, pigs and humans in Eastern Europe. Free-living lagomorphs and deer were both serologically and bacteriologically negative. Waterfowl were bacteriologically negative, and only one serological titre was found.

INTRODUCTION

Free-living animals are known to be carriers and disseminators of leptospires in many countries. Although a high level of endemic leptospiral infection is present in domestic stock in New Zealand,[2,21,32] and the disease is an important zoonosis,[29] only limited information is available on the prevalence of infection in wildlife.

A three-year study was carried out to investigate the prevalence of leptospiral infection in free-living species in the North Island of New Zealand. Apart from the Australian brush-tailed possum, the majority of free-living mammals present in New Zealand were introduced during colonial times[40] and have successfully colonised vacant ecological niches. The pattern of leptospiral infection in these species in New Zealand provides an interesting comparison with the pattern of infection in the same species in Great Britain, their country of origin.

MATERIALS AND METHODS

Free-living mammals (9 species) and waterfowl (3 species) were collected from farmland, forest and urban environments throughout the North Island of New Zealand (Table 1). Methods of collection are described elsewhere.[19] All animals were weighed at the time of collection, sexed, and classified as being sexually immature or mature.

Serologic examinations were conducted at a minimum serum dilution of 1:24 using a modification of the microscopic agglutination test (MAT) described by Cole.[12] Sera were tested against 12 live antigens, *viz: australis, autumnalis, ballum, bataviae, biflexa, canicola, copenhageni, grippotyphosa, hardjo, pomona, pyrogenes* and *tarassovi.* Sera from possums *(Trichosurus vulpecula)* were also tested against *balcanica.*

Whole kidneys for culture were homogenised in a Coleworth Stomacher[2] using the technique

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TABLE 1. Serological and bacteriological prevalence of leptospiral infection in free-living animals and birds from the North Island of New Zealand.

Species	No. Sera	No. titres						Sero.* prev. (%)	No. cultured	No. pos.	Cultural prev. (%)	Isolate Identification	
		ballum	*copen.*	*hardjo*	*pom.*	*tara.*	others					Serogroup	Serovar
House mouse *(Mus musculus)*	39	3	—	—	—	—	1	10	70	11	16	Ballum	ballum
Norway rat *(Rattus norvegicus)*	168	6	—	—	1	2	3	5	245	63	26	Ballum	ballum
Ship rat *(Rattus rattus)*	29	8	1	—	—	1	4	38	63	21	33	Ballum	ballum
Possum *(Trichosurus vulpecula)*	754	11	2	403	4	2	5	56	161	59	37	Hebdomadis	balcanica
Hedgehog *(Erinaceus europaeus)*	25	9	4	—	4	1	5	40	27	5	19	Ballum	NT
Hare *(Lepus europaeus)*	5	—	—	—	—	—	—	0	5	0	0	—	—
Rabbit *(Oryctolagus cuniculus)*	9	—	—	—	—	—	—	0	9	0	0	—	—
Red deer *(Cervus elaphus)*	27	—	—	—	—	—	—	0	3	0	0	—	—
Sika deer *(Cervus nippon)*	4	—	—	—	—	—	—	0	2	0	0	—	—
Grey duck *(Anas superciliosa)*	29	—	—	—	1	—	—	3	29	0	0	—	—
Mallard duck *(Anas platyrynchos)*	29	—	—	—	—	—	—	0	29	0	0	—	—
Pukeko *(Porphyrio melanotus)*	34	—	—	—	—	—	—	0	—	—	—	—	—

*excludes multiple titres in individual serum samples.

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described by Hathaway.[20] Aliquots of 0.3 ml from each of three dilutions of kidney homogenate (1:50, 1:500, 1:5000) were inoculated into semisolid (0.15% agar) EMJH[3] medium containing 200 μg/ml of 5-fluorouracil.[4] In the case of rats and mice the two series of media used for kidney culture contained 200 μg/ml of 400 μg/ml of 5-fluorouracil respectively. The stomacher could not be used for very small amounts of tissue; therefore primary kidney homogenates from mice were made by forcing kidneys through a 2 ml, sterile disposable syringe. Cultures from both liver and kidneys were made from ducks.

Cultures were examined by dark-field microscopy at two-week intervals for three months. The serogroup of each isolate was determined by cross-agglutination against standard antisera using the MAT. Representative isolates from each species of animal were identified by cross-agglutination absorption at the WHO/FAO Leptospirosis Reference Centre, CDC, Atlanta, Georgia, USA.

