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The epidemiology of leptospirosis in North Queensland

II. Further observations on the hosts in the Mossman district

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The studies of leptospiral infections in North Queensland recorded in Part I of this series (Emanuel, Mackerras & Smith, 1964) were undertaken mostly in the Johnstone and Mulgrave shires, and they consequently included little information about two serotypes which had been isolated only from human patients in the Mossman district (16° 26′ S., 145° 22′ E.) of the Douglas shire farther to the north. Serotype *medanensis* was recovered from two brothers (Ives), canefarmers, in 1951 (Smith *et al.* 1954), and *grippotyphosa* from a cane-cutter (Valbuzzi) in 1954 (Smith & Brown, 1955). The survey reported here was made in July and August, 1962, primarily to obtain information on the animal hosts of these two serotypes.

MATERIALS AND METHODS

The topography of the district has been described in Part I, and details of the areas trapped are shown in Fig. 1. All were within 10 miles of the town of Mossman.

Animals were trapped by the methods described by Harrison (1962); 2750 trap-nights produced 221 animals, of which 216 were in a state suitable for examination. A further 30 animals were captured by schoolchildren and are listed under Miscellaneous in Table 1. Some of the areas were selected with reference to known cases of leptospirosis, e.g. the farms where Ives and Valbuzzi worked, and Schild's farm that was the source of the soil which probably infected a Brisbane scientist with *grippotyphosa* (Tonge & Smith, 1961). Others were chosen with a view to their suitability for certain scrub typhus investigations that were performed concurrently, and are listed under names of occupiers of farms.

Two hundred and forty-six animals were examined, comprising 85 bandicoots of two species and 161 rodents of seven species. Their distribution in relation to species and habitats is set out in Table 1. Although the number of such species was small, the habitats observed were similar to those described by Harrison (1962), with two exceptions. Four *I. macrourus* and 6 *M. lutillus* were caught in rain-forest. However, the various types of rain-forest encompassed here were small pockets in or adjacent to sugar cane and grassland. As the survey was conducted at the height of the cane burning and cutting season, disturbance and movement of

species could be expected. No animal trapped at Dayman Point, forest and grassland, showed evidence of infection with leptospires.

At a temporary field laboratory established at the Mossman District Hospital, the traps were removed from the cloth bag and suspended over clean white enamel trays for the collection of urine. The traps were kept in a place that was under

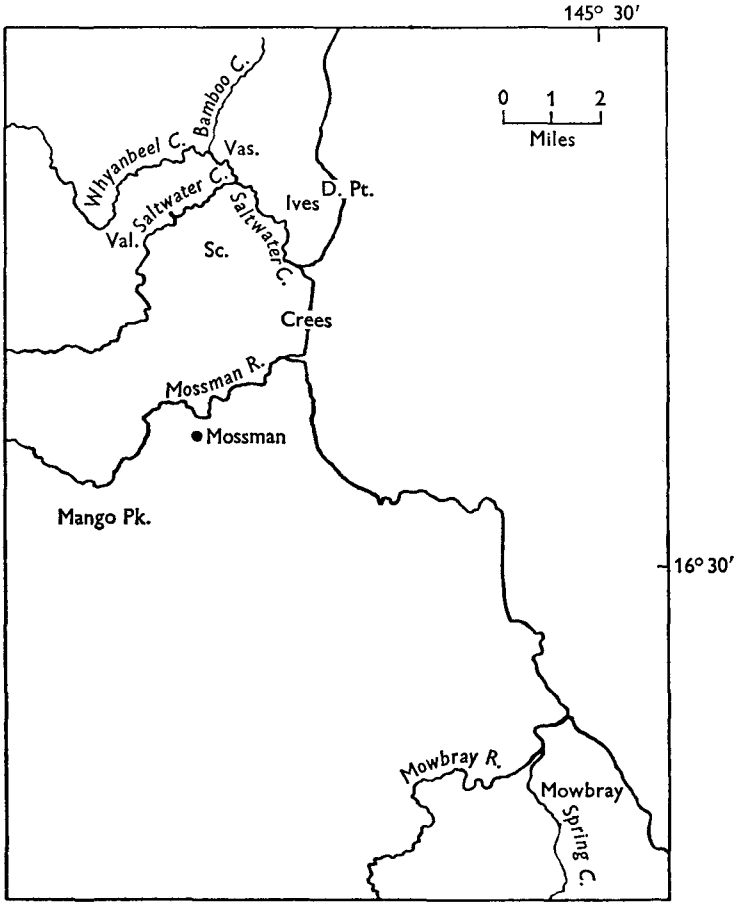

Fig. 1. Map of the Mossman district, showing trapping areas: Vas., Vassalini; Val., Valbuzzi; Ives; D. Pt., Dayman Point; Sc., Schilds; Crees; Mango Park; Mowbray.

