Seroepidemiology of Scrub Typhus and Murine Typhus in the Philippines*

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SUMMARY

There is little information on the prevalence and distribution of scrub and murine typhus in the Philippines. In biomedical surveys conducted in several areas sera collected were tested for antibodies to both *Rickettsia tsutsugamushi* and *Rickettsia mooseri* by the indirect flourescent antibody test (IFAT); titers equal to or greater than 1:40 were considered positive. The prevalence of antibody titers for scrub typhus was as follows: Sorsogon 6%, Capiz 16%, Palawan 7% and Cebu 8%. Only one person each in Pangasinan and Misamis Oriental was positive. Antibodies associated with murine typhus were found in all areas tested; Misamis Oriental 23%, Capiz 15% Palawan 13%, Sorsogon 9%, and Pangasinan 9%. Nearly 30,000 sera were tested by the: Bureau of Research and Laboratories from 1950 to 1980 by the Well-Felix reaction and titers of 1:80 and. above found in 0.5% and 8.8%, respectively, for OXK and OX19. Both tsutsugamushi disease and epidemic typhus are occuring in the Philippines but are apparently unrecognized. Most cases are probably being treated as a fever of unknown origin. *[Phil J Microbiol Infect Dis 1981; 10(1):25-34]*

Key Words: R. tsutsugamushi, R. mooseri, typhus fever, Weil-Felix test

Rickettsiae, until recently, were thought to be related to viruses because of their small size and the requirementof intracellular growth, but today are considered, true bacteria since they possess typical bacterial characteristics. A number of the rickettsia species infect man and cause diseases such as epidemic typhus, murine typhus, scrub typhus, Rocky Mountain spotted fever, boutonneuse fever, African tick typhus, North Asian tick-borne rickettsiosis, Queensland tick typhus, rickettsialpox, trench fever and Q fever. Except for Q fever, all are transmitted by arthropods and when introduced into man, characteristically cause fever, headache, malaise and a skin rash. Although scrub typhus and murine typhus have been reported in the Philippines, little is known about the prevalence and distribution of these rickettsiosis.

During biomedical surveys conducted throughout the archipelago, we tested sera from several populations for antibodies to scrub typhus and murine typhus and have identified some areas endemic for these diseases. In addition, data are also available from routine serological examinations done by the Bureau of Research and Laboratories.

This paper will review past information on scrub and murine typhus in the Philippines and present a summary of more recent findings.

Scrub Typhus

Background Review

Scrub typhus also known as tsutsugamushi disease, chigger-borne typhus, mite-borne typhus, or Japanese river fever is caused by *Rickettsia tsutsugamushi* and is transmitted by the larval stage or chigger or trombiculid mites of the genus Leptotrombidium. The organism is unique in that is transmitted transovarially in mites from one generation to the next (transtadial)

and is not acquired from the rodent host upon which the chigger feeds. The chigger is the only stage of the acarine that feeds but it does not suck blood. When firmly attached in the skin of the vertebrate host, the chigger injects a digestive fluid that lyses cells and the resulting fluid is utilized as food. The digestive fluid causes an itch and the reaction, which occurs leads to a necrotic ulcer that becomes covered with a black crust; the primary lesion or eschar. Mites and the rodent hosts may be found in a variety of habitats such as in areas of scrub or secondary jungle vegetation, mountain slopes with kogan grass or along coralline coastal areas.

Scrub typhus has been known to occur in Asia for several hundred years. Chinese writing refers to red "sna lice" or mites associated with illness characterized by fever and a pustule at the site of injury (Ashburn and Craig, 1908). In Japan a similar illness, "tsutsuga," was known for many years and linked to mites or "mushi" leading to the name tsutsugamushi disease (Philips, 1964).

The geographic range of this chiggerborne rickettsiosis extends from the southeastern tip of Siberia, Korea, Japan, Taiwan, China, Vietnam, Laos, Kampuchea, Burma, Thailand, Malaysia, Indonesia, Papua New Guinea, Australia, Bangladesh and India. During World War II, the disease was a problem to Japanese, Australian, British and American troops, especially in New Guinea, Burma and the Philippines. Prior to World War II, there were few reports of tsutsugamushi disease in the Philippines. Ashburn and Craig (1908), probably observed two cases in American soldiers from a Samar Army Camp; during the liberation this area of Samar was also found to be highly endemic (Philips, 1946). In a later report, De Roda (1937) tested sera of 500 patients hospitalized in Manila for antibodies to scrub typhus by the Well-Felix reactions using proteus strains OX19, OX2 and OXK and found high titers in 47 persons. The disease was encountered by American troops during the invasion of Leyte and later in Samar, Mindoro, Luzon, Negros and Mindanao. Nearly 400 cases with 12 deaths were recorded in the Philippines while in the South Pacific and the China, Burma, India areas, over 15,000 cases and 600 deaths were attributed to infection with *R. tsutsugamushi.*

