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Survey of rickettsial antibodies at two local sites and review of rickettsiosis in Papua New Guinea

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SUMMARY

The status of rickettsial infection in Papua New Guinea (PNG) is unknown although several reports of typhus-like illnesses infecting predominantly white settlers and the Allied troops during World War 2 were published between 1930 and 1945. We performed a serological survey for evidence of rickettsial infection by measuring rickettsia-specific antibody levels in the blood of 191 non-randomly selected Papua New Guineans living in Port Moresby (n=93) and in the highland villages of Samberigi (n=98). Antibodies were measured by a microimmunofluorescence method using a panel of rickettsial antigens of a number of species and strains. In addition, we have reviewed the current status of rickettsiosis in PNG. Overall, we were able to demonstrate significant titres of antibodies to two groups of rickettsiae, the scrub typhus group (STG) and the spotted fever group (SFG). All positive individuals (7/191) were residents of Port Moresby. None from the highlands showed any significant levels of antibodies to rickettsiae. The strains detected within each group were Gilliam and Karp for STG and, for SFG, *Rickettsia honei, R. conorii, R. sibirica, R. rickettsii, R. australis and R. akari.* No significant antibody titres to typhus group infection were detected in either Port Moresby or highland volunteers. These findings were not surprising given previous reports of typhus-like illnesses and favourable environmental characteristics for rickettsiae in some parts of PNG. Until a definite status of this disease is known, we suggest that rickettsial infection be included as a differential diagnosis for patients presenting with acute febrile illness in Port Moresby and surrounding areas.

Introduction

Rickettsial infection (rickettsiosis) is caused by obligate intracellular bacteria called rickettsiae. Molecular biology techniques have recently separated rickettsial diseases into two main groups (1). The first is the scrub typhus group (STG), which contains the scrub typhus rickettsia, originally named *Rickettsia tsutsugamushi*, but now reclassified into a new genus as *Orientia tsutsugamushi* (2). The new genus name reflects the geographic distribution of scrub typhus in the Orient, including East Asia, Southeast Asia and the Northern Territory of Australia. The second group represents the genus *Rickettsia* and is itself divided into two groups, the spotted fever group (SFG) and the typhus group (TG).

The SFG includes many 'species' which are closely related genetically and antigenically. They should not qualify for separate 'species' status but historically distinct names have been used because the diseases occur in defined geographic locations. Examples of some of the

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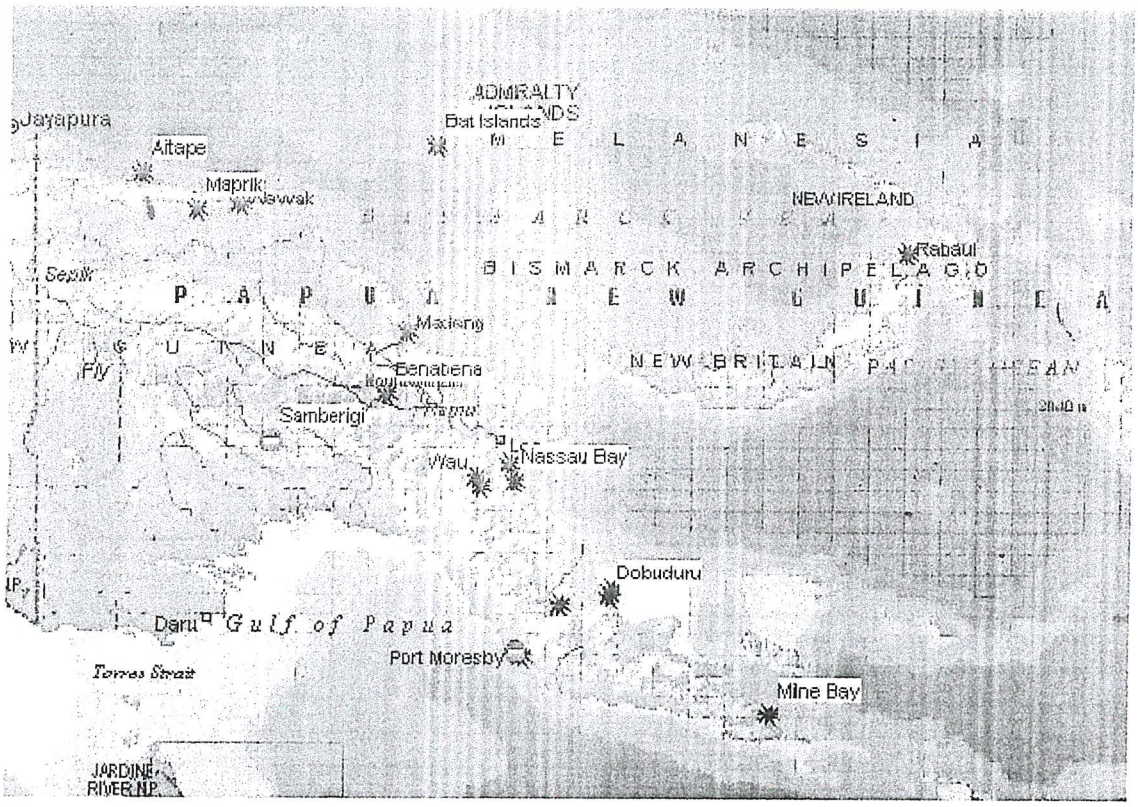


