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Source: Journal of Wildlife Diseases, 43(3) : 492-497

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.3.492

SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 43(3), 2007, pp. 492–497

Leptospirosis Serology in the Common Brushtail Possum (*Trichosurus vulpecula*) from Urban Sydney, Australia

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ABSTRACT: The common brushtail possum (*Trichosurus vulpecula*) is indeed a common marsupial in major cities of Australia. This species is known to be susceptible to leptospirosis and often lives in close contact with humans, raising concerns about the potential for transmission of this disease in urban areas. A total of 192 brushtail possum blood samples were collected from 136 individuals in suburban areas of metropolitan Sydney from November 2002 to November 2004. Sera were screened against a reference panel of 21 *Leptospira* spp. using the microscopic agglutination test. Leptospiral antibodies were detected in 9.6% (13/136) of tested brushtail possums and represented two serovars; antibodies to *Leptospira interrogans* serovar Hardjo were most frequently identified (11/136). A representative of the exotic serogroup Ballum, most likely serovar Arborea, was found in two of 136 brushtail possums. Exposure to leptospirosis seemed to be associated with age, as older animals had a higher incidence, but there was no distinction in relation to gender. Antibody prevalence varied between the different sampling sites and seropositive animals were clustered and restricted to a few sites. These data support the possible role of brushtail possums as a maintenance host for *Leptospira spp.* in urban environments and also identified them as a previously unknown and potential source of serovar Arborea.

Key words: Common brushtail possum, leptospirosis, marsupial, microscopic agglutination test, serosurvey, serovar, *Trichosurus vulpecula*, urban wildlife.

Leptospirosis is a zoonotic bacterial disease of worldwide importance that can affect humans and domestic and wild animals (Levett, 2001). These bacteria are spread through the urine of infected animals and infections range from asymptomatic or subclinical to acute and fatal. Transmission occurs directly from a mammalian host or through indirect transmission via contact with *Leptospira*-contaminated water or soil. In Australia, leptospirosis in humans is a notifiable disease, and in temperate regions, such as Sydney, serovar Hardjo is the predominant serovar infecting humans (Biosecurity Australia, 2000). Some *Leptospira* serovars are indigenous to Australia and native animals need to be recognized as a source of infection (Slack et al., 2006). Contact with kangaroos, wallabies, native rats, bandicoots, and possums was reported in some human cases of leptospirosis.

The common brushtail possum (*Trichosurus vulpecula*) is the most familiar of the Australian possums because it often cohabits with people in urban areas (Matthews et al., 2004). This species is considered a maintenance host for *Leptospira interrogans* serovar Balcanica in New Zealand (Hathaway, 1981; Hathaway et al., 1981), where it was introduced in the late 1800s for fur trade. Infection with serovar Balcanica in brushtail possums is characterized by high susceptibility to infection, temporary subclinical effects, low pathogenicity to the host, long-term leptospiruria, and natural transmission within the host species (Hathaway et al., 1981; Day et al., 1997). In New Zealand, seroprevalence varies greatly; up to 80% of adults test positive on the North Island, whereas leptospirosis appears to be absent from brushtail possums on the South Island (Horner et al., 1996). In Australia, antibodies to serovar Hardjo were detected in the state of Victoria (Durfee and Presidente, 1979) and in southeastern Australia (Milner et al., 1981). To date there is no data on the leptospirosis prevalence in urban brushtail possums in Australia or New Zealand. These data are needed to assess the possible effect on both human health and urban brushtail possum populations. The aim of this study was to investigate the seroprevalence of *Leptospira spp.* in urban brushtail possums and to analyze any association between seroprevalence and geographic location, sex, age, and body condition.

Brushtail possum collection sites, handling and sampling techniques, and statistical procedures are described in Eymann et al. (2006). Briefly, brushtail possums were live-trapped from five suburban localities within the Sydney metropolitan area, New South Wales, Australia, namely Beecroft (33°45′S, 151°04′E), Chatswood West (33°47′S, 151°09′E), North Epping (33°45′S, 151°05′E), Pymble (33°45′S, 151°07′E), and Scotland Island (33°38′S, 151°17′E). A total of 192 blood samples from 136 individuals were sampled from November 2002 to November 2004 and analyzed.

Methods used for serological examination are described in Smythe et al. (2002). The sera were screened using the microscopic agglutination test (MAT) against a reference panel of 21 leptospiral serovars of *L. interrogans*, *Leptospira borgpeterseni*, *Leptospira weilii*, and *Leptospira kirschneri* species previously isolated in mainland Australia; serovar Balcanica also was included. Titers of 50 or higher were regarded as evidence of past or present exposure. Sera with antibody titers to more than one serovar were assessed for known cross-reactions, and a positive result was recorded against only the most likely serovars.