NAMRU-2 has had an interest in scrub typhus for many years and carried out extensive studies on Taiwan and some of the offshore islands (Cooper et al, 1964, Gale et al, 1974, Kundin et al, 1974, Fang et al, 1974,), Van Peenen et a1, 1976, Bourgeois et al, 1977, Olson et al, 1977, Coolbaugh et al, 1978, Olson et al, 1979). In 1969-1970, six cases of scrub typhus were experienced by U.S. Air Force personnel attending the Pacific Air Force Jungle Survival School located at Clark Air Force Base in the Philippines (Reisen et al, 1973). NAMRU-2 was asked by the Air Force to assist in epidemiological studies in the area and sent a team to help examine rats for chiggers and to attempt isolation of *R. tsutsugamushi* from these animals. The organism was subsequently isolated from *Rattus mindanensis*, but not from two vector species of mites, *Leptotrombidium akamushi* and *L. deliensis*, recovered from the rats. A few years later, NAMRU-2 carried out additional studies in the same area at the Clark Air Force Base. Blood was collected from a Negrito population living there and the sera tested for antibodies to three strains of *R. tsutsugamushi* (Karp, Kate and Gilliam) by the indirect flourescent antibody test (IFAT). Sera from 32 males and 22 females, 4 to 57 years of age, were examined and 21 (39%), 14 males, 7 females in all age groups were found positive (Joseph, unpublished data). Sera from rats trapped in the area were also tested for antibodies by IFAT and *R. tsutsugamushi* was recovered from the spleens of rats that were IFA positive (Van Peenen, et al 1977).

More recently, to determine the prevalence of scrub typhus in the general population, we have included the testing of sera collected in biomedical surveys for antibodies to Karp, Kate and Guilliam strains of *R. tsutsugamushi.* Antigens slides were prepared by placing small amounts of diluted infected chick yolk sac onto microscope slides. The antigens were supplied by the U.S. Naval Medical Research Institute in Bethesda, Maryland. The slides were air dried, fixed in acetone and frozen (-20°C) until used. The IFAT used was according to the method of Bozeman and Elisberg (1963). The sera were screened at dilution of 1:40 and those examples that failed to give specific fluorescence were considered negative (Bourgeois et al 1977).

Results of Serologic Survey

Sera collected from volunteer population groups in the areas of Mangaldan, Pangasinan, Sorsogon, Sorsogon; Pontevedra, Capiz; Toledo, Cebu; El Salvador, Misamis Oriental and the Iwahig Penal Colony, Palawan were tested for the rickettsial antibodies (Figure 1). The prevalence of positive antibodies titers in Pangasinan and Misamis Oriental was very low (only one person from each area was found to be seropositive), while in Sorsogon, Capiz, Palawan and Cebu, the prevalences were 6%, 16%, 7% and 8%, respectively (Table 1). The seropositivity rates between males and females were not significant even for Palawan where only 79 females were examined compared to 1,928 males. Significant differences, according to age were noted for Sorsogon ($p < 01$) and Capiz ($p < .05$) where the positivity rates increased with age. The lowest rates were in those below 20 years of age. In Cebu, however, the highest rate was in those between 10 and 19 years of age and although there is variation between young and old, the difference was only slightly significant ($p < .05$). In Palawan, there was no difference by age but this data may be biased since not all age groups were equally represented.

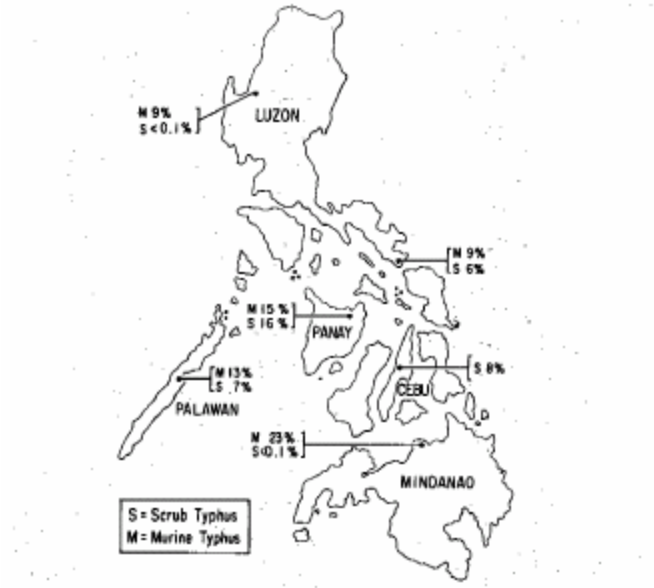

Figure 1. Sera collected from volunteer population groups in the Philippines.