RESULTS

Serologic examinations revealed evidence of leptospiral infection in several species. Titres to *ballum* were the most common, with the prevalence of titres of 1:24 or greater in house mice *(Mus musculus),* Norway rats *(Rattus norvegicus),* ship rats *(Rattus rattus)* and hedgehogs *(Erinaceus europaeus)* ranging from 4% to 36% (Table 1). Titres in house mice, ship rats and hedgehogs ranged from 1:24 to 1:384, whereas *ballum* titres in Norway rats had a maximum of 1:48. *Ballum* titres were also found in 2% of possum sera.

Titres to *hardjo,* an antigen from the Hebdomadis serogroup, were present in 53% of possum sera. Titres ranged from 1:24 to 1:12,288. Titres to *balcanica,* also of the Hebdomadis serogroup, were present in 377 of 754 possum sera (50%), and ranged from 1:24 to 1:3072.

Only a few titres, all less than 1:192, were detected against antigens from other than the Ballum and Hebdomadis serogroups. Low titres to several antigens were detected in some sera from hedgehogs with *ballum* titres and these were attributed to cross-agglutination. No titres were found in sera from rabbits *(Oryctolagus cuniculus),* hares *(Lepus europaeus),* or deer *(Cervus elaphus* and *Cervus nippon).* Only one titre (1:96 to *pomona)* was found in sera from 3 species of waterfowl.

Leptospiral isolates were obtained from only five species, all mammals. Isolates of the Ballum serogroup were obtained from house mice (16%), Norway rats (26%), ship rats (33%) and hedgehogs (19%). Individual isolates from the three species of rodents were identified by cross-agglutination absorption as serovar *ballum.* Although 2% of possum sera had *ballum* titres, no isolations from this serogroup were made from possums.

Isolates belonging to the Hebdomadis serogroup were obtained from 37% of possums. Eight isolates subjected to cross-agglutination absorption were all identified as serovar *balcanica.* Despite the presence of Hebdomadis serogroup *(hardjo* and *balcanica)* titres of up to 1:12,288 in possum sera, there was virtually no evidence of cross-agglutination with antigens from other serogroups.

There were marked differences in the age-specific prevalences of infection in culture-positive species (Table 2), with most isolates being obtained from mature animals. Isolations from sexually-immature animals were made from only two Norway rats and one house mouse. Differences in prevalence

[3] Difco, Detroit, Michigan, USA.

[4] Sigma Chemical Co., St. Louis, Missouri, USA.

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TABLE 2. Bacteriological prevalence of leptospiral infection in immature and mature possums, rodents and hedgehogs in New Zealand.

Species	Immature Animals			Mature Animals			Overall Prevalence (%)
	No.	No. positive	Prevalence (%)	No.	No. positive	Prevalence (%)	
House mouse	29	1	3	41	10	24	16
Norway rat	26	2	8	219	61	28	26
Ship rat	4	0	0	59	21	36	33
Possum	41	0	0	120	59	49	37
Hedgehog	10	0	0	17	5	29	19

of infection by sex were not significant ($P > 0.5$).

Isolates often were recovered from sero-negative rodents. Titres were not detected in 83% of house mice, 89% of Norway rats and 67% of ship rats from which Ballum serogroup isolates were recovered.

DISCUSSION

Leptospiral infection in free-living and domestic animals embraces a wide range of host-parasite relationships. A particular serovar may be host-specific and localized geographically; less host-specific and more widely distributed; or non-host-specific with a cosmopolitan distribution. Serovar *ballum* is in this last category and has been isolated from a wide variety of free-living and domestic animals and man in different parts of the world.[22,23] In the present survey, infection with leptospires of the Ballum serogroup was found to be endemic in house mice, ship rats and hedgehogs and it is considered that these species constitute maintenance hosts for *ballum* in New Zealand. Serial cross-sectional sampling of infected populations inhabiting different ecosystems demonstrated that endemic infection was maintained in different species for at least the three years of the present study. It appeared that interspecies transmission was not necessary for the maintenance of a nidus of infection in an individual species. There was very little contact between mice, rats and hedgehogs in the ecosystems investigated[19] and ecological studies have shown that the rodent species that live in the same farmland or forest ecosystem in New Zealand inhabit distinct and separate biotopes.[40]

Endemic *ballum* infection in three free-living species inhabiting the same geographic region has not been described in other countries, although house mice have often been found to have a high prevalence of infection.[9,11,39] The high prevalence of *ballum* infection in ship rats and hedgehogs in New Zealand may represent unique type-ecosystems for this serovar. Vacant ecological niches have been colonised by different introduced species of mammals which, perhaps due to the absence of other serovars, have become maintenance populations for *ballum*. Ballum serogroup infection in rodents and hedgehogs has also been reported by other workers in New Zealand[5,6,7] and sporadic infections have been reported in domestic stock and man.[1,30]

Ballum infection was only present in Norway rat populations inhabiting refuse dumps where population densities were extremely high. The inability of the Norway rat to maintain *ballum* infection outside of synanthropic foci indicates that this species does not constitute a natural maintenance host for *ballum*.[4]

