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Leptospiral Antibodies in Flying Foxes in Australia

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ABSTRACT: The sera of 271 pteropid bats (or flying foxes) collected from Queensland, New South Wales, Western Australia, and the Northern Territory were screened against a reference panel of 21 *Leptospira* spp. using the microscopic agglutination test (MAT). Sera were collected from December 1997 through August 1999. The MAT panel represented those serovars previously isolated in Australia, as well as exotic serovars found in neighboring countries. Leptospiral antibodies were detected in 75 (28%) of the sera and represented seven serovars, one of which, *L. interrogans* serovar cynopteri has been regarded as exotic to Australia. Sixty sera were reactive to one serovar, 12 sera were reactive to two serovars, and three sera were reactive to three serovars. The *L. kirschneri* serovar australis was most frequently identified (60.2%). The findings suggest a previously unrecognized role of pteropid bats in the natural history of leptospirosis. The potential exists for establishment of infection in new host species, the transmission of new serovars to known host species, and for changes in virulence of leptospires as a result of passage through these species.

Key words: bats, chiroptera, flying foxes, *Leptospira interrogans,* leptospirosis, *Pteropus conspicillatus,* serosurvey, transmission.

In recent years bats (order Chiroptera) have attracted considerable research focus in relation to emerging and zoonotic diseases in Australia and southeast Asia. This focus is largely a consequence of the identification of the role of pteropid bats (suborder Megachiroptera; commonly called flying foxes) as a natural host of Hendra virus, the previously undescribed agent responsible for the deaths of 16 horses and two humans in Queensland since September 1994 (Murray et al., 1995; Rogers et al., 1996; Field et al., 2000). Subsequent investigations of three recently recognized viruses have identified a probable role of flying foxes in the natural history of Australian bat lyssavirus and Menangle virus in Australia (Philbey et al., 1998) and Nipah virus in Peninsular Malaysia (Field et al., 2001; Johara et al., 2001).

Surveys of flying foxes and other wildlife species for evidence of leptospiral infection followed an increased incidence of human infections between 1991 and 1999 (Smythe et al., 1997). Many cases were reported from the horticultural industry of northern Queensland where flying foxes seasonally feed on fruit crops. It was hypothesized that flying foxes could be a reservoir of infection, responsible for either direct or indirect transmission to humans or other animal species. In addition, there had been limited previous investigation of the role of flying foxes as maintenance hosts for pathogenic *Leptospira.* Early studies reported isolation of serovars from bats in Indonesia (Alston and Broom, 1958). Of eight bat species surveyed in previous Australian studies, only the spectacled flying fox (*Pteropus conspicillatus*) had leptospiral antibodies (Emanual et al., 1964). Of 33 spectacled flying fox sera tested, six had titers of 1:100 or more to serovar australis. However no leptospires were isolated from any of the animals (Emanual et al., 1964).

There are four species of flying fox in mainland Australia (Fig. 1): grey-headed flying foxes (*P. poliocephalus*) occur in coastal eastern Australia from Victoria to southern Queensland; black flying foxes (*P. alecto*) are found along the coastal areas of northern New South Wales, Queensland, Northern Territory and northern Western Australia; spectacled flying foxes (*P. conspicullatus*) are restricted to the wet tropics of northern Queensland; and little red flying foxes (*P. scapulatus*) have been recorded over a large part of the eastern,

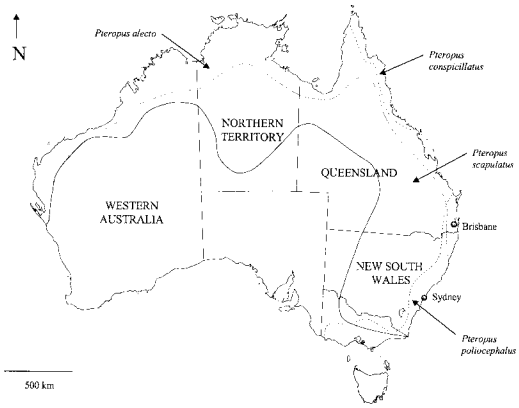

FIGURE 1. Distribution of mainland flying fox species and sampling locations of bats in Western Australia (December 1998), the Northern Territory (August 1999), Queensland (December 1997, May 1998, April 1999), and New South Wales (December 1997, February 1998).

northern and western parts of the Australian continent (Hall, 1987; Mickleburg et al., 1992).

Wild populations of flying foxes were non-randomly sampled in Western Australia (three locations), Northern Territory (one location), Queensland (four locations), and New South Wales (one location) (Fig. 1) over 3 yr commencing in December 1997. Flying foxes were either wild-caught by mist-netting or shooting or were diagnostic (opportunistic) specimens submitted to the Animal Research Institute (Queensland Department of Primary Industries). Sampling was part of a wider disease surveillance program targeting recently recognized viruses associated with flying foxes.