Table 1. Prevalence* of Antibodies to Scrub typhus in Some Areas of the Philippines by Age and Sex

Area** Province	Number Tested	Age						Sex		
		0-9	10-19	20-29	30-39	40-49	50+	Male	Female	Average
Sorsogon	1744	1%	5%	6%	9%	6%	9%	6%	6%	6%
Capiz	1366	6	12	19	19	16	16	15	17	16
Palawan	2007	0	6	8	7	7	8	7	6	7
Cebu	959	7	15	8	6	7	7	9	8	8

*Percentage to nearest whole number
**Pangasinan - one 10-year old female
**Misamis Oriental one 67-year old male

The reason for these differences are not known nor do we have an explanation for differences between areas except that environmental and ecologic differences between positive

and negative areas was evident. For example, Mangaldan, Pangasinan would be considered an urban area and, therefore, the population examined probably was not readily exposed to infection. E1 Salvador, Misamis Oriental was coastal, but the environment did not appear to be the type habitat associated with scrub typhus. The endemic areas of Sorsogon, Capiz, Palawan and Cebu, on the other hand, were rural with much of the populations living in areas that would be considered suitable habitats for sylvan rodents and trombiculid mites.

Antibodies to all three strains of *R. tsutsugamushi*, Karp, Guilliam and Kato used in IFAT, were detected in all positive population groups. Although more individuals and antibodies tested positive to the Guilliam strain, a small number demonstrated antibodies singly to the other strains or to two or sometimes all three strains. In a study of monkeys from the Philippines, Kitaoka (1972) also found compliment fixing antibodies more frequently to the Guilliam strain than to the other two strains.

Murine Typhus

Background Review

Murine typhus also called shop typhus, urban typhus, endemic typhus, rat typhus or flea-borne typhus is caused by *R. mooseri* and is transmitted from rodents to human by the rat flea *Xenospsylla cheopis* and the rat louse. *Polyplax spinulosa*. The organism is maintained in nature through a rat-flea-rat or rat-louse-rat cycle. The arthropod acquires the infection from rodent blood and the rickettsia is excreted in the arthropod feces while feeding. The organisms are subsequently rubbed into the skin by scratching the bite. Murine typhus is a disease of rats that occasionally occurs in man. It is usually a mild disease with fever and rash and the mortality rate is low.

The disease has been reported from all parts of the world but in the Philippines, it has only been reported from Mindanao (Foster, 1915) and Manila (Steel, et al 1973). In studies by NAMRU-2 in other parts of Asia, serologic evidence of the disease has been found in Vietnam (Berman, 1973), Taiwan (Kundin, et al 1974) and Indonesia (Van Peenen, et al 1977).

Results of Serologic Survey

The methods used to detect antibodies to R. mooseri were similar to those Used for scrub typhus. The sera were screened by IFAT and a titer of 1:40 was considered significant. In our serologic surveys in the Philippines, evidence of murine typhus was found in all areas where sera were tested (Figure 1). The highest prevalence for IFA antibodies was found in sera of persons from Misamis Oriental (23%) followed by Capiz (15%), Palawan (13%), Sorsogon (9%) and Pangasinan (9%). Males and females were equally infected with evidence of infections increasing with age in all areas except in Palawan where there was no significant difference by age (Table 2). The data from Palawan may be biased, since not all groups were equally represented.

Murine typhus is known from both rural and urban areas where there is an abundance of rodents. Wild rodent populations are high in all parts of significant number of persons does not seem unusual. It is also apparent that infections are continuously occuring in the population since antibody levels were detected with increasing age.

Weil-Felix Reaction

From 1950 through 1980, the Bureau of Research and Laboratories tested sera from patients with fever or for antibodies to proteus strain OXK and OX19. A total of 29,847 sera from patients at the Veterans Memorial Hospital, the Philippine Army Station Hospital, Philippine

Navy Hospital and V. Luna General Hospital were tested and titers of 1:80 greater were found in 137 (0.5%) against OXK and 2,617 (8.8%) against OX19 (Table 3).