Antibodies to *Leptospira* spp. were found in 9.6% (13/136) of individuals and in 8.9% (17/192) of all serum samples (Table 1). Antibodies to serovar Hardjo were most common, with 85% (11/13) of the seropositive brushtail possums showing reactivity; titers ranged from 50 to 3,200. Cross-reactions occurred with the serovars Balcanica, Szwajizak, and Medanensis and individual sera reacted with up to four different serovars. Positive reactions with Medanensis are the result of antigen sensitivities and simple cross-reactions; hence Medanensis is not shown in Table 1.

After discounting Medanensis, sera from eight brushtail possums reacted to more than one serovar (Table 1). This is most likely the result of cross-reactions between different serogroups, and in most cases the highest antibody titers were observed with serovar Hardjo. Cross-reactions are common, and the highest detected antibody titer may not necessarily represent the infective serovar (Levett, 2001). In a captive study by Hathaway et al. (1981), brushtail possums infected with Balcanica showed higher or equivalent antibody titers to Hardjo. Therefore, in the absence of additional samples, which would allow isolation of the causative leptospires, there remains some uncertainty as to which serovar or serovars infected some of the brushtail possums. The isolation of *Leptospira* from urine of animals can prove difficult, and culture of actual kidney samples is more successful. This approach would have involved killing the study animals, which would have impacted further investigations of tagged animals and would not have been ethically acceptable in our study.

Two animals (15% of seropositive animals) had a single reaction to the exotic serovar Ballum, serogroup Ballum. From other studies conducted in Australia by the World Health Organization (WHO)/ Food and Agricultural Organization (FAO)/ Office International des Epizooties (OIE) Collaborating Centre for Reference and

TABLE 1. Antibodies to *Leptospira* spp. in brushtail possums from the Sydney metropolitan area of Australia.

Host no.	Sex	Age class[a]	Date	Location[b]	Weight (kg)	*Leptospira* spp. serovar (titer)			
						Hardjo	Balcanica	Ballum	Szwajizak
1	Male	—	2 November 2002	B1	2.30	800	200	—	100
3	Male	—	6 November 2002	B1	2.40	200	—	—	—
		7	15 August 2003	B1	2.65	400	—	—	—
39	Female	—	20 February 2003	P21	2.25	—	—	50	—
		5	3 September 2003	P21	2.35	—	—	100	—
54	Female	—	6 March 2003	P24	2.40	1,600	200	—	—
		4	3 September 2003	P24	2.60	3,200	100	—	—
55	Male	—	6 March 2003	P24	2.75	3,200	200	—	—
		4	3 September 2003	P24	2.90	3,200	50	—	400
110	Male	4	9 September 2003	P19	2.45	—	—	50	—
115	Male	3	16 September 2003	P16	2.40	—	—	—	—
		3	11 August 2004	P13	2.60	100	—	—	50
123	Female	—	28 March 2003	P13	2.20	—	—	—	—
		3	18 September 2003	P13	2.20	—	—	—	—
		3	10 August 2004	P13	2.55	50	—	—	—
124	Male	5	18 September 2003	P13	3.50	—	—	—	—
		5	10 August 2004	P13	3.05	100	—	—	100
126	Male	3	19 September 2003	P13	2.70	400	—	—	—
161	Male	2	18 August 2004	P16	2.15	1,600	400	—	100
162	Female	3	19 August 2004	P16	2.55	200	100	—	—
164	Male	5	24 August 2004	P16	2.90	100	50	—	100

[a] Age class 2 = 1.0–1.5 yr (mean true age 1.2 yr); age class 3 = 0.9–3.6 yr (1.7 yr); age class 4 = 2.6–5.4 yr (3.7 yr); age class 5 = 4.2–9.3 (6.2 yr); age class 7 = 7.3–11.5 (9.6 yr).

[b] B = Beecroft; P = Pymble.

Research on Leptospirosis, Brisbane, Australia, we suggest that these two animals are likely to have been exposed to the serovar Arborea. This Ballum serogroup representative appears to be newly established in Australia and is an emerging source of leptospirosis in humans (Slack et al., 2006). Isolation studies are needed to confirm the identity of the serovar or serovars in the Ballum serogroup found in brushtail possums. In the absence of this information, future references for the serovar Ballum identified in this study will describe only the serogroup.