constant observation and microscopic examination was carried out within a few minutes of the passing of urine. Most rodents would pass a few drops of urine if excited by blowing in the face, but the marsupial bandicoots seldom provided specimens. Urine was collected from the bladder of these as soon as the sterile dissection was completed. One or two drops of urine were placed on a clean, glass slide, covered with a 22 mm. square coverslip and examined for leptospires using dark ground illumination and a magnification of approximately 400. The presence of leptospires was recorded and graded from + for only an occasional organism to + + + + for a heavy infection.

The animals were transferred to a metal box with glass lid and killed with an

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Table 1. *Distribution of animal species in relation to trapping areas and habitat*

Species	Area and habitat																				Total
	Vassalini		Mowbray		Mango Park		Dayman Point	Crees	Schilds		Ives		Valbuzzi		Miscellaneous						
	Secondary woodland and grassland	Rain forest	Secondary woodland and grassland	Rain forest	Secondary woodland and grassland	Rain forest	Secondary woodland and grassland	Farm	Farm	Rain forest	Farm	Secondary woodland and grassland	Farm	Rain forest	Farm and grassland						
Perameles nasuta	4	4	—	—	—	—	7	—	—	—	—	—	—	—	—						15
Isoodon macrourus	1	—	—	1	2	2	—	11	3	1	2	—	18	1	28						70
Hydromys chrysogaster	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1						2
Uromys caudimaculatus	5	—	—	—	1	—	—	—	—	—	3	—	2	1	—						7
Melomys cervinipes	10	10	3	5	—	—	1	—	2	—	3	1	2	—	—						36
M. lutillus	1	6	5	7	3	2	12	2	4	1	16	1	16	1	—						76
M. spp. (young)	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—						2
Rattus rattus	—	—	—	—	1	—	2	—	—	—	—	—	—	—	1						4
R. assimilis	—	1	—	—	—	1	1	—	—	—	—	—	—	—	—						3
R. sordidus conatus	—	—	—	—	1	6	—	3	—	—	—	2	13	—	—						31
Total	22	21	8	13	8	11	23	16	9	2	27	3	50	3	30						246

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ether-soaked swab of cotton-wool and gauze. Immediately after death sterile dissection was carried out and media inoculated with a very small piece of kidney cortex. Three $\frac{1}{4}$ oz. McCartney bottles, each containing 3 ml. of media, were inoculated from each animal. The media used were:

(*a*) 'TPB', Tryptose phosphate broth (0·2 %) plus rabbit serum (10 %).

(*b*) 'TPA$_1$', Tryptose phosphate broth (0·2 %) and agar (0·15 %) plus rabbit serum (10 %).

(*c*) 'TPA$_2$', Tryptose phosphate broth (0·2 %) and agar (0·15 %) plus rabbit serum (20 %).

A haemoglobin supplement and cyclohexamide were added to each medium.

Cultures were incubated at 30° C. and examined microscopically with dark-ground illumination on the 7th, 14th, 21st and 28th days. If there was no growth, they were then discarded. Positive cultures were subcultured and forwarded by air to the Laboratory of Microbiology and Pathology, Brisbane, for identification.

Table 2. *Comparison of survey methods in bandicoots and rodents examined by all three methods*

Species	No. examined	No. of infections determined by			
		Culture	Dark-ground examination	Serology	All methods
P. nasuta	15	2	1	4	5
I. macrourus	59	4	4	24	25
H. chrysogaster	2	1	0	2	2
U. caudimaculatus	7	4	1	5	5
M. cervinipes	36	3	1	5	6
M. lutillus	57	0	0	0	0
R. rattus	4	0	0	0	0
R. assimilis	3	0	0	0	0
R. s. conatus	30	7	8	8	9
Total	213	21 (10 %)	15 (7 %)	48 (23 %)	52 (24 %)

Leptospires were isolated from twenty-one animals, but as five of these had both kidneys cultured, there were twenty-six positive kidneys. The results of the three media are:

leptospires were grown from 17 kidneys in 'TPB',

leptospires were grown from 18 kidneys in 'TPA$_1$',

leptospires were grown from 19 kidneys in 'TPA$_2$',

this suggests that no medium is better than the others, but that the more media inoculated, the greater the chance of growing leptospires.

