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PREVALENCE OF ANTIBODIES TO SPOTTED FEVER GROUP RICKETTSIAE IN DOGS FROM SOUTHEASTERN AUSTRALIA

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Abstract. Recent epidemiologic data suggests that *Rickettsia australis*, the cause of Queensland tick typhus, is present in southeastern Australia. In order to further confirm this observation, a canine serosurvey was undertaken to determine if naturally occurring antibodies were present in pet and farm dogs from this newly-recognized endemic area. Thirty-five of 312 surveyed dogs (11.2%) had indirect immunofluorescent antibody titers of 1:64 or greater against *R. australis* antigen. Positive control sera were obtained from two dogs experimentally inoculated with *R. australis*. One of these dogs was serially sampled and a rickettsemia could not be documented. None of 26 control sera obtained from dogs from South Australia, New Zealand, western Victoria, or North Carolina had antibody titers ≥ 1:64. These results suggest that spotted fever group rickettsiae are present in Southeastern Australia.

Serologic evidence of canine infection with spotted fever group (SFG) rickettsiae is a useful indirect marker for the presence of these microorganisms in specific geographic regions. In fact, free-roaming and pet dogs from areas endemic for Rocky Mountain spotted fever and Mediterranean spotted fever frequently have antibodies against SFG rickettsiae.[1–14]

We reasoned that if dogs develop convalescent antibodies after experimental infection with *Rickettsia australis*, the presence of naturally occurring antibodies in canines would indirectly support recent epidemiologic data suggesting that *R. australis* is present in southeastern Australia.[15–18]

MATERIALS AND METHODS

Sera were collected from 312 pet and farm dogs from coastal New South Wales, eastern coastal Victoria, Flinders Island, and the Tasmanian mainland (Figure 1). Canine sera were obtained from veterinarians and from household pets on Flinders Island. One hundred-fifty of 312 dogs (48%) were clinically well at the time of serologic sampling. Ten dogs had tick paralysis, 38 had acute or chronic arthritis, six had fever and anorexia, three had heartworms, three had cutaneous abscesses, and one had unexplained lymphadenopathy. No clinical information was available for 101 dogs (32%). Information was not collected concerning the breed or age of individual dogs.

Two dogs of mixed breed (one male and one female), both seven months of age, and weighing 15 and 14 kg respectively, were inoculated with *R. australis* grown in Buffalo Green monkey kidney (BFMK) cells in RPMI-1640 media with antibiotic-free 10% fetal calf serum, and incubated at 35°C in an atmosphere of 5% CO_2 in air. Rickettsia-infected tissue culture cells were harvested by collecting cells from the supernatant fluid after centrifugation (12,000 *g* for 20 min), and from tissue culture monolayers by freezing and thawing in a sucrose phosphate glutamate (SPG) solution. The final pooled rickettsia-containing preparation was stored at −70°C in SPG solution until it was used for intraperitoneal injections. The rickettsial titer of this preparation was between 1 and 5×10^6 tissue culture infectious doses ($TCID_{50}$) per ml. Dog #1 was injected intraperitoneally on day 1 with $1–5 \times 10^2$ $TCID_{50}$ of *R. australis;* on day 58, a second dose of *R. australis* was given intramuscularly in the form of a single homogenized infected spleen taken from a mouse inoculated intraperitoneally six days previously with *R. australis*. On day 88, the dog was killed and autopsied. Dog #2 received a homogenized infected mouse spleen intramuscularly on day 1 and a similar second dose on day 44. It was killed and necropsied on day 74.