There was a significant difference in the number of seropositive animals observed in each location (χ^2=19.8, P=0.0006, df=4), ranging from 0% (Chatswood West, n=14; North Epping, n=21; Scotland Island, n=35), to 18% (11/62) in Pymble and 50% (2/4) in Beecroft. There was no apparent relationship with gender; 8% of females (4/52) and 11% of males (9/84) tested seropositive (binomial P=0.27). The age distribution of exposed brushtail possums was as follows: 1/21 in age class 2 (1.0–1.5 yr), 4/45 in age class 3 (0.9–3.6 yr), 3/29 in age class 4 (2.6–5.4 yr), 3/11 in age class 5 (4.2–9.3 yr), and 1/3 in age class 7 (7.3–11.5 yr). Age was not determined for 27/136 of adult animals from initial trapping. There was an indication that older animals have a higher incidence of leptospirosis, but this difference between age groups was not significant (χ^2=5.6, P=0.24, df=4). In New Zealand, it has been observed that the proportion of seropositive animals increases with age. Primary infections are thought to be acquired with sexual maturity and serovar Balcanica infection is predominantly found in brushtail possums over 18 mo of age (Durfee and Presidente, 1979; Cowan et. al, 1991).

There was no evidence that seroconversion resulted in a decline in body weight (apart from animal 124, Table 1), suggesting there was no loss in body condition associated with exposure. However, two animals that were both seropositive for serogroup Ballum (39 and 110) showed severe signs of dermatitis, a condition known to affect stressed urban brushtail possums (Hemsley and Canfield, 1994). The recapture data indicate that seropositive animals can demonstrate MAT titers for a long time (up to 9 mo as recorded for animal 3). We did not observe large changes in antibody titers between serum samples from the same animal (Table 1). Experimental infection with serovar Balcanica in captive brushtail possums resulted in a peak in antibody titers within the first 2 wk of infection (Hathaway et al., 1981). Titers then fell rapidly, reaching a plateau about 2 mo postinoculation; titers were maintained at almost constant levels for the remaining 11 mo of the experiment. These patterns imply that we did not detect any recent exposures in our study.

Seropositive animals were usually captured on the same properties in Beecroft and Pymble (Table 1). This was very apparent on two Pymble properties (Pymble 13 and 16), located within 120 m (approximately 390 feet) of each other, where seven seropositive brushtail possums were detected. Between March 2003 and August 2004 the number of seropositive individuals steadily increased, with seroconversions detected in some recaptured animals (animals 115, 123, and 124; Table 1) and other 'new' brushtail possums testing positive. During this period, one individual (animal 115; Table 1) was captured alternating between these properties, showing a connection between the two sites. After the major breeding season in 2004, numbers of seropositive brushtail possums in these backyards increased sharply, probably because of increased social contacts. Previous research in New Zealand suggests that Balcanica transmission occurs with sexual activity in the breeding season (Day et al., 1998) and that seroprevalence may be elevated during the breeding season months (Cowan et al., 1991). A recent study showed that contacts between wild brushtail possums are related to mating and associated behavior and therefore mainly occur in the peak mating season (Ji et al., 2005). Transmission of leptospiral infection in brushtail possums may be density dependent (Caley and Ramsey, 2001), and density is potentially elevated in some urban areas (Matthews et al., 2004).

Our results show that brushtail possums are exposed to *Leptospira* spp. in an urban environment. The increase in seropositive animals in few specific locations over time suggests local foci of exposure and supports that transmission of leptospirosis may be the result of affiliate or sexual behavior. Most exposed animals had antibodies to serovar Hardjo, but some cross-reaction with serovar Balcanica occurred. Two animals had been exposed to the exotic serogroup Ballum. Brushtail possums are an accidental host for serogroup Ballum; leptospiruria is not associated with infection (Hathaway et al., 1981) and severe illness can occur. Indirect contact with free-living maintenance host species such as the black rat (*Rattus rattus*) and house mouse (*Mus musculus*) are the likely source of serogoup Ballum and we occasionally encountered black rats in our brushtail possum traps. The impact of serogroup Ballum on brushtail possum populations is unknown, but because leptospires do not seem to be shed by brushtail possums infected with Ballum, public health concerns would be minimal. In contrast, some seropositive animals may have been exposed to serovar Balcanica, and therefore could pose a potential source for contaminating the urban environment with leptospires. For example, rainwater tanks may enable interspecies transmission. In Australia, the use of rainwater tanks is increasing, and tank water is generally collected from roof tops which are frequently used by urban brushtail possums as runways. In Hawaii, human cases of leptospirosis have been linked with household use of rainwater catchment systems contaminated by rat urine (Sasaki et al., 1993). The potential role that possums could have in leptospirosis disease transmission to humans in urban Australia requires further investigation.