Table 2. Prevalence* of Antibodies to Murine typhus in Some Areas of the Philippines by Age and Sex

		Age						Sex		
Area** Province	Number Tested	0-9	10-19	20-29	30-39	40-49	50+	Male	Female	Average
Sorsogon	1744	2%	6%	12%	8%	14%	14%	8%	10%	9%
Capiz	1366	4	10	12	22	16	17	13	16	15
Palawan	2007	3	11	12	15	15	14	13	13	13
Pangasinan	1486	3	6	16	10	14	15	7	10	9
Misamis Oriental	1297	6	13	20	23	34	33	23	22	23

*Percentage to nearest whole number.

Table 3. Sera Tested by the Bureau of Research and Laboratory by the Weil-Felix Reaction for Antibodies to Proteus Strains OXK and OX19, 1950 through 1980*

	Titer						
	1/20	1/40	1/801	1/160	1/320	1/640	1/1280
Strains	Number reactive						
OXK	156	110	81	38	8	10	0
OX19	880	505	1941	173	171	323	9

29,847 sera tested

Comment

It is questionable whether scrub typhus and murine typhus are health problems in the general Philippine population. The diseases are not is reported with any regularity since infections may be inapparent and the symptoms not always characteristic. Fever, headache, rash, myalgia lymphadenopathy and hepatosplenomegaly could be attributed to a number infectious diseases, but the presence eschar, as in scrub typhus, however, is considered pathognomonic. Laboratory diagnosis of the disease is not available and most physicians consider the symptoms as a fever of unknown (FUO) origin, and elect to initiate treatment since chloramphenicol, tetracycline or doxycycline (Brown, et al, 1978) are highly effective.

Laboratory diagnosis can be made by isolating the organisms from the blood by mouse inoculations. The rickettsiae can be found in peritoneal exudates or in spleen cells. Positive Weil-Felix reactions and the complement fixation test are of value when other tests are not available. Paired sera should be tested and fourfold rises in antibody titers is considered significant. The indirect flourescent antibody tests using Karp, Guilliam and Kato strains is sensitive and specific with titers of equal to or greater than 1:40 considered positive.

In flea-borne typhus, the symptoms are usually mild with fever and with a rash that appears, and fades quickly. The symptoms are often mild enough for the illness to go unrecognized and untreated. Most individuals that acquire these rickettsiosis and develop symptoms are ones who usually have not had previous exposures to the agents. Transmigrants moving into a new area and working new fields often become exposed and suffer ill effects. Both diseases are of military importance and in recent years most reports of infections have been with military populations in Taiwan (Gale et al, 1974, Fang et al, 1974, Bourgeois et al 1977) Vietnam, (Berman et al, 1973, Miller et al, 1974) and the Philippines (Reisen et al, 1973). Most positive OXK and OX19 agglutination titers detected in persons with fever by the Bureau of Research and Laboratories in the past few years were in sera obtained from Philippine Military Personnel.

Serologic evidence of scrub and murine typhus in the surveys reported here, certainly, suggest rickettsial activities. Little disease has been reported, however, and it is likely that those with antibody titers may have been infected with strains of the rickettsiae that are immunogenic yet not pathogenic. It is also conceivable that infections experienced at an early age confer immunity but when "immunological virgins, such as young military personnel, enter an endemic area, clinical disease may occur.

Murine typhus is apparently much more widespread than scrub typhus. This is undoubtedly due to the distribution of the rodent hosts and the vectors. Both commensal and compestral rodents are involved in the ecology of murine typhus and fleas usually accompany their rat host.

Scrub typhus often occurs in zoonotic islands with the vector chiggers associated with rodents in sylvan or less disturbed areas. Furthermore, while *R. mooseri* is transmitted from fleas to rodents to fleas, *R. tsutsugamushi* is only transmitted by the chiggers to rodents and not from rodents to chiggers. *R. tsutsugamushi* is perpetuated in the chigger populations only by transovarial transmission.

These data are only preliminary in nature and hopefully the findings presented here will encourage further research into these two rickettsiosis in the Philippines. There is a paucity of information on prevalence, distribution and pathogenesis of the organisms as well as the strains of the rickettsiae that may be present in the country. Details on the vectors and vertebrate hosts are also meager.

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REFERENCES

Ashburn PM, Craig CF. A computative study of tsustsugamushi disease and spotted or tick fever of Montana. Phil J Sci 1908; 3:1-23.

Berman SJ, Kundin WD. Scrub typhus in South Vietnam: A study of 87 cases. Ann Intern Med 1973; 79:26-30.

Berman SJ, Irving GS, Kundin WD, Gunning JJ, Watten RH. Epidemiology of acute fevers of unknown origin in South Vietnam: Effect of laboratory support upon clinical diagnosis. Am J Trop Med Hyg 1973; 22:795-801.