Heart blood was collected, the serum separated and forwarded to the Laboratory of Microbiology and Pathology for serological investigation. The sera were screened by the microscopic agglutination test against the following serotypes: *ictero-haemorrhagiae*, *canicola*, *broomi*, *zanoni*, *robinsoni*, *australis*, *bratislava*, *pomona*, *grippotyphosa*, *medanensis*, *kremastos*, *mini*, *hyos*, *celledoni* and *autumnalis*. Serological results were interpreted with the conservatism described in Part I.

Table 2 records the number of infections determined by culture, dark-ground

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examination and serology in 213 animals in which all three procedures were used. The percentage of infections, proved by culture, in this series was double that of Part I. However, the ratio of culture to dark-ground examination was the same for both series, 2:1. This suggests that the higher culture rate was probably due to a focus of infection in the district rather than improved culture media.

Omitted from this series is a group of 20 *M. lutillus* and *Melomys* (young) in which organisms resembling leptospires were seen in the urine but not grown by kidney culture. Their sera did not show any leptospiral antibodies even when tested with an extended range of thirty-two serotypes representing eighteen groups. As tissues had been preserved in formalin for other purposes, sections of two kidneys from animals with a + + + and + + + + excretion in their urines were cut and stained but no leptospires were seen. It is probable that the organisms seen in their urine were not leptospires; they are certainly not comparable with the '*pomona*-like' strains mentioned in Part I.

RESULTS

Cultures and sera

Twenty-two leptospiral strains were cultured from 21 of 246 animals tested (Table 2). Strains of two serotypes, *medanensis* and *celledoni*, were grown from the kidney of one short-nosed bandicoot, *I. macrourus*, which also showed serological evidence of infection with *grippotyphosa* and *hyos*. The strains were distributed amongst seven serotypes, namely *grippotyphosa* (8), *medanensis* (5), *zanoni* (4), *hyos* (2), *celledoni* (1), *australis* (1) and *bindjei* (1).

Sera from 233 animals were tested. Forty-eight, twenty-eight bandicoots and twenty rodents, showed the presence of antibodies to a titre of 1:100 or more (Table 2). Thirty-six of these exhibited a serological pattern characteristic of infection with organisms of a single serogroup and twelve showed evidence of multiple infection.

Seven of the 8 *grippotyphosa* strains were isolated from *R. s. conatus*, the cane-field rat. Six of these strains were isolated from animals trapped in the Valbuzzi area and one trapped in the Ives area. The eighth *grippotyphosa* strain was cultured from an *I. macrourus* trapped in the Valbuzzi area. All the *grippotyphosa* strains were grown from animals caught in sugar cane. Ten animals, in addition to those from which *grippotyphosa* isolates were obtained, showed serological evidence of *grippotyphosa* infection. Seven of these, all *I. macrourus*, were trapped in sugar cane; 2, a *P. nasuta* and a *U. caudimaculatus*, were caught in rain forest; the 10th was an *I. macrourus* trapped on the pineapple farm (Schilds) that provided the soil which probably infected the Brisbane scientist quoted above. Thirteen of the animals showing evidence of *grippotyphosa* infection came from the same area as the patient Valbuzzi, from whom *grippotyphosa* was first isolated in Australia. The titres ranged from 1:100 to 1:30,000. Three of the animals from which *grippotyphosa* was isolated had antibody titres of only 1:100.

Two of the five *medanensis* strains were isolated from the long-nosed bandicoot, *P. nasuta*. Both of these were trapped in the Vassalini area. The remaining three

Table 3. *Occurrence of infections in animals as determined by culture and serology*

Species	No. examined	Isolations: *bindjei*	*zanoni*	*australis*	*grippotyphosa*	*medanensis*	*hyos*	*celledoni*	Total	Antibodies: *canicola* group	*pyrogenes* group	*australis* group	*pomona*	*grippotyphosa*	*hebdomadis* group	*hyos*	*celledoni*	Total
P. nasuta	15	—	—	—	—	2	—	—	2	2	—	—	—	1	2	1	1	7
I. macrourus	59	—	—	—	1	3*	—	1*	4	2	4	1	8	9	5	1	1	31
H. chrysogaster	2	—	—	—	—	—	1	—	1	—	1	—	—	—	—	1	1	3
U. caudimaculatus	7	—	2	1	—	—	1	—	4	2	2	1	3	1	—	1	—	10
M. cervinipes	36	1	2	—	—	—	—	—	3	1	2	—	2	—	—	—	—	5
M. lutillus	75	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	0
M. spp. (young)	2	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	0
R. rattus	4	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	0
R. assimilis	3	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	0
R. s. conatus	30	—	—	—	7	—	—	—	7	—	1	—	1	7	—	—	—	9
Total	233	1	4	1	8	5	2	1	22/21	7	10	2	14	18	7	4	3	65

* Double infection.