Blood taken from dog #1 on days 2, 4, 7, 9,

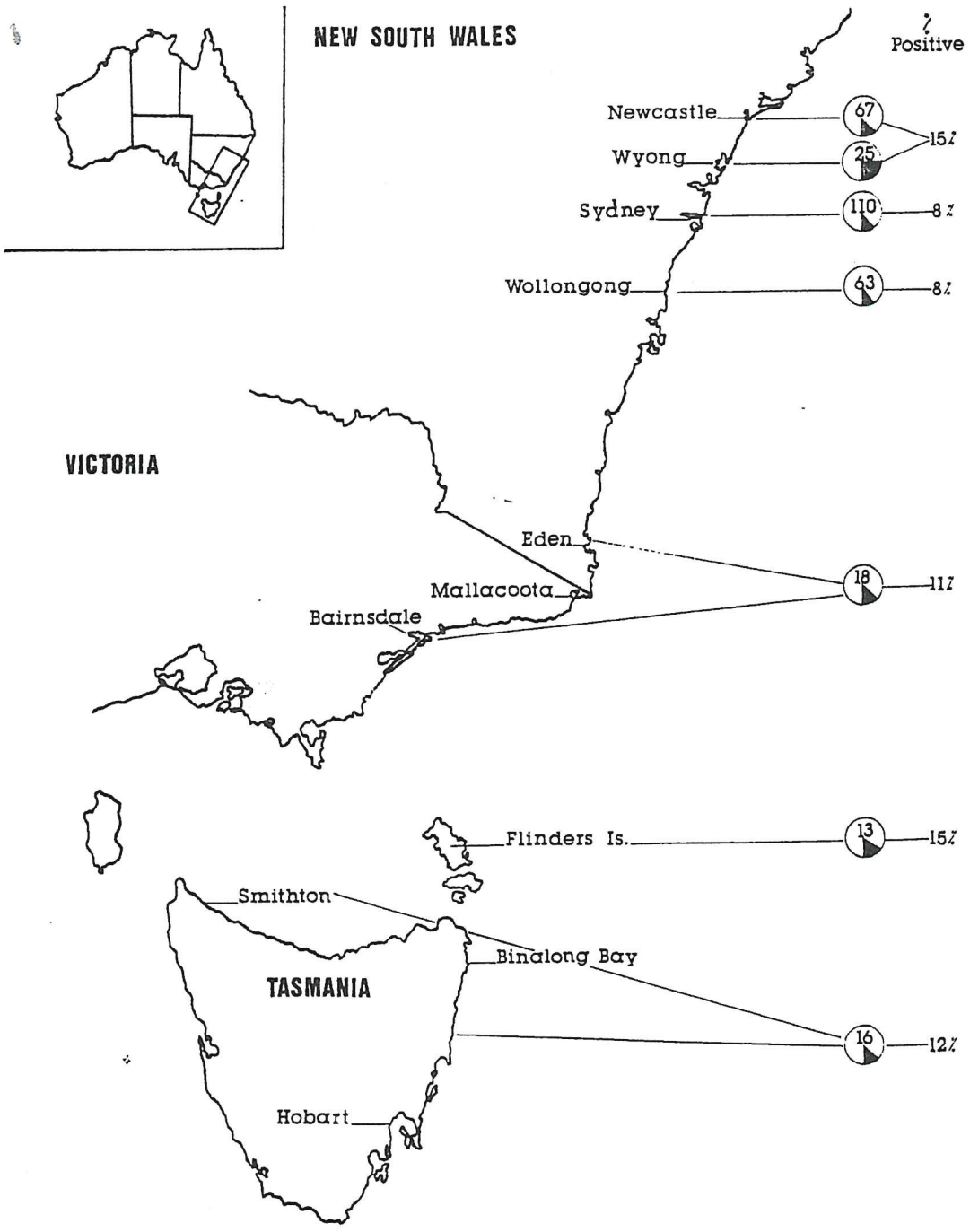

FIGURE 1. Areas of southeastern Australia surveyed in this study, showing the frequencies of canine sera positive for antibodies to *Rickettsia australis*.

11, 14, and 18 after the original injection with *R. australis* was allowed to clot and was stored at −70°C before being inoculated into neonatal mice, and later into BGMK tissue culture cells. Mice were bled 4–8 weeks after inoculation. Their sera were examined for antibodies to *R. australis* by microimmunofluorescence.[18]

Control era were obtained from six normal dogs from New Zealand, eight dogs from South Australia, two dogs from western Victoria, and 10 laboratory-reared dogs from the North Carolina State University School of Veterinary Medicine.

Canine antibody titers against *R. australis* antigens were determined using the microimmunofluorescence technique.[19] All endpoints were read by two individuals who were blinded to results of the other. Agreement within one dilution occurred in all cases. The lowest titer was selected in those cases in which the endpoint varied by one dilution. An antibody titer of 1:64 or greater was considered positive.

Each dog had daily clinical examinations for one week after inoculation and three times a week thereafter until they were killed. Rectal body temperatures were measured. Levels of C-reactive protein, blood urea nitrogen, electrolytes, and hemoglobin, erythrocyte sedimentation rates, liver functions, white blood cell counts, and platelet concentrations were determined serially for 35 days following the first inoculation.