Bourgeois AL, Olson JG, Ho CM, Fang RCY, Van Peenen PFD. Epidemiology and serological study of scrub typhus among Chinese military in the Pescadores Islands of Taiwan. Trans R Soc Trop Med Hyg 1977; 71:338-342.

Bozeman FM, Elisberg BL. Serological diagnosis of scrub typhus by indirect immunofluorescence. Proc Soc Exp Biol Med 1963; 112:568-573.

Brown GW, Saunders JP, Singh S, Huxsoll DL, Shirai A. Single dose doxycycline therapy for scrub typhus. Trans R Soc Trop Med Hyg 1978; 72: 412-416.

Coolbaugh JC, Ho CM, Fang RCY. Diagnosis of scrub typhus by culture of blood monocytes. In: Current Concepts in the Diagnosis and Treatment of Parasitic and Other Tropical Diseases in Southeast Asia, 1978. pp. 102-106.

Cooper WC, Lien JC, Hsu HC, Chen WF. Scrub typhus in the Pescadotes Islands. An epidemiology and clinical study. Am J Trop Med Hyg 1964; 13:388-838.

De Roda AF. Typhus fever in the Philippines I. Weil-Felix reaction of 500 febrile cases. J Phil Med Assoc 1937; 17:147-158.

Fang RCY, Lin WP, Chao PS, Kuo NT, Chin CM. Clinical observations of scrub typhus on Penghu (The Pescadores Islands). Trop Geogr Med 1975; 27:143-150.

Foster GB. Endemic typhus in the Philippines. Observations based on a study of twenty-three cases occuring among Filipinos at Camp Keithley, Mindanao, P.I. with results of animal inoculations. Arch Intern Med 1915; 16:363-381.

Gale JL, Irving GS, Wang HC, Lien JC, Chen WF, Cross JH. Scrub typhus in Eastern Taiwan, 1970. Am J Trop Med Hyg 1974; 23:679-684.

Kitaoka M. Serological survey of scrub typhus on monkeys imported from Southeast Pacific area. J Hyg Epidemiol Microbiol Immunol 1972; 18:257-260.

Kundin WD, Irving GS, Raulston GS. A team approach to a disease survey on an aboriginal island (Orchid Island, Taiwan) V. Arboviral, rickettsial and brucella antibodies on Orchid Island. Chinese J Microbiol 1974; 7:42-46.

Miller MB, Bratton JL, Hunt J, Blankenship R, Lohr DC, Reynolds R. Murine typhus. Vietnam Mil Med 1974; 139:184-186.

Olson JG, Bourgeois AL. *Rickettsia tsutsugsmushi* and scrub typhus incidence among Chinese military personnel in the Pescadores Islands. Am J Epidemiol 1977; 106:172-175.

Philip CB, Woodward TE, Sullivan RR. Tsutsugamushi disease (Scrub or mite-borne typhus) in the Philippines Islands during American re-occupation in 1944-45. Am J Trop Med Hyg 1946; 26:229-242.

Philip CB, Woodward JE. Tsutsugamushi disease (scrub or mite-borne typhus) in the Philippine Islands during American re-occupation in 1944-45. II Observation on trombiculid mites. J Parasitol 1946; 32:502-513.

Phillips CB. Scrub typhus and scrub itch. In: Preventive medicine in World War II. Vol. VII. Communicable diseases arthropod-borne diseases other than malaria, 1964. pp. 275-348.

Reisen WK, Pollard TJ, Tardy WJ. Some epidemiological considerations of scrub typhus (*Rickettsia tsutsugamushi*) a natural focus on the Zambales mountains, Luzon, Republic of the Philippines. Am J Trop Med Hyg 1973; 22:503-508.

Steel JH, Arambulo PV, Becan GW. The epidemiology of zoonoses in the Philippines. Arch Environ Hlth 1973; 26 :330-339.

Van Peenen, P.F.D., Lein, J.C., Santana, F.J. and See, R. 1976. Correlation of chigger abundance with temperature at a hype-rendemic focus of scrub typhus. J. Para-sitol. 62: 653-654.

Van Peenen PFD, Ho CM, Bourgeois AL. Indirect immunofluorescence antibodies in natural and acquired *Rickettsia tsutsugamushi* infections of Philippine rodents. Infect Immunol 1977; 15:813-816.

Van Peenen PFD, Koesharjono C, See R., Bourgeois AL, Irving GS. Antibodies against murine typhus in sera from Indonesia. Trans R Soc Trop Med Hyg 1977; 71:297-299.