Table 4. *Infections in animals from different habitats*

Species	Rain forest: No. examined	*canicola* group	*pyrogenes* group	*australis* group	*pomona*	*grippotyphosa*	*hebdomadis* group	*hyos*	*celledoni*	Secondary woodland and grassland: No. examined	*canicola* group	*pyrogenes* group	*pomona*	*hebdomadis* group	Cultivation: No. examined	*canicola* group	*pyrogenes* group	*australis* group	*pomona*	*grippotyphosa*	*hebdomadis* group	*hyos*	*celledoni*
P. nasuta	4	—	—	—	—	1	1	1	1	11	2	—	—	1	0	—	—	—	—	—	—	—	—
I. macrourus	4	1	—	—	1	—	1	—	—	4	—	1	2	—	62	1	3	1	5	9	4	1	1
H. chrysogaster	1	—	1	—	—	—	—	—	—	0	—	—	—	—	1	—	—	—	—	—	—	1	1
U. caudimaculatus	7	2	2	1	3	1	—	1	—	0	—	—	—	—	0	—	—	—	—	—	—	—	—
M. cervinipes	10	1	—	—	2	—	—	—	—	19	—	1	—	—	7	—	1	—	—	—	—	—	—
M. lutillus	6	—	—	—	—	—	—	—	—	32	—	—	—	—	38	—	—	—	—	—	—	—	—
M. spp. (young)	0	—	—	—	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—
R. rattus	1	—	—	—	—	—	—	—	—	2	—	—	—	—	1	—	—	—	—	—	—	—	—

strains of *medanensis* were grown from *I. macrourus* caught in the Valbuzzi area. These sera reacted with all three serotypes of the *hebdomadis* group, but their *medanensis* titres were at least ten times greater than *kremastos* and *mini* titres. Two other animals showed serological evidence of infection with the *hebdomadis* group; an *I. macrourus* trapped at Vassalini reacted only with *medanensis*; and an *I. macrourus* caught on a farm by a schoolchild showed higher titres to *kremastos* and *mini* than to *medanensis*. The type of country varied from rain forest to grassland and sugar cane.

A strain of *bindjei* was grown from a *M. cervinipes* trapped in rain forest in the Vassalini area. Antibodies to the serotypes in the *canicola* group were found in six other sera from the species *P. nasuta* (2), *I. macrourus* (2) and *U. caudimaculatus* (2).

Strains belonging to serotypes *zanoni*, *hyos* and *celledoni* were also isolated (Table 3). Antibodies to serotype *pomona* were demonstrated in the sera of 14 animals. Six of these were *I. macrourus* trapped by schoolchildren on various farms in the district. The others were *I. macrourus* (2), *U. caudimaculatus* (3), *R. s. conatus* (1), *M. cervinipes* (2) which were trapped in rain forest and grassland. No strains of *pomona* were isolated. A similar finding in relation to apparent *pomona* infections in *R. assimilis* was reported in Part I.

The association of infected animals and habitats are set out in Table 4. It is interesting that *M. lutillus* (76 trapped) showed no evidence of infection.

Multiple infections

A high incidence of multiple infections was noted in both rodents (30%) and bandicoots (21%). This differs from the results of the earlier survey in which antibody patterns in rodents indicated relatively few multiple infections. The species showing evidence of infection with more than one serotype were:

In 2 *P. nasuta:* 1 *canicola* group + *medanensis**, 1 *medanensis** + *hyos* + *celledoni*.

In 4 *I. macrourus:* 1 *canicola* group + *hebdomadis* group, 1 *grippotyphosa* + *medanensis**, 1 *pyrogenes* group + *grippotyphosa* + *medanensis**, 1 *grippotyphosa* + *medanensis** + *hyos* + *celledoni*.

In 1 *H. chrysogaster: hyos** + *celledoni*.

In 4 *U. caudimaculatus:* 1 *zanoni** + *pomona*, 1 *canicola* group + *hyos**, 1 *australis** + *pomona*, 1 *zanoni** + *pomona* + *grippotyphosa*.

In 1 *R. s. conatus: pyrogenes* group + *grippotyphosa**.

Isolations indicated by *.

New host records

Isolation and identification of strains extended the proven host range of six serotypes (Table 5). The isolation of *zanoni* from *U. caudimaculatus* and *M. cervinipes* extends the host range of this serotype to all rodents trapped in North Queensland except *H. chrysogaster*.

Maintaining and incidental hosts

The excretion index (Emanuel *et al.* 1964) was calculated for each association between serotype and host where the numbers permitted. The samples were small, but the figures may be considered to give an indication of which animals are the maintaining hosts of the two main serotypes, *grippotyphosa* and *medanensis*, under investigation.