We thank all householders who gave access to their properties and M. Herberstein for statistical advice. This research was supported by an international Macquarie University Research Scholarship and funded in part by the Higher Degree Research Fund. It was approved by the Macquarie University Animal Ethics Committee (permit 2002/022) and the New South Wales Department of Environment and Conservation (permit S11336).

LITERATURE CITED

Biosecurity Australia. 2000. A scientific review of leptospirosis and implications for quarantine policy. Agriculture, Fisheries and Forestry – Australia, Canberra. Australian Capital Territory, Australia, 108 pp.

Caley, P., and D. Ramsey. 2001. Estimating disease transmission in wildlife, with emphasis on leptospirosis and bovine tuberculosis in possums, and effects of fertility control. Journal of Applied Ecology 38: 1362–1370.

Cowan, P. E., D. K. Blackmore, and R. B. Marshall. 1991. Leptospiral infection in common brushtail possums (*Trichosurus vulpecula*) from lowland podocarp/mixed hardwood forest in New Zealand. Wildlife Research 18: 719–727.

Day, T. D., J. R. Waas, and C. E. O'connor. 1997. Effects of experimental infection with *Leptospira interrogans* serovar *balcanica* on the health of brushtail possums (*Trichosurus vulpecula*). New Zealand Veterinary Journal 45: 4–7.

———, C. E. O'connor, J. R. Waas, A. J. Pearson, and L. R. Matthews. 1998. Transmission of *Leptospira interrogans* serovar *balcanica* infection among socially housed brushtail possums in New Zealand. Journal of Wildlife Diseases 34: 576–81.

Durfee, P. T., and P. J. A. Presidente. 1979. A sero-epidemiological study of *Leptospira interrogans* serovar *balcanica* in four brush-tailed possum populations in Victoria, Australia. Ajebak 57 (2): 191–201.

Eymann, J., C. A. Herbert, D. W. Cooper, and J. P. Dubey. 2006. Serologic survey for *Toxoplasma gondii* and *Neospora caninum* in the common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia. Journal of Parasitology 92 (2): 267–272.

Hathaway, S. C. 1981. Leptospirosis in New Zealand: An ecological view. New Zealand Veterinary Journal 29: 109–112.

———, D. K. Blackmore, and R. B. Marshall. 1981. Experimental infection of the possum (*Trichosurus vulpecula*) with *Leptospira interrogans* serovar *balcanica*. I. Characteristics of infection. New Zealand Veterinary Journal 29: 121–125.

Hemsley, S., and P. Canfield. 1994. Dermatitis in free-living common brushtail possums (*Trichosurus vulpecula*). Australian Veterinary Practitioner 24: 147–155.

Horner, G. W., D. D. Heath, and P. E. Cowan. 1996. Distribution of leptospirosis in possums from New Zealand and its offshore islands. New Zealand Veterinary Journal 44: 161.

Ji, W., P. C. L. White, and M. N. Clout. 2005.

Contact rates between possums revealed by proximity data loggers. Journal of Applied Ecology 42: 595–604.
LEVETT, P. N. 2001. Leptospirosis. Clinical Microbiology Reviews 14: 296–326.
MATTHEWS, A. D. LUNNEY, K. WAPLES, AND J. HARDY. 2004. Brushtail possums: "Champion of the suburbs" or "our tormentors"? *In* Urban wildlife: More than meets the eye, D. Lunney and S. Burgin (eds.). Royal Zoological Society of New South Wales, Mosman, New South Wales, Australia, pp. 159–168.
MILNER, A. R., C. R. WILKS, D. M. SPRATT, AND P. J. PRESIDENTE. 1981. The prevalence of anti-leptospiral agglutinins in sera of wildlife in southeastern Australia. Journal of Wildlife Diseases 17: 197–202.
SASAKI, D. M., L. PANG, H. P. MINETTE, C. K. WAKIDA, W. J. FUJIMOTO, S. J. MANE, R. KUNIOKA, AND C. R. MIDDLETON. 1993. Active surveillance and risk factors for leptospirosis in Hawaii. American Journal of Tropical Medicine and Hygiene 48: 35–43.
SLACK, A. T., M. L. SYMONDS, M. F. DOHNT, AND L. D. SMYTHE. 2006. The epidemiology of leptospirosis and the emergence of *Leptospira borgpetersenii* serovar Arborea in Queensland, Australia, 1998–2004. Epidemiology and Infection 134: 1217–1225.
SMYTHE, L. D., H. E. FIELD, L. J. BARNETT, C. S. SMITH, M. F. DOHNT, M. L. SYMONDS, M. R. MOORE, AND P. F. ROLFE. 2002. Leptospiral antibodies in flying foxes in Australia. Journal of Wildlife Diseases 38: 182–186.

Received for publication 7 June 2006.