Jpn. J. Infect. Dis., 56, 26-28, 2003

Short Communication

Detection of Antibodies against Spotted Fever Group *Rickettsia* (SFGR), Typhus Group *Rickettsia* (TGR), and *Coxiella burnetii* in Human Febrile Patients in the Philippines

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(Received August 8, 2002. Accepted February 7, 2003)

SUMMARY: A total of 157 sera from febrile patients in the Philippine General Hospital in Manila, Luzon, and the Northern Samar Provincial Hospital, the Philippines, were used. Serum antibodies against spotted fever group *Rickettsia* (SFGR) and typhus group *Rickettsia* (TGR) were detected by indirect immunofluorescence test. Antibody positive rates were 1.3% for SFGR (*Rickettsia japonica*) and 2.5% for TGR (*R. typhus*), respectively. Rickettsial antibodies in humans in the Philippines were found for the first time. These results underscore the need for further epidemiological study of clinical rickettsioses in the Philippines.

Rickettsioses, arthropod-borne zoonoses, are notably some of the major human febrile illnesses in the Asia-Pacific region (1,2). *Rickettsia* organisms are obligate intracellular bacteria that are antigenically divided into spotted fever group *Rickettsia* (SFGR) and typhus group *Rickettsia* (TGR) (1-3). In Asia, a novel SFGR, *Rickettsia japonica* was first isolated in Japan (4). Subsequent serosurveys in humans have revealed that SFGR and TGR are prevalent in neighboring Asian countries including Thailand, Indonesia, Malaysia, Taiwan, and China (5-7). In the Philippines, human rickettsioses are non-reportable diseases. Our recent survey showed a SFGR seroprevalence in dogs (9.2%) and rats (12.2%) in selected areas of Luzon and Samar (8). A survey of Q fever has not been performed in humans. This situation including our findings has invited testing on rickettsial infection and Q fever in humans in the Philippines.

A total of 172 human sera were used in this study. Using blood sampling filter paper (Toyo-Roshi, Tokyo), approximately 100 μl of each 152 whole blood sample were allowed to absorb and dry. After the blood-absorbing area was cut into several pieces which were soaked in 600 μl of PBS containing 0.1% sodium azide in a tube. The tube was left overnight at 4°C in order to extract antibody components. This solution was regarded as a 16-fold diluent of the sera, and stored at 4°C prior to use. Twenty and 117 whole blood samples were obtained from febrile patients in the Philippine General Hospital (PGH) in Manila, Luzon, and in the Northern Samar Provincial Hospital (NSPH), respectively. Fifteen whole blood samples were taken from afebrile volunteers living in the same area as the patients in Samar. All of the whole blood was obtained between January and August 2001. In addition, 20 stored sera of febrile patients, those the PGH had collected between 1992-1998, were absorbed onto the filter paper. Antibody components were eluted as the same manner when the whole blood was treated. These samples were regarded as a sevenfold diluent of sera. Detailed data of individual patients and volunteers were not presented due to concerns regarding their privacy. Only age and sex data were recorded. *R. japonica* YH strain was prepared and maintained on Vero E6 cells (provided by Prof. Uchida of the Virology School of Medicine, Tokushima University, Tokushima). *R. typhi* Wilmington strain was prepared and maintained on BSC-40 (provided by Dr. Kaiho, Chiba Prefecture Public Health Laboratory, Chiba). *Coxiella burnetii* Nine Mile II (ATCC VR-616) was prepared and maintained in BGM cells. After incubation at 35°C for 7 to 9 days, each infected cells was trypsinized and collected. After the centrifugation of the cell, the pellets were resuspended in proper volume of PBS. Ten microliters of the suspension were dispensed onto each well of 12-well assay glass slides. The slides which contained each of the three strains were used as antigens. An indirect immunofluorescence test was performed as described by Morita et al (9,10). Antibody titers equal to or above 1:64 were read as positive (6,10,11).

Table 1 shows the positive rates and antibody titer readings of the sera against *R. japonica*, *R. typhi*, and *C. burnetii*. The overall seropositive rate of the patients for SFGR is 1.3% (2/157) and 2.5% (4/157) for TGR. Seropositive patients for SFGR were found in both Luzon and Samar. This survey revealed that a seropositive patient was already present in 1992 in Luzon. An aspect of the seroprevalence of TGR was similar to that of SFGR. None of the patients yielded positive to *C. burnetii*. All the sera from volunteers were negative to the three antigens used.

This study is the first to demonstrate that the presence of rickettsial antibodies (SFGR, 1.3% and TGR, 2.5%) in human febrile patients in the Philippines. It is likely that SFGR and TGR have been infecting humans in Luzon and Samar, the Philippines. These findings have complemented the serosurvey that had demonstrated 9.2% of the dogs and 12.2% of the rats examined in Luzon and Samar had SFGR anti-

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Table 1. Seropositive rates to SFGR, TGR, and *C. burnetii* in humans, and age, sex and antibody titer of the seropositive human in the Philippines

Hospital (District)	Condition	Numbers tested	Percentages and numbers of the seropositive human <Age, sex and antibody titer of the human>		
			SFGR (%)	TGR (%)	*C. burnetii*
PGH[1)] (Luzon)	Febrile	40	2.5 (1/40) <male, 1:64[3)]>	2.5 (1/40) <male, 1:64[3)]>	0
NSPH[2)] (Samar)	Febrile	117	0.9 (1/117) <7 year-female, 1:128>	2.6 (3/117) <19 year-male, 1:128> <32 year-female, 1:64> <49 year-female, 1:128>	0
Subtotal of febrile patients		157	1.3 (2/157)	2.5 (4/157)	0
Samar	Afebrile volunteers	15	0	0	0

[1)]: Philippine General Hospital in Manila
[2)]: Northern Samar Provincial Hospital
[3)]: Age was not recorded. Sera from these two patients were obtained in July, 1992.

bodies (8). Comparing other Asian countries including Thailand, Malaysia, Taiwan, and Japan (5-7), seropositive rates were low in the Philippines. It could be a reason of the absence of a reported clinical rickettsiosis in the Philippines. A lack of awareness of rickettsioses might have led to it being overlooked as a human infection, since this study showed that the sera obtained in 1992 were antibody- positive for SFGR and TGR. In addition, the extensive prevalence of scrub typhus in the Philippines may include the presence of rickettsioses. Although the numbers of afebrile volunteers, who lived the same area as the patients, examined were very few, no specific antibody was found in them. So, the fevers presented by the patients may be caused by SFGR and/or TGR infection. In this study, it was confirmed that none of them was Q fever. However, rickettsioses may mimic other exanthemantous febrile illnesses such as dengue fever (12). The lack of diagnostic tools may have missed actual encounters with the disease. It should be noted that despite the rarity of seropositive humans, rickettsiosis may have been present in the Philippines. Clinicians are therefore advised to consider rickettsiosis in their differential diagnoses in the event of either usual or unusual cases of infectious febrile, exanthemantous ailments.

ACKNOWLEDGMENTS

Special thanks to Dr. Ignacio Pacho, Provincial Health Officer of the Northern Samar Provincial Hospital and to Dr. Myrna Mendoza, Head of Infectious Disease Section of the Philippine General Hospital for their generous approval in collecting human blood and sera samples. To the doctors and medical technologist staff of the Northern Samar Provincial Hospital; to Ms. Concepcion Ang and to Dr. Raul Destura of the Infectious Disease Section, Philippine General Hospital for the case selection and assistance in blood and sera sampling.

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