Infections with *grippotyphosa* were recorded from *R. s. conatus* and *I. macrourus*. *R. s. conatus* exhibited an infection rate of 23 %, and an excretion index of 1·0, all animals with evidence of infection being excretors. It could thus be an important maintaining host in the area. On the other hand, *I. macrourus* had an infection rate of 13 % and excretion index of 0·07, so it may well be only an incidental host, as *R. rattus* presumably was in the earlier survey.

Table 5. *New host records of serotypes*

Host species	Serotype
P. nasuta	*medanensis* (2)
I. macrourus	*medanensis* (3)
	grippotyphosa (1)
H. chrysogaster	*hyos* (1)
U. caudimaculatus	*zanoni* (2)
	australis (1)
M. cervinipes	*bindjei* (1)
	zanoni (2)
R. s. conatus	*grippotyphosa* (8)

Figures in parentheses indicate the number of strains isolated.

Infections with *medanensis* were recorded from *P. nasuta* and *I. macrourus*. Their infection rates of 12 and 7 %, respectively, for *hebdomadis* group infections, almost exclusively *medanensis* in this instance, and excretion indices of 1·0 and 0·7, respectively, suggest that both are maintaining hosts. It is noteworthy that here, as in the earlier investigations, no rodents were infected with members of the *hebdomadis* group.

Infection rates for *bindjei* cannot be determined because of serological cross reactions in the *canicola* group. However, *M. lutillus* and *M. cervinipes* inoculated with *bindjei* became urinary carriers (Emanuel *et al.* 1964) and the serotype has been isolated only from these rodents. On these grounds it may be considered that *Melomys* species are the maintaining hosts of the serotype in this area.

DISCUSSION

The ready isolation of *grippotyphosa* and *medanensis* strains from animals in an area where the only human strains were isolated years previously, emphasizes the focal nature of the distribution of some serotypes.

Whilst *R. s. conatus* is an undoubted maintaining host of *australis*, an association responsible for a preponderance of human infections in Division 3 of Mulgrave shire, it appears to play a similar important role in relation to *grippotyphosa* in the

Douglas shire. The bandicoots, *P. nasuta* and *I. macrourus*, here fill the role, as maintaining hosts of *medanensis*, that they play in association with the other *hebdomadis* group serotypes, *kremastos* and *mini*, in more southerly districts of the sugar belt.

There is some serological evidence that both *grippotyphosa* and *medanensis* occur outside the Douglas shire, even in New South Wales. Since 1954, ten patients have submitted multiple specimens of sera which showed titres of 1:300 or more for *grippotyphosa* (unaccompanied by a pattern of cross reaction indicative of infection with other serotypes). Five of these lived on the North Queensland coastal plain, one came from Mackay on the central coastal plain, and four lived and worked in south-eastern Queensland and northern New South Wales. Furthermore, Forbes, Keast, Wannan & Lawrence (1955) reported a series of fifteen bovine sera which contained antibodies to *grippotyphosa*. These sera were collected from cattle in south-eastern New South Wales. Since 1954 also nine patients have had multiple specimens of sera showing titres of 1:300 or more for *medanensis* and lower titres for other serotypes in the *hebdomadis* group. Of these, five lived on the coastal plain north of Innisfail and four lived in south-eastern Queensland. However, no survey of native animals for leptospiral infection has been made by us outside North Queensland.

The isolation of *bindjei* from *M. cervinipes* provides the second recorded rodent host of this serotype in the shire. The animal was trapped in the Vassalini area on Whyanbeel Creek, about 5 miles downstream from the farm on which *bindjei* had been isolated previously from *M. lutillus* and where a human infection had occurred, as recorded in Part I.

Significant infection rates for *pomona* in *I. macrourus* (11 %), *M. cervinipes* (6 %), *U. caudimaculatus* (4 %), and *R. s. conatus* (3 %) were not accompanied by evidence of urinary excretion. The relative frequency of *pomona* infections is lower (22 %) than that (53 %) reported from the Douglas shire by Emanuel *et al.* (1964). *U. caudimaculatus* is a host for a wide range of serotypes in this area, and there is some evidence that it might prove to be a maintaining host for *zanoni*, *hyos* and *australis*.

SUMMARY

Investigations in the Mossman districts of North Queensland showed the maintaining hosts of leptospiral serotype *medanensis* to be *Perameles nasuta* and *Isoodon macrourus* in canefields, secondary woodland and rain forest. *Rattus sordidus conatus* is the maintaining host of *grippotyphosa* in canefields. The host range of six serotypes was extended by the cultural studies.

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