In Great Britain, infection of wildlife with leptospires of the Ballum serogroup appears to be rare. Serovar *ballum* has

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been recovered from two long-tailed field mice *(Apodemus sylvaticus),* one bank vole *(Clethrionomys glareolus)* and one short-tailed vole *(Microtus agrestis).*[8] Only one *ballum* isolate was recovered from 108 Norway rats in Scotland.[28] Twenty *icterohaemorrhagiae* and one *canicola* isolates were also recovered from this series. Stating that "the association of *Leptospira ballum* with house mice is well known," Twigg[37] related infection with *ballum* in domestic stock in Britain to an ecological association with house mice, however, he did not isolate this serovar from mice. A survey of house mice in Northern Ireland also failed to demonstrate *ballum* infection[24] and extensive surveys in the South of England by one of the authors (S.C. Hathaway, unpubl.) have failed to find evidence of *ballum* infection in house mice and other free-living species. In Denmark, the mouse is reported to be a maintenance host for *sejroe.*[18]

Although *ballum* has been isolated from the hedgehog in Europe[41] such infections are rare. The hedgehog is recognised as a maintenance host for *bratislava* in Europe, including Great Britain.[25,41] No evidence of infection with this serovar was found in hedgehogs in New Zealand.

This survey demonstrated the inadequacy of serology as a diagnostic method for the detection of Ballum serogroup infection in rodents. The inability of organisms in this serogroup to stimulate high or sustained titres has been reported in several species.[6,31,35] This inability appears to be especially pronounced in the Norway rat.

The finding of a high prevalence of infection with *balcanica* in possums from a number of forest and farmland environments in New Zealand extends the known geographical distribution of this serovar, which also has been isolated from possums in Australia.[16] Isolations have been reported from cattle, pigs and humans in Bulgaria and the USSR; however, no recoveries have been made from wildlife in these countries.[3,27,33]

Serovar *balcanica* is closely related to serovar *hardjo,* another member of the Hebdomadis group, and it is evident by the failure to isolate *hardjo* from possums that the high prevalence of *hardjo* titres is due to cross-reacting agglutinins stimulated by infections with *balcanica.*

Serovar *hardjo* is endemic in cattle in New Zealand. Previously all Hebdomadis serogroup titres in domestic animals in New Zealand have been considered as infection with *hardjo.* Possums have a close ecological association with cattle and sheep under New Zealand farming conditions; therefore, the possibility of sporadic interspecies transmission of *balcanica* to domestic animals must be considered in future epidemiological investigations. The independent existence of two or more closely related serovars from one serogroup in different free-living or domestic animal species sharing an ecosystem has been described by several workers.[10,17,26] This emphasises the importance of definitive identification of serovars when investigating the epidemiology of leptospirosis.

Lagomorphs were serologically and bacteriologically negative in the present study but in earlier work Blackmore[5] isolated a leptospire belonging to the Ballum serogroup from one of 17 rabbits, further illustrating the ubiquitous nature of this serovar under New Zealand conditions. Although serological surveys of Lagomorpha in Great Britain have revealed titres to *bratislava, bataviae* and *sejroe,*[25,33] the significance of these titres remains undetermined.

Serovar *copenhageni* has previously been isolated from both Norway rats and domestic stock in New Zealand[7,15,30] but no isolates were obtained during this survey. Apparently this serovar is limited in distribution to certain areas in

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the northern half of the North Island where it is maintained by Norway rats.

Deer in New Zealand, both serologically and bacteriologically negative in other studies,[13,14] do not appear to be commonly infected with leptospires. This also appears to be the situation in Great Britain, where serological surveys have revealed only a very low prevalence of titres.[38]

Only one titre to *pomona* was found in sera from 58 ducks, all of which were trapped in and around a piggery where *pomona* is endemic in pigs. No isolations were made, despite the fact that ducks were observed to feed in effluent drains from which *pomona* was isolated. Pukeko's *(Porphyrio melanotus)* one of the most common wading birds inhabiting farmland in New Zealand, also were serologically negative. These results provide further evidence of the general insusceptibility of birds to leptospiral infection.[36]

It is evident that the same species of animals inhabiting distinct ecological niches in different countries may represent different type-ecosystems for specific leptospiral serovars. The reasons for the predominance of *ballum* infection in small free-living species in New Zealand remains a matter for conjecture; however, it may be significant that only three different serovars *(ballum, balcanica* and *copenhageni)* have been isolated by various investigators from the limited number of free-living species that are present in New Zealand. In comparison, there are many more free-living species in Great Britain, and these species are infected with a wide range of serovars.[33] The establishment of *ballum* infection in several free-living species in New Zealand may have been due to the absence of infection with other serovars.

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