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TRANSMISSION OF *LEPTOSPIRA INTERROGANS* SEROVAR *BALCANICA* INFECTION AMONG SOCIALLY HOUSED BRUSHTAIL POSSUMS IN NEW ZEALAND

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ABSTRACT: *Leptospira interrogans* serovar *balcanica* is a potential vector being investigated for spreading a biological control agent among introduced brushtail possums (*Trichosurus vulpecula*) in New Zealand. As previous studies have shown that possums are unlikely to contract leptospirosis through a contaminated environment alone, the objective was to determine whether *L. interrogans* serovar *balcanica* could be transmitted between sexually mature, socially housed possums. Possums were infected experimentally with *L. interrogans* serovar *balcanica* and housed in pairs or groups with uninfected possums for either 70 or 140 days, during the breeding or non-breeding seasons. No transmission occurred between any infected and uninfected possums during the non-breeding season. However, transmission occurred between females that had been socially housed in pairs or groups in the breeding season. Mixed sex transmission also occurred in pairs and groups, both from males to females and from females to males. Mixed sex transmission usually occurred rapidly (<44 days) and was not associated with the production of offspring. No transmission occurred between males during the breeding or the non-breeding seasons. Transmission probably occurs as a result of affiliative or sexual behaviour, but is unlikely to occur through fighting. The social transmission pathways determined in this study suggest that *L. interrogans* serovar *balcanica* may have the transmission attributes desired in a vector for biological control.

Key words: Biological control, brushtail possum, disease transmission, *Leptospira interrogans* serovar *balcanica*, social behaviour *Trichosurus vulpecula*.

INTRODUCTION

In New Zealand, brushtail possums (*Trichosurus vulpecula*) cause severe damage to native forests by selective browsing (Nugent, 1995) and, through competition and predation, adversely affect native fauna (Innes, 1995). Possums also are vectors in maintaining and spreading bovine tuberculosis (Tb) to cattle and deer (Livingstone, 1991). Biological control may be the only affordable option for achieving a long-term major reduction in possum numbers throughout New Zealand (Jolly et al., 1992). An ideal biological control agent should be humane, infect and survive only in possums, and have a possum-specific mode of action (Jolly, 1993). The modes of transmission of a biological control agent will influence both its specificity and its prevalence and dictate the speed and success with which it spreads throughout the population.

Leptospira interrogans serovar *balcanica* is among potential organisms presently being investigated for use as a vector of a biological control agent. The possum is considered to be the maintenance host for *L. interrogans* serovar *balcanica* infection in New Zealand (Hathaway, 1978). A lack of clinical signs (Day et al., 1997a) and no evidence of renal damage or kidney lesions (Hathaway, 1981) indicate that it has low pathogenicity in possums. Among captive possums, *L. interrogans* serovar *balcanica* appears to be readily spread and is found predominantly in animals >18 mo of age (Pearson and Ashby, 1995).

Transmission of different types of leptospirosis in maintenance host species usually occurs directly, when body fluids from an infected animal pass directly to another animal (e.g., during sexual contact or suckling; Faine, 1994). Indirect transmission also may occur occasionally between maintenance hosts or from maintenance hosts to other species, as a result of contact with

environments containing infected urine. It is not known whether indirect transmission of *L. interrogans* serovar *balcanica* occurs from possums to other species in New Zealand. However, in experimental trials this leptospire does not appear to be transmitted between possums by contact with contaminated environments (Day et al., 1997b). Furthermore, no "vertical" transmission of *L. interrogans* serovar *balcanica* has been seen between infected mothers and their offspring (Hathaway, 1978). A very low seroprevalence of *L. interrogans* serovar *balcanica* in immature possums (Pearson and Ashby, 1995) suggests that the acquisition of infection may be associated with sexual maturity (Hathaway, 1978; Durfee and Presidente, 1979). The present study was designed to determine whether *L. interrogans* serovar *balcanica* could be transmitted directly between sexually mature possums and to identify likely behaviours associated with transmission.