RESULTS

Microimmunofluorescent antibody titers of all control dogs were < 1:64. Five control dogs had antibody titers of 1:32, 12 of 1:16, 7 of 1:8 and 2 < 1:8. An antibody titer of 1:64 or greater was found in 35 of 312 dogs (11.2%) from southeastern Australia (Figure 1). The highest titer observed was 1:256; eight dogs had titers of 1:128 and 26 had titers of 1:64. Thirty-eight dogs had titers of 1:32. Two of 35 seropositive dogs had tick paralysis, three were tick infested when tested, and three had arthritis. Twenty seropositive dogs were healthy when tested; no clinical information was available on the remaining 10 seropositive dogs. No significant differences in the rate of seropositivity were observed among dogs sampled in coastal New South Wales, Victoria, Flinders Island, and Tasmania. (Figure 1).

Rickettsia australis was not isolated from dog #1 after experimental inoculation. Both dogs remained well throughout the observation period; neither developed a fever above 38.8°C, and results of all laboratory studies remained unchanged from their pre-inoculation values. Mice injected with blood from dog #1 remained seronegative to *R. australis* antigen. No abnormalities were found in either dog at necropsy. Both experimentally-infected dogs lacked detectable anti-rickettsial antibodies prior to inoculation; they both seroconverted, each developing a peak antibody titer of 1:128.

DISCUSSION

Presumably, the dogs sampled in this survey acquired their infection from naturally infected ticks. The two known vectors of *R. australis*, *Ixodes holocyclus* and *I. tasmanii*, bite both humans and dogs. *Ixodes holocyclus* is a well-known cause of tick paralysis, and is widely distributed along eastern coastal Australia from North Queensland to southern Victoria, but is not known to exist on Flinders Island.[20] One of the three isolates of *R. australis* obtained by Campbell and Domrow was from a pool of *I. holocyclus* removed from a dog in Queensland.[21] Unfortunately this dog was not tested for *R. australis* antibody. *Ixodes tasmanii* is found throughout eastern Australia and is present on Flinders Island,[20] and it may have been the source of seropositivity in dogs from Flinders Island and the Tasmanian mainland.

Limited information is available on the prevalence of *R. australis* in Australian ticks. The few published studies examining this question relied on experimental inoculation of pooled ticks into laboratory animals.[21, 22] Most of these experiments gave negative results. However, the laboratory animals used may not have been suitable for the detection of all SFG rickettsial strains; non-pathogenic SFG species or weakly virulent strains of *R. australis* may have been overlooked.[15]

Recent epidemiologic and clinical studies suggest SFG that rickettsiae infect humans in southeastern coastal Australia from southern New South Wales to Flinders Islands in the Bass Straits.[15–18] Our data show that dogs from these newly recognized endemic areas acquired antibody to SFG rickettsial antigens, whereas none of 26 control dogs from New Zealand, western Victoria, South Australia, or North Carolina had antibody titers ≥ 1:64.

TABLE 1

Serologic studies of canine infection with spotted fever group rickettsiae

Location	Technique	Minimum positive titer	Study dogs		Control dogs		Comments
Long Island and Maryland (1)	CF**	>1:4	38/75	(51%)	0/22	(0)	6/75 study dogs had recent illness, 8/75 were from neighborhood of recent human RMSF cases.
Massachusetts (2)	IF*** CF	Not stated	6/130	(5%)	None		All 6 positive dogs were from neighborhood or households of recent human RMSF cases.
Mississippi (3)	CF MA***	>1:8 >1:8	53/116 56/108	(46%) (52%)	1/21 2/21	(5%) (9%)	Stray and pet dogs tested. 8 seropositive dogs were contacts of human RMSF cases. Dogs >1 yr. old were more often seropositive, seropositive dogs <1 yr. old had higher geometric mean titers.
Maryland (4)	IF	>1:40	48/288	(16%)	0/40	(0)	Two dogs with high titers had clinical illness diagnosed as RMSF.
Ohio (5)	IF	>1:16	12/16	(75%)	1/375	(0.2%)	All study dogs had been in close contact with humans with RMSF.
Eastern USA (6)	IF	>1:40	149/467	(32%)	None		Dogs housed indoors had 1/2 the seropositivity rate of those housed outdoors.
Connecticut (7)	IF	>1:64	31/1,576	(2%)	None		More dogs were positive against *R. rhipicephali* than *R. rickettsii* antigens.
Columbus, Ohio (8)	IF	>1:8	33/73	(45%)	1/137	(0.7%)	Study dogs were from a known focus of human RMSF.
Costa Rica (9)	IF	>1:16	12/36	(33%)	None		Study dogs were from an area with human RMSF.
Sicily (10)	IF	Not stated	88/108	(81%)	None		73 of 108 dogs were infested with ticks containing *R. rickettsiae* using the hemolymph test.
North Carolina (11)	IF	>1:64	179/600	(30%)	None		Prevalence rate (as determined by choosing highest titer to each of 4 antigens) was 5% to *R. rickettsii*, 15% to *R. montana*. 11% to *R. rhipicephali*. Older dogs were more often seropositive.
Louisiana (12)	IF	>1:64	9/86 12/86 11/86	(10%) (14%) (13%)	None		27/86 dogs were ill and had thrombocytopenia.
Japan (13)	IF	>1:16	14/134	(10%)	4/189	(2%)	Most dogs tested were household pets. "Positives" had to have titers >1:16 against both antigens.
France (14)	IF	1:32	383/481	(80%)	None		317/481 sera tested were from military dogs. No data available on the health of the other study dogs.