The sera were screened against a reference panel of 21 leptospiral serovars using a microscopic agglutination test (MAT) (Stallman, 1982). The panel represented those serovars of *Leptospira interrogans, L. borgpeterseni, L. weilii,* and *L. kirschneri* genospecies previously isolated in mainland Australia [australis (strain Ballico), bulgarica (Nicolaevo), canicola (Hond Utrecht IV), celledoni (Celledoni), copenhageni (M 20), grippotyphosa (Moskva V), hardjo (Hardjoprajitno), kremastos (Kremastos), medanensis (Hond HC), pomona (Pomona), robinsoni (Robinson), szwajizak (Szwajizak), tarassovi (Perepelitsin), zanoni (Zanoni)], and the exotic serovars, of genospecies *L. borgpeterseni, L. interrogans, L. kirschneri, L. noguchi,* and *L. santarosai,* ballum (Mus 127), bataviae (Swart), cynopteri (3522 C), djasiman (Djasiman), javanica (Veldrat Batavia 46), panama (CZ 214), and shermani (1342 K). Titers of 1:50 or higher were regarded as evidence of past or current infection. This approach is consistent with that of previous studies of leptospiral infections in wild animal populations in Australia (Emanual et al., 1964; Durfee et al., 1979; Mason et al., 1998), and of unpublished findings of the reference laboratory.

A total of 271 flying foxes were surveyed. Species, sex, age, capture method, capture location, and capture year are presented in Table 1. Serologic reactivity with one or more leptospiral serovars was identified in 75 individuals from all four species of flying fox (Table 1). After discounting known and observed serovar cross reactions (Wolff, 1954), sera from 87% (65/75) of the bats had reactions suggestive of infection caused by a single serovar, 12% (9/75) had reactions suggestive of infection caused by two serovars, and 1% (1/75) had reactions suggestive of infection with three serovars (Table 2). This latter flying fox may have been infected with serovars australis, hardjo and pomona. The most common infecting serovar was australis, with 75% (56/75) of the seropositive bats showing reactivity. Of the exotic serovars, reactivity to serovar cynopteri was found in 17% (9/75) of bats (Table 2).

Age was the only variable significantly associated ($P < 0.001$) with leptospiral antibody status by logistic regression analysis. The frequency distribution of MAT titers from 65 bats with serologic reactions consistent with infection caused by a single serovar is presented in Figure 2.

Flying foxes are nocturnal fruit and nectar-eating mammals whose primary ecological role is pollination of flowers and dis-

TABLE 1. Characteristics of 271 Australian flying foxes surveyed for agglutinating antibodies to *Leptospira* spp. between 1997 and 1999.

Variable	Number (%) of flying foxes tested	
	Total tested	MAT seropositive[a] (%)
Pteropus species		
P. poliocephalus	79	12 (15)
P. scapulatus	51	10 (20)
P. alecto	88	32 (36)
P. conspicillatus	53	21 (40)
Total	271	75 (28)
Sex		
Male	162	40 (25)
Female	107	34 (32)
Unknown	2	1 (50)
Age[b]		
Immature	91	7 (8)
Mature	177	66 (37)
Unknown	3	2 (67)
Method of capture[c]		
Wild-caught	208	54 (26)
Opportunistic	63	21 (33)
Year of capture		
1997	53	21 (40)
1998	156	34 (22)
1999	62	20 (32)
Location of capture[d]		
NSW	29	3 (10)
WA	77	22 (29)
QLD	118	34 (29)
NT	47	16 (34)

[a] Microscopic agglutination test titers ≥50 to at least one serovar were classified as positive.

[b] Age classes were determined on the basis of physiologic criteria indicating sexual maturity (attained at 2–2.5 yr) and included weight and forearm length, dentition wear, and mammary development.

[c] Flying foxes were either wild-caught by mist nets set adjacent to roosting sites of free-living populations or were opportunistic samples presented to the Animal Research Institute for diagnostic purposes.

[d] NSW = New South Wales, WA = Western Australia, QLD = Queensland, NT = Northern Territory.

persal of seeds of eucalypt and rainforest tree species. Notwithstanding a preference for native tree species (Marshall, 1985; Richards, 1990), flying foxes are commonly regarded as orchard pests because of their habit of feeding on commercial fruit crops when natural foods are scarce. During the day flying foxes roost communally in dedicated tree canopy "camps," with populations frequently numbering in the thousands, and seasonally, in the tens or hundreds of thousands.