MATERIALS AND METHODS

Possums used in this study were box trapped between December 1995 and January 1997 from Kawau Island (36°26′S, 174°50′E) and Nelson (41°20′S, 172°43′E), New Zealand. Possums from both areas are reportedly free of *L. interrogans* serovar *balcanica* infection (Horner et al., 1996). Animals were transported to the Animal Behaviour and Welfare Research Centre (AgResearch, Ruakura, Hamilton, North Island, New Zealand). All possums were aged by tooth wear (Winter, 1980) and sexual maturity was determined during routine veterinary checks. All procedures used in the present study were approved by the AgResearch Ruakura (Hamilton, New Zealand) and University of Waikato (Hamilton, New Zealand) Animal Ethics Committees.

Serological testing for any evidence of *L. interrogans* serovar *balcanica* infection was conducted prior to the beginning of the experiments using a modified version of the Microscopic Agglutination Test (MAT) by the Central Animal Health Laboratory (AgResearch Wallaceville, Wellington, North Island, New Zealand). Cross absorption procedures (Faine, 1982) were used to discriminate between *L. interrogans* serovar *balcanica* and other serovars. Positive tests were reported as the greatest serum dilution at which serum showed a reaction. If no reaction was seen in a 1:50 dilution, results were reported as negative. Mid-stream urine also was collected from possums before the experiments and examined under dark-field microscopy (400×) for the presence of leptospires. None of the captured possums used in this study had serological or urinary evidence of infection; therefore they were considered to be uninfected with *L. interrogans* serovar *balcanica* prior to the commencement of the experiments.

Twenty three sexually mature experimentally infected possums (mean age ± SE = 4.2 ± 0.3 yr; 12 female, 11 males) were used in this study. Possums were anaesthetised and inoculated intraperitoneally with 0.5 ml *L. interrogans* serovar *balcanica* (about 1×10^8 organisms). The inoculum was cultured from an infected possum at the Department of Veterinary Pathology and Public Health (Massey University, Palmerston North, New Zealand) and confirmed as New Zealand possum *L. interrogans* serovar *balcanica* isolate, using restriction endonuclease analysis and DNA verification. The characteristics of experimental and free-living *L. interrogans* serovar *balcanica* infection in possums have been thoroughly examined (Hathaway, 1981), and do not appear to differ significantly. Using MAT serology, all experimentally infected possums were shown to be infected with *L. interrogans* serovar *balcanica* at 12 days post inoculation (pi).

Each experimentally infected possum was housed with other animals in a grass enclosure from day 12 pi, either with one uninfected possum (in pairs) or with 3 uninfected animals (in groups). Each of the eight enclosures used was individually fenced (ranging in size from 51m^2 to 130m^2), contained at least two wooden nest boxes and two climbing logs and was subject to natural weather conditions. Possums had *ad libitum* access to fresh pellets (Northern Rolling Mill, Auckland, New Zealand), apples and water in their enclosure.

Seventeen pairs of sexually mature possums (four female/female, eight male/female, five male/male pairs), each containing one of the infected animals, were housed in the enclosures for a 70 day period, during the "breeding" or "non-breeding" seasons. Thirteen pairs were housed together in the breeding season (three female/female, six male/female, four male/male) and four pairs in the non-breeding season (one female/female, two male/female, one male/male). In four of the mixed sex pairs a male was infected and in the other four pairs a female was infected. Serum was sampled and tested for evidence of *L. interrogans* serovar *balcanica* infection from each infected possum on day 0 (12 pi) and day 70 (82 pi) of social

housing, and from uninfected possums on days 30, 44 and 70. Mid-stream urine was sampled from infected possums at day 30 (42 pi) and examined under dark field microscopy (400×) for the presence of leptospires. All females also were examined for evidence of offspring (pouch young) at days 30, 44 and 70.

Four groups of four sexually mature possums (one all female, two mixed sex, one all male group), each containing one of the infected animals, were also housed in separate enclosures for a 140 day period that encompassed both the non-breeding season (January to early March 1997) and the breeding season (mid March to May 1997). Two additional groups of four possums (one all female, one mixed sex), also containing an infected possum, were housed in the enclosures for a 70 day period during the breeding season only. Serum was collected from each infected possum on day 0 (12 pi) and day 70 (82 pi) for the breeding season groups, and day 0 (12 pi) and day 140 (152 pi) for the groups housed together in both seasons. Uninfected possums in all groups had serum collected on days 21, 30, 44 and 70, plus days 84, 98, 112, 126 and 140 for the groups housed in both seasons. Mid-stream urine was sampled from infected possums between days 77 and 82 pi or days 142 and 152 pi and examined for the presence of leptospires. Females from all social groups were examined for pouch young when each serum sample was collected.

