Serological prevalence study of exposure of cats and dogs in Launceston, Tasmania, Australia to spotted fever group rickettsiae

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A sero-epidemiological study of cats and dogs in the Launceston area of Tasmania, Australia was undertaken to determine the prevalence of antibodies to spotted fever group (SFG) rickettsiae. Results showed that 59% of cats and 57% of dogs were positive for antibodies, but there was no correlation between the animal's health and seropositivity at the time of testing, suggesting that rickettsial exposure is unrelated to ill-health in these two species of domestic animals.

Keywords Australia; cats; dogs; rickettsial disease; spotted fever group rickettsiae; serology

Abbreviations FISF, Flinders Island spotted fever; FITC, fluorescein isothiocyanate; IFA, microimmunofluorescence assay; PBS, phosphate-buffered saline; SFG, spotted fever group

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The rickettsiae are obligate intracellular coccobacilli bacteria of the α-proteobacteria class.[1] The genus *Rickettsia* is molecularly divided into two groups: the typhus group and the spotted fever group (SFG).[2] With the exception of *R. felis* and *R. akari*, the SFG rickettsiae are transmitted by tick vectors, which are also the natural reservoir of these bacteria.[2]

The SFG rickettsiae cause a number of human diseases in Australia, including Queensland tick typhus (*R. australis*),[3] Flinders Island spotted fever (FISF; *R. honei*)[4] and, more recently, a variant form of FISF (*R. honei* strain *marmionii*).[5] These three diseases have similar symptoms of malaise, headaches, chills, fever and rash, and all are treatable with appropriate antibiotics.[6–8] Additionally, *R. honei* strain *marmionii* has been isolated from patients with chronic illness, including fatigue.[9]

There are only three published cases of human SFG rickettsial disease on mainland Tasmania,[5,10,11] although there have been a number of published reports of disease from the surrounding islands.[4,12,13] *Rickettsia honei* has been isolated from the tick species *Bothriocroton* (formally *Aponomma*) *hydrosauri* on Flinders Island[14] and *B. hydrosauri* is found in most of the south-eastern area of Australia,[15] where human cases of FISF are known to occur.[16]

The present study focused on Launceston, which is the second largest city in Tasmania, with over 63,000 people living in the city itself and approximately 100,000 in the surrounding area.[17] As well as significant areas of farming land, Launceston is surrounded by eucalypt forest with medium-to-dense undergrowth that supports a wide variety of fauna, making it a suitable tick habitat. The magnitude of the domestic dog and cat population is unknown.

Determining the seroprevalence of SFG antibodies in domestic dogs and cats can indirectly indicate the presence of the SFG rickettsiae within a particular region.[18,19]

Materials and methods

Sample and data collection

Serum samples from 368 dogs and 150 cats were collected from a veterinary hospital in Launceston, rickettsiae over a 16-month period in 2004 and 2005. All tested dogs and cats resided within a 50-km radius of the town centre.

Information collected for each animal included age, sex, geographical origin and clinical state (sick or healthy). Sickness was determined by the presence of one or more clinical markers, including fever, weight loss, lameness, asthenia and swollen lymph nodes.[20]

Detection of antibodies to SFG rickettsiae

Each serum sample was tested for the presence of antibodies to the SFG rickettsiae using a microimmunofluorescence assay (IFA),[12] which involved titrating each sample by a series of doubling dilutions in a 2% casein–phosphate-buffered saline (PBS) solution on antigen slides consisting of a mixture of SFG rickettsiae (*R. akari*, *R. australis*, *R. conorii, R. honei, R. rickettsii* and *R. siberica*) produced by the Australian Rickettsial Reference Laboratory, Geelong, Victoria, Australia. The slides were incubated at 37°C for 30 min, followed by three 5-min washes in PBS. Positive and negative cat and dog sera were included in each assay. Control dog sera were obtained from a previous study[19] and the cat control sera were produced by Helen Owen from Murdoch University, Perth, Western Australia using *R. australis* antigen. A 1:50 diluted fluorescein isothiocyanate (FITC)-labelled goat anti-dog IgG antibody or a FITC-labelled goat anti-cat IgG antibody (Kirkegaard & Perry Laboratories, USA) was then added to the slides and incubated at 37°C for 30 min before being washed three times for 5 min each in PBS. Results were obtained by viewing the slides with a Leica DM LS microscope (Leica, Germany) with an ultraviolet fluorescence illuminator.

All samples were screened at a 1:50 dilution, which is similar to both the standard human screening dilution and that used by others.[20,21] Positive samples were titrated until an end-point was obtained.