TABLE 2. Reactivity of *Leptospira* serovars in 75 Australian flying foxes tested positive by microscopic agglutination to one or more serovars.

Leptospira spp. serovar[a]	Number of positive tests[b]	Frequency
australis	47	63%
cynopteri	9	12%
hardjo	4	5%
australis/hardjo	4	5%
australis/cynopteri	4	5%
bulgarica	3	4%
tarassovi	2	3%
australis/hardjo/pomona	1	1%
pomona/canicola	1	1%

[a] Only most likely infecting serovars are listed and likely cross-reactions are not included.

[b] Microscopic agglutination test titers ≥50 were classified as positive. Sera from 65 bats reacted to one serovar, nine reacted to two serovars, and one reacted to three serovars.

Transmission of urine-borne pathogens such as *Leptospira* is likely to occur readily within flying fox populations. The size and density of most camps make exposure to the urine of a conspecific a frequent event. In addition, the grooming behavior of flying foxes typically involves urination and the licking of urine through the coat and wings. Transmission between populations is likely to be facilitated by the seasonally nomadic nature of many flying fox populations and the sharing of roosting camps.

After controlling for confounding factors, age is the only variable significantly associated with the presence of anti-leptospiral antibodies. A higher seropreva-

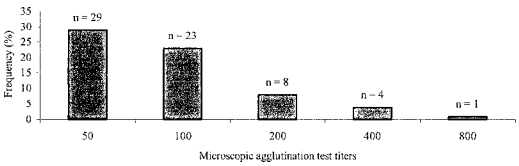

FIGURE 2. Frequency distribution of the antibody titers of 65 flying foxes reactive to a single *Leptospira* spp. serovar.

lence in older animals is consistent with a pattern of primarily horizontal transmission, whereby older animals have had a longer time to be exposed. The absence of any significant association between species, location of capture or year of capture, and leptospiral serostatus on multi-variate analysis is consistent with leptospiral infection being endemic in Australian flying foxes. The unadjusted seroprevalence in flying foxes (28%) approaches that recorded in native rodents (43%) determined as part of a field study into the potential health risks and possible control strategies of rodents in the fruit growing areas of North Queensland during 1999 (L. D. Smythe, unpubl. data).

Leptospira spp. serovars have been grouped into serogroups based on their degree of cross reactivity (Stallman, 1982). Our study identified many flying foxes with titers to multiple serovars. While some were consistent with known cross-reactions, we are uncertain whether the remainder represent multiple infections or unknown cross-reactions (Emanual, 1964). Studies are proposed to isolate the bacteria from flying foxes in part to address this uncertainty.

Cross-reactivity with known serovars has been excluded as the basis of serovar cynopteri reactivity (Table 2). Given its absence in human cases to date, the serologic evidence of infection of Australian flying foxes poses several questions. Is serovar cynopteri infection restricted to flying foxes in Australia, or has the serovar only recently arrived in Australia in flying foxes, with insufficient time for it yet to establish in other reservoir species and cause human infections? Additional studies are necessary to determine the spatial and temporal distribution of infection with this serovar in Australian flying foxes.

Three of the four flying fox species found on mainland Australia are also known to occur in New Guinea and nearby islands, with the distribution of one, the black flying fox, extending to the eastern islands of the Indonesian archipelago. However, while seasonal foraging movements of flying foxes between Papua New Guinea and numerous Torres Strait islands have been observed, large-scale movements of flying foxes in and out of Australia are not believed to occur (Hall, pers. comm.). Nonetheless, it is appropriate to explore the level of effective contact between geographically overlapping flying fox populations throughout southeast Asia and the significance of this contact in the transmission of disease between the populations. This scenario has been addressed in the investigation of the natural history of Hendra virus and Australian bat lyssavirus (Halpin et al., 2000; Field et al., 2001). Given the previous isolation of serovar cynopteri from bats in Indonesia (Alston and Broom,1958), the current evidence of its presence in Australian flying foxes may indicate effective contact.

For ground-dwelling species residing under flying fox roosts, heavy urine contamination of their environment represents an increased likelihood of exposure to leptospiral infection and suggests the possibility of a previously unidentified flying fox-rodent cycle. The potential exists for establishment of infection in new host species, the transmission of new serovars to known host species, and for changes in virulence of leptospires as a result of passage through these species. Concerns of possible enhanced virulence are prompted by an increased prevalence of particular serovars in human cases and by more serious clinical manifestations associated with these serovars (Smythe et al., 1997). That these observations reflect changes in the natural history or the dynamics of reservoir populations is possible. Further studies of the role of flying foxes in the transmission of leptospirosis in humans are needed.

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