Paired t-tests were used to analyze changes in serological *L. interrogans* serovar *balcanica* titers over time. Differences in transmission between the breeding and non-breeding season were analyzed using one-tailed Fisher's exact tests in the Genstat 5 statistical analysis package (Release 3.1; Lawes Agricultural Trust, Rothamsted Experimental Station Hertfordshire, UK).

RESULTS

All of the 23 experimentally infected possums had positive serological *L. interrogans* serovar *balcanica* titers by day 12 pi (mean ± SE, 1:420 ± 70; range, 1:100 to 1:1,600). Leptospires were detected in the urine of 19 of the infected possums at either day 42, 82 or 152 pi (4 with motile and 15 with non-motile leptospires). At day 82 pi, the mean *L. interrogans* serovar *balcanica* titre had decreased (1:200 ± 40; range, 1:50 to 1:800; $t(17) = 5.31$, $P < 0.001$), but was still present in all but one female possum. However, in this female, non-motile urinary leptospires were still detected on day 82 pi. At day 152 pi, *L. interrogans* serovar *balcanica* titers were still positive (1:180 ± 90; range, 1:50 to 1:400) in the four infected possums.

TABLE 1. Observed transmissions of *L. interrogans* serovar *balcanica* between different types of socially housed possum pairs over a 70 day period during either the breeding or non-breeding seasons.

Season	Pair type	Number of pairs	Observed transmission	% Transmission
Breeding	Female	3	1[a]	33
	Mixed sex	6	3[b]	50
	Male	4	0	0
Non-breeding	Female	1	0	0
	Mixed sex	2	0	0
	Male	1	0	0

[a] Transmission after 160 days social housing.
[b] Two transmissions from male to female and one from female to male.

Transmission occurred between possums in three of six male/female pairs during the breeding season, but in neither of the two male/female pairs outside the breeding season (Table 1). Infections passed both from males to females and from females to males, with all transmission occurring by day 44 of association. One transmission also occurred between two females after 160 days during the breeding season. No transmission was recorded between males (Table 1). Although no transmission was observed in the non-breeding season, there was not a statistically significant difference in transmission rate between seasons ($P = 0.30$), due to the relatively low rate of transmission overall in the breeding season (31%). The initial serological *L. interrogans* serovar *balcanica* titres of possums that became naturally infected during social housing were similar to those seen after recent experimental infections (mean ± SE, 1:550 ± 170; range, 1:200 to 1:800; $t(3) = -0.78$, $P > 0.05$). All possums that became infected in the pairs continued to have detectable *L. interrogans* serovar *balcanica* titres until the end of the experiment (70

TABLE 2. Serological reactions of the six possums naturally infected with *L. interrogans* serovar *balcanica* as a result of social contact with experimentally infected animals. Titres given are the greatest serum dilution at which serum showed a reaction.

	Day of social housing						
Possum	0	30	44	70	98	126	160
1	Neg[a]	1:200	1:100	1:50	—[b]	—	—
2	Neg	1:800	1:400	1:100	—	—	—
3	Neg	Neg	1:400	1:200	—	—	—
4	Neg	Neg	Neg	Neg	—	—	1:800
5	Neg	1:1,600	1:200	Neg	Neg	—	—
6	Neg	1:12,800	1:200	1:50	1:50	Neg	—

[a] Negative with no reaction at 1:50 serum dilution.
[b] — indicates not tested.

days; Table 2). No offspring were produced by the females housed in pairs.

No transmission occurred in the groups during the non-breeding season. During the breeding season, transmission occurred between an infected female and uninfected male and between two females (Table 3). No transmission occurred between males during the breeding season. Again, there was no statistically significant difference in transmission between seasons ($P = 0.35$). The two possums that became infected in the groups initially had high titres (1:1,600 and 1:12,800 respectively) which quickly decreased (Table 2). In one of these possums, the *L. interrogans* serovar *balcanica* titre was no longer detected from 40 days after infection and in the other, a titre was no longer detected after 90 days of infection. Non-motile leptospires were detected in the urine of these animals between 46 and 51 days after the first positive *L. interrogans* serovar *balcanica* titre.