The samples used were clinical specimens (for various unrelated tests) that were otherwise to be discarded. Ethical approval was given by the

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Table 1. Seropositivity for spotted fever group rickettsiae in dog and cat serum samples tested at 1/50, 1/100 and 1/200 dilutions

	Seropositive		Seronegative		Total		% positive		P value	
	Dog	Cat	Dog	Cat	Dog	Cat	Dog	Cat	Dog	Cat
Seroreactivity at 1/50 dilution									0.44	0.25
Clinically sick	106	53	88	42	194	95	55%	56%		
Well	102	36	72	19	174	55	59%	65%		
Total	208	89	160	61	368	150	57%	59%		
Seroreactivity at 1/100 dilution									0.25	0.23
Clinically sick	56	37	138	58	194	95	29%	39%		
Well	60	27	114	28	174	55	34%	49%		
Total	116	64	252	86	368	150	32%	43%		
Seroreactivity at 1/200 dilution									0.48	0.51
Clinically sick	23	29	171	66	194	95	12%	31%		
Well	25	14	149	41	174	55	14%	25%		
Total	48	43	320	107	368	150	13%	29%		

There was no statistical relationship (P > 0.05) between clinically sick animals and seropositive animals.

Australian Rickettsial Reference Laboratory Foundation Ltd Animal Care and Ethics Committee, and for use of the cat control sera by Murdoch University Animal Ethics Committee.

Statistical analysis of serological results

Statistical analysis was undertaken to determine if there was any correlation between the animal's clinical state and rickettsial seropositivity at three different serological values (1/50, 1/100 and 1/200). P values were determined for each 2 × 2 cell to determine whether there was a statistically significant correlation between the degree of seropositivity and the animal's sickness. Chi-square analysis was used to determine P values (Table 1), with $P < 0.05$ considered statistically significant.

Results

Serology results

The results were analysed using three different serum dilution cut-off points to test for any correlation between the level of rickettsial seropositivity and sickness in the animals (Table 1). the dog total seropositivity was 57%, 32% and 13% at 1/50, 1/100 and 1/200 respectively, and for the cats it was 59%, 43% and 29%, respectively. Overall, 53% of the dogs and 63% of the cats tested were defined as sick (Table 1).

Statistical analysis

Using a level of significance of P<0.05, none of the cells showed statistical significance (Table 1). There was no correlation between dogs and cats being seropositive for SFG rickettsiae at the three endpoints and being unwell at the time of serum sampling.

Discussion

The results from this study confirm that a very high proportion (>50%) of the cats and dogs in the Launceston area of mainland Tasmania has serological evidence of exposure to SFG rickettsiae at a 1/50 serum dilution cut-off. There was, however, no statistically significant correlation between animal sickness and rickettsial seropositivity at this or the higher dilutions, which could be related to either frequent low-level exposure to a SFG rickettsia or exposure to a rickettsia that produces mild or no symptoms. Clinical symptoms may have been present earlier, but were not severe enough to warrant veterinary attention.

A previous study undertaken on the south-east coastal area of Australia found that only 12% of the dogs tested in Tasmania showed exposure to a SFG rickettsia.[19] However, our current study had a much larger sampling pool from a specific area and may more truly represent the level of rickettsial exposure of domestic animals in Tasmania.

As our serosurvey involved single sampling of animals without convalescent serum or DNA samples being obtained, we were unable to determine whether the animals were currently infected with a SFG rickettsia or had been exposed in the past.

This study shows that cats and dogs in the Launceston area are exposed (perhaps frequently) to SFG rickettsiae. The causative rickettsia and the invertebrate vector for its transmission are still unknown, although mainland Tasmania has a number of species of ticks that would be suitable vectors. *Ixodes* spp. are common and abundant in Tasmania and are known to feed on marsupials and other mammals, including dogs, cats, wombats, wallabies and rats; some are also known to bite humans (e.g. *I. tasmani*).[13,22] The vector for FISF (*Bothriocroton hydrosauri*) is known to bite humans and is found throughout mainland Tasmania.[14] We currently do not have any comparable data on either the level of SFG rickettsial antibodies or the tick bite frequency in the human population within this region.

During a 17-year period between 1973 and 1989 there were 26 reported human cases of FISF.[4,12] As Flinders Island had a population of only 1000 people at that time, this was a high incidence of rickettsial infection. Flinders Island is part of Tasmania, located 100 km off the north-east coast, and recently FISF has been reported as more widespread

through the eastern half of Australia.[5,16] To date, however, there have been only three published cases of human SFG rickettisal infection on mainland Tasmania,[5,10,11] which suggests significant under-detection.

We can only hypothesise as to the reason for the rarity of reported human cases of SFG infection in Tasmania. Firstly, the infection may be undiagnosed or misdiagnosed because of the low awareness of the disease in Australia or because it may only present as a mild infection with a low-virulent strain of rickettsia. Secondly, the vector for this rickettsia, which causes seropositivity in cats and dogs, may not bite humans and therefore the rickettsial agent is unlikely to pass to a human host. This hypothesis is less plausible, because most ticks that bite other mammals will also bite humans opportunistically.

Future studies could include a sero-epidemiological study of cats and dogs in other parts of Tasmania, and be expanded to include native animals (e.g. possums, wombats and Tasmanian devils). Blood samples from those animals could be also used to attempt isolation of the rickettsial agent(s) responsible.

Such a study could be further expanded to encompass a sero-epidemiology study of the human population of Tasmania to determine whether they have a high seroprevalence to SFG rickettsiae, as was previously demonstrated in the recognition of FISF.[12]

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