RMSF = Rocky Mountain spotted fever.
Number positive/number tested (percent positive).
* Complement fixation.
** Immunofluorescent antibody test.
*** Microagglutination test.

We were unable to demonstrate rickettsemia in one dog after experimental infection with *R. australis*. It is possible that freezing and thawing resulted in a loss of infectivity and spuriously negative results. Specific experiments to study the limits of detection of *R. australis* using suckling mice have not been done.[23] Thus it is possible that low grade or transient rickettsemia could have been missed by the isolation methods we used. Despite the absence of documented rickettsemia, both dogs developed increased antibody titers following inoculation. For unknown reasons, antibody titers observed in these dogs were not as high as those observed in American dogs infected with *R. rickettsii*.[24, 25]

The presence of antibodies against *R. australis* does not prove that the dogs we surveyed were naturally infected with this agent, since both pathogenic and nonpathogenic strains of SFG rickettsiae cross react, as determined by the microimmunofluorescence method. Spotted fever group rickettsiae other than *R. australis* have not been detected in Australia; however, the widespread presence of nonpathogenic rickettsiae elsewhere implies they could exist on this island continent. Studies using appropriate methods to detect nonpathogenic strains have not been done. If nonpathogenic SFG rickettsiae exist in Australia, our results may overestimate the prevalence of *R. australis* infection in Australian dogs. Thus, studies using appropriate methods to investigate this possibility should be undertaken.

Since Australian dogs naturally acquire SFG rickettsial antibodies in New South Wales, Victoria, and Tasmania, similar antibodies are likely to be present in native animals from coastal southeastern Australia. Complement-fixing antibodies against *R. australis* have been detected in wild rats and bandicoots in Queensland;[22] similar studies have not been reported from southeastern coastal Australia or Flinders Island, but are currently underway. The unavailability of specific conjugated immunoglobulins for most indigenous Australian animals makes such serologic surveys difficult. However, a competitive enzyme-linked immunosorbent assay specific and sensitive for *R. australis*, using sera from dogs, rabbits, mice, and rats, should facilitate serosurveys for *R. australis* antibodies in wild animals.[26] However, until such techniques become more widely available, it remains easier to use canine seropositivity to detect the presence of rickettsiae in specific regions.

Many questions persist about the epidemiology of Queensland Tick Typhus (QTT). Since it is often a mild disease with nonspecific features, QTT is easily confused with a myriad of viral or other self-limiting illnesses. Thus, QTT may be frequently overlooked by clinicians and, in fact, may have a wider distribution in Australia than currently recognized. Further studies examining canine sera for SFG rickettsial antibodies could indirectly define the geographic limits and prevalence of *R. australis* by detecting localized or hyperendemic foci of canine seropositivity. Such findings could, in turn, lead to better surveillance for human illness and better diagnostic evaluation of people with clinical illness compatible with QTT.

Dogs have been used as surrogate markers for SFG rickettsial infection in Central America, Europe, Japan, and the United States (Table 1). Our data further support the utility of using canine serology to demonstrate antibodies against SFG rickettsiae in areas of uncertain or unproven rickettsial activity.

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