TABLE 3. Observed transmissions of *L. interrogans* serovar *balcanica* in different types of socially housed possum groups over 70 day periods during the breeding and non-breeding seasons.

Season	Transmission type	Potential transmission	Observed transmission	% Transmission
Breeding	Female	8	1	13
	Mixed sex	6	1	17
	Male	4	0	0
Non-breeding	Female	4	0	0
	Mixed sex	4	0	0
	Male	4	0	0

Pouch young were found in three females from the mixed sex groups during the breeding season, two of which had positive titres during the period in which mating would have occurred. The other female with a pouch young remained uninfected throughout the experimental period.

DISCUSSION

This study showed that *L. interrogans* serovar *balcanica* can be transmitted between sexually mature brushtail possums when housed together during the breeding season. Previous work has shown that contact with contaminated environments alone is insufficient to allow indirect transmission of this leptospire between possums (Day et al., 1997b), and therefore some aspect of direct contact must be important for transmission.

The experimentally infected possums in this study had characteristics of infection (*L. interrogans* serovar *balcanica* titres, leptospiruria) similar to those described previously (Hathaway, 1981). However, one of the experimentally infected possums and two of the socially infected animals no longer had detectable *L. interrogans* serovar *balcanica* titres between 30 and 90 days after the establishment of in-

fection. Non-motile leptospires were detected in the urine of all of these animals between 46 and 82 days after infection (between 8 days before and 16 days after the negative titre was detected), but no transmission occurred in the group containing the experimentally infected female that ceased to have a detectable titre. This type of transient serological response to *L. interrogans* serovar *balcanica* has been described previously in a possum after experimental infection (titer lasting only 30 days, but *L. interrogans* serovar *balcanica* was recovered from kidneys 70 days after infection; Hathaway, 1981). In many maintenance hosts for *Leptospira* spp., seronegative animals that are still infected with leptospirosis are found. This infers that leptospires, which are generally localized in the renal tubules, exert only minor antigenic stimulus on maintenance hosts (Hathaway, 1978). Therefore, the animals in this study that no longer had *L. interrogans* serovar *balcanica* titres were likely to still be infected, as is suggested by the presence of non-motile leptospires in the urine around the time that negative titres were observed.

Based on the observation that *L. interrogans* serovar *balcanica* infection occurs primarily in sexually mature animals (Pearson and Ashby, 1995), and the lack of transmission during the non-breeding season in this study, direct contact associated with sexual maturity and possibly the breeding season appears to be important in the acquisition of infection. In the pairs, no successful mating (as measured by presence/absence of pouch young) occurred, so it is unknown whether the mixed sex transmission occurred as a result of unsuccessful mating or through some other behaviour. Successful mating occurred in the groups between infected and uninfected possums, although did not inevitably result in transmission of *L. interrogans* serovar *balcanica*. The timing of a behaviour (such as mating) relative to the stage of infection in the possum, may be critical for the transmission of the leptospire. Therefore, the role of mating itself in transmission of *L. interrogans* serovar *balcanica* remains uncertain, as does the potential for transmission to occur in the non-breeding season.

Transmission between females, which must have resulted from behaviour other than mating, also occurred in this study. However, female to female transmission also only occurred during the breeding season, suggesting that transmission is associated with particular behaviours occurring during this period. In female to female and mixed sex contacts between captive possums, affiliative behaviours (e.g., den sharing, allogrooming, food sharing) are common during the breeding season (Day, 1996), but occur less frequently in the non-breeding season. These behaviours may be important for the transmission of *L. interrogans* serovar *balcanica* between possums. Furthermore, male possums very rarely show affiliative behaviours towards each other (Day, 1996) and no transmission occurred between males in this study. In laboratory mice, the transmission of another leptospire (*L. interrogans* serovar *ballum*) also is dependant on sexual maturity, but not on mating. Transmission can occur between mature pairs of female mice and mature mixed-sex pairs, but does not occur between sexually mature male mice (Hathaway, 1978).

The rate of transmission of *L. interrogans* serovar *balcanica* in this study was lower in the groups than in the pairs. While the reasons for this are unknown, it is possible that in the groups, each uninfected possum had less opportunity for contact with the infected animal than they would have had if housed in pairs. Therefore, transmission that is reliant on close contacts such as affiliative or sexual behaviours (as our data suggest), may occur relatively slowly in a group situation. However, the transmission rate observed in the groups is comparable to the rate of transmission found previously in groups of captive possums (Pearson and Ashby, 1995).

