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Original Article

Prevalence of Spotted Fever Rickettsial Antibodies in Dogs and Rodents in the Philippines

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SUMMARY: Antibodies against spotted fever group rickettsiae have been detected in blood samples of dogs and rodents obtained from selected areas in the Philippines. In this serosurvey, the positive percentage rates are 8.3% (11/132) in dogs and 12.2% (6/49) in rats. Positive results were read from samples tested with *Rickettsia japonica* antigen. No positive result was obtained in blood samples of rats and house mice using *R. akari* antigen. The findings of this study are the first to confirm the detection of spotted fever group rickettsial antibodies in the Philippines.

INTRODUCTION

Spotted fever rickettsiosis has been regarded historically as an arthropod-borne disease of North and South America until Japanese researchers first isolated *Rickettsia japonica*, a new member of the spotted fever group rickettsiae (SFGR), in 1984 (1). This organism was found to be antigenitically distinct from other SFGR, and thus the disease caused by this newcomer was officially named Japanese spotted fever (2,3). Serological surveys after its initial isolation in Japan have been extended to other neighboring Asian countries including Taiwan, Indonesia, and Thailand where antibodies against SFGR among rodents in those countries have been detected (4-6). This study was therefore undertaken to document the occurrence of SFGR in the Philippines. Potential mammalian vectors including those of dogs, rats, and mice were sampled in order to detect the presence of antibodies against SFGR pathogens .

MATERIALS AND METHODS

Two-hundred-and-twenty-eight (228) blood samples from dogs and rodents (132 dogs; 47 mice, *Mus musculus*; 49 rats, *Rattus rattus* spp.) were obtained from selected areas in the Philippines using 'blood sampling filter paper' (Toyo-Roshi, Tokyo) on which approximately 100 μl of blood was allowed to absorb and dry. After drying, the blood was eluted in 0.6 ml phosphate buffered saline (PBS) (pH 7.5; Nissui Pharmaceutical Co. Ltd., Tokyo). After the blood absorbing areas of the filter-paper was cut into several parts, the sample was soaked in 600 μl PBS. This eluted serum was examined at a 16-fold dilution. *R. japonica* antigens were used in this study, and were prepared from Vero-E6 cells infected with YH strain of *R. japonica* (provided by Prof. T. Uchida of the Department of Virology, School of Medicine, Tokushima University, Tokushima) and *R. akari* (provided by Dr. Kaiho, Department of Virology, Chiba Prefectural Public Health Laboratory, Chiba) which were maintained in Vero-E6 cells. Similar procedures of indirect immunofluorescent antibody testing as described by Morita et al. (4-6) was utilized in this study. A 2-fold dilution of each serum was added to individual antigens and was incubated for 45 min at 37°C. After washing, fluorescein isothiocyanate conjugated anti-rat IgG goat serum (Organon Teknika Corp., Durham, N.C., USA) was added. Slides were incubated, washed, and examined under a fluorescent microscope. Antibody titer equal to or above 64 ($\geqq$ 1:64) was read as positive.

RESULTS

The Figure shows the map of the Philippines and the areas where blood samples were obtained. The samples were taken in selected areas of Luzon, Samar, and Mindanao islands. Outlined in the Table are the species sampled, the sampling sites, and the number and percentage positive for SFGR. Shown in the Table are the positive percentage figures of antibody titer readings equal to or above 64 ($\geqq$ 1:64) as observed in samples from dogs and rats. These positive results were obtained using *R. japonica* antigen. Antibodies against *R. akari* were not detected in the blood samples of the mice and rats examined. Of the three species of mammals examined, all of the mice blood samples yielded a negative antibody titer reading against the two antigens used. The overall positive rates were 8.3% (11/132) in dogs and 12.2% (6/49) in rats.

DISCUSSION

The data gathered in this study confirmed the presence of spotted fever group rickettsial antibodies in the blood of dogs and rats in the Philippines. Whether these species of dogs and rats serve as the mammalian reservoir of SFGR remains to be ascertained, as current proof of interisland variabilities in the positive detection of antibodies poses a question as to its natural vector or host in the country. Only antibodies against

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Table. Seroprevalence of *R. japonica* antibodies in dogs and rodents in selected islands in the Philippines

Location of samples collected	Animals			Total (%)
	Dogs	Mice	Rats	
Luzon Island[1]	11/81[4]	–[5]	–	11/81 (13.5)
Samar Island[2]	0/39	0/47	6/49	6/135 (4.4)
Mindanao Island[3]	0/12	–	–	0/12 (0.0)
Total (%)	11/132 (8.3)	0/47 (0.0)	6/49 (12.2)	17/228 (7.5)

[1,2,3] Blood samples were collected from Luzon (Manila and Laguna), from Samar (Northern, Western and Eastern), and from Mindanao (Cotabato) Islands.
[4] Numerators and denominators indicate numbers of antibody-positive samples and total number of samples examined, respectively.
[5] - means no sample was obtained.

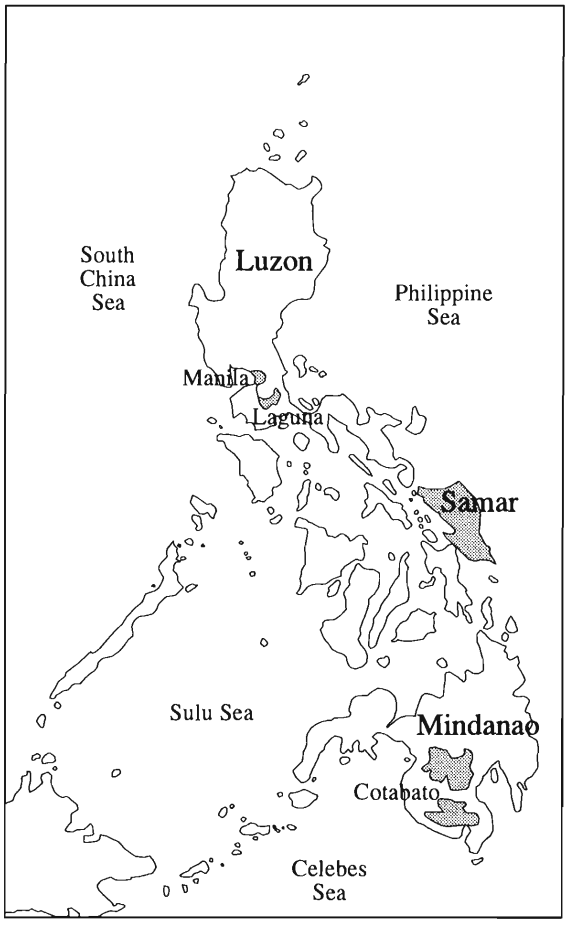

Figure. Map of the Philippines showing the sampling sites (in shaded areas).

R. japonica were detected in the present study. No positive antibody reading has been noted in blood samples of rats and mice against *R. akari* antigen. In addition, none of the rat samples showed evidence of antibody against *R. typhi* (typhus group rickettsia) antigen (data not shown).

While a confirmed antibody titer against *R. japonica* has been detected in dogs in the Luzon area, a zero prevalence was surprisingly noted in dogs in Samar and Mindanao provinces. Moreover, rats sampled in the three provinces of Samar showed positive antibody activity against *R. japonica* (SFGR) antigen. All local house mice examined yielded a negative antibody titer against the three antigens including *R. typhi*. These observed differences in geographic prevalence and in the affected mammalian species invite further in-depth studies now that the actual arthropod vector in each locale in the Philippines has been incriminated. Thus the issues presented in this survey could be complemented with further investigation.

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