Social transmission of *L. interrogans*

serovar *balcanica* between possums is desirable from the perspective of biological control, as it would be more likely to be species-specific (Jolly et al., 1992). An agent that is transmitted by sexual or other behaviours that primarily occur during the breeding season (e.g., affiliative behaviours) may be most useful for biological control. Possums actively seek mates during the breeding season (Jolly et al., 1992), which would allow an agent to continue spreading as possum density reduced (Barlow, 1994). The data collected in this study have shown that *L. interrogans* serovar *balcanica* appears to be transmitted most effectively during the breeding season and may be associated with affiliative or sexual behaviour. Therefore, *L. interrogans* serovar *balcanica* appears to have transmission properties that are suited for use in biological control.

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LITERATURE CITED

BARLOW, N. D. 1994. Predicting the effect of a novel vertebrate biocontrol agent: a model for viral-vectored immunocontraception of New Zealand possums. Journal of Applied Ecology 31: 454–462.

DAY, T. D. 1996. *Leptospira interrogans* serovar *balcanica* transmission in the brushtail possum (*Trichosurus vulpecula*). M.Sc. Thesis, University of Waikato, Hamilton, New Zealand, 98 pp.

———, J. R. WAAS, AND C. E. O'CONNOR. 1997a. Effects of experimental infection with *Leptospira interrogans* serovar *balcanica* on the health of brushtail possums (*Trichosurus vulpecula*). New Zealand Veterinary Journal 45: 4–7.

———, J. R. WAAS, C. E. O'CONNOR, P. W. CAREY, L. R. MATTHEWS, AND A. J. PEARSON. 1997b. Leptospirosis in brushtail possums: Is *Leptospira interrogans* serovar *balcanica* environmentally transmitted? Journal of Wildlife Diseases 33: 254–260.

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. Australian Journal of Experimental Biology and Medical Science 57: 191–201.

FAINE, S. 1982. Guidelines for the control of leptospirosis. Offset publication No. 67, World Health Organisation, Geneva, Switzerland, 193 pp.

———. 1994. *Leptospira* and leptospirosis. CRC Press, Boca Raton, Florida, 334 pp.

HATHAWAY, S. C. 1978. Leptospirosis in free living animals in New Zealand, with particular reference to the possum (*Trichosurus vulpecula*). Ph.D. Thesis, Veterinary Pathology and Public Health, Massey University, Palmerston North, New Zealand, 434 pp.

———. 1981. Experimental infection of the possum (*Trichosurus vulpecula*) with *Leptospira interrogans* serovar *balcanica* I. Characteristics of infection. New Zealand Veterinary Journal 29: 121–125.

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: 162.

INNES, J. 1995. The impacts of possums on native fauna. *In* Possums as conservation pests; proceedings of a workshop on possums as conservation pests, C. F. J. O'Donnell, (ed.). National Science Strategy Committee, Christchurch, New Zealand, pp. 11–15.

JOLLY, S. E. 1993. Biological control of possums. New Zealand Journal of Zoology 20: 335–339.

———, G. HICKLING, AND N. BARLOW. 1992. Biological control of brushtail possums: key issues. Contract Report LC9293/2, Landcare Research New Zealand Ltd., Christchurch, New Zealand, 26 pp.

LIVINGSTONE, P. G. 1991. Tuberculosis in New Zealand — current status and control policies. Surveillance 19: 14–18.

NUGENT, G. 1995. Effects of possums on the native flora. *In* Possums as conservation pests; proceedings of a workshop on possums as conservation pests, C. F. J. O'Donnell, (ed.). National Science Strategy Committee, Christchurch, New Zealand, pp. 5–10.

PEARSON, A. J., AND M. G. ASHBY. 1995. The incidence and spread of leptospirosis among possums of the Ruakura Colony 1982–1984. Contract report, March 1995, Ministry of Agriculture and Fisheries, Policy Division, Wellington, New Zealand, 23 pp.

WINTER, J. W. 1980. Tooth wear as an age index in a population of the brush-tailed possum, *Trichosurus vulpecula* (Kerr). Australian Wildlife Research 7: 359–363.

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