Figure 1. Map of Papua New Guinea showing regions where typhus-like illnesses have been reported between 1930 and 1945 (marked by an asterisk) and the two locations for the current survey (white bars).

notable SFG species and infections they cause are: *R. rickettsii* – Rocky Mountain spotted fever; *R. australis* – Queensland tick typhus; *R. conorii* – Mediterranean spotted fever; *R. honei* – Flinders Island spotted fever; *R. japonica* – Japanese spotted fever; *R. africae* – African tick bite fever; and *R. akari* – rickettsialpox. The typhus group includes *R. prowazekii*, the louse-borne rickettsia causing classical rickettsial infection or epidemic typhus and *R. typhi* causing murine or endemic typhus.

Rickettsial disease has a worldwide distribution and various arthropods serve as ectoparasite vectors for transmission of rickettsiae. In Papua New Guinea (PNG), the last report of rickettsial-like infections was published 58 years ago (3). Since then no further cases of rickettsial infection have been reported in the literature. We performed a serological survey of anti-rickettsial antibodies in two groups of non-randomly selected local people living in highland villages and Port Moresby and review the current status of rickettsiosis in PNG.

Materials and Methods

Sample collection

Serum samples were collected from healthy volunteers, 93 permanent residents in the coastal city of Port Moresby and 98 residents in the rural highland villages of Masiki, Pawale, Popouateke, Sao and Pawabi in the Samberigi area in October 1997. Samberigi is a rural area situated at an altitude of 400-600 metres above sea level and located in the middle of thick tropical rainforest in the Southern Highlands (Figure 1). Port Moresby is

TABLE 1

RICKETTSIAL ANTIGENS USED IN THE MICROIMMUNOFLUORESCENCE TEST

Spotted fever group microdot

R. rickettsii	(Rocky Mountain spotted fever)
R. conorii	(Mediterranean spotted fever)
R. sibirica	(North Asian tick typhus)
R. australis	(Queensland tick typhus)
R. honei	(Flinders Island spotted fever)
R. akari	(rickettsialpox)

Typhus group microdot

R. prowazekii	(epidemic typhus)
R. typhi	(endemic typhus)

Scrub typhus group microdot

O. tsutsugamushi	(strain Karp*)
O. tsutsugamushi	(strain Kato)
O. tsutsugamushi	(strain Gilliam)
O. tsutsugamushi	(strain Litchfield)

*originally isolated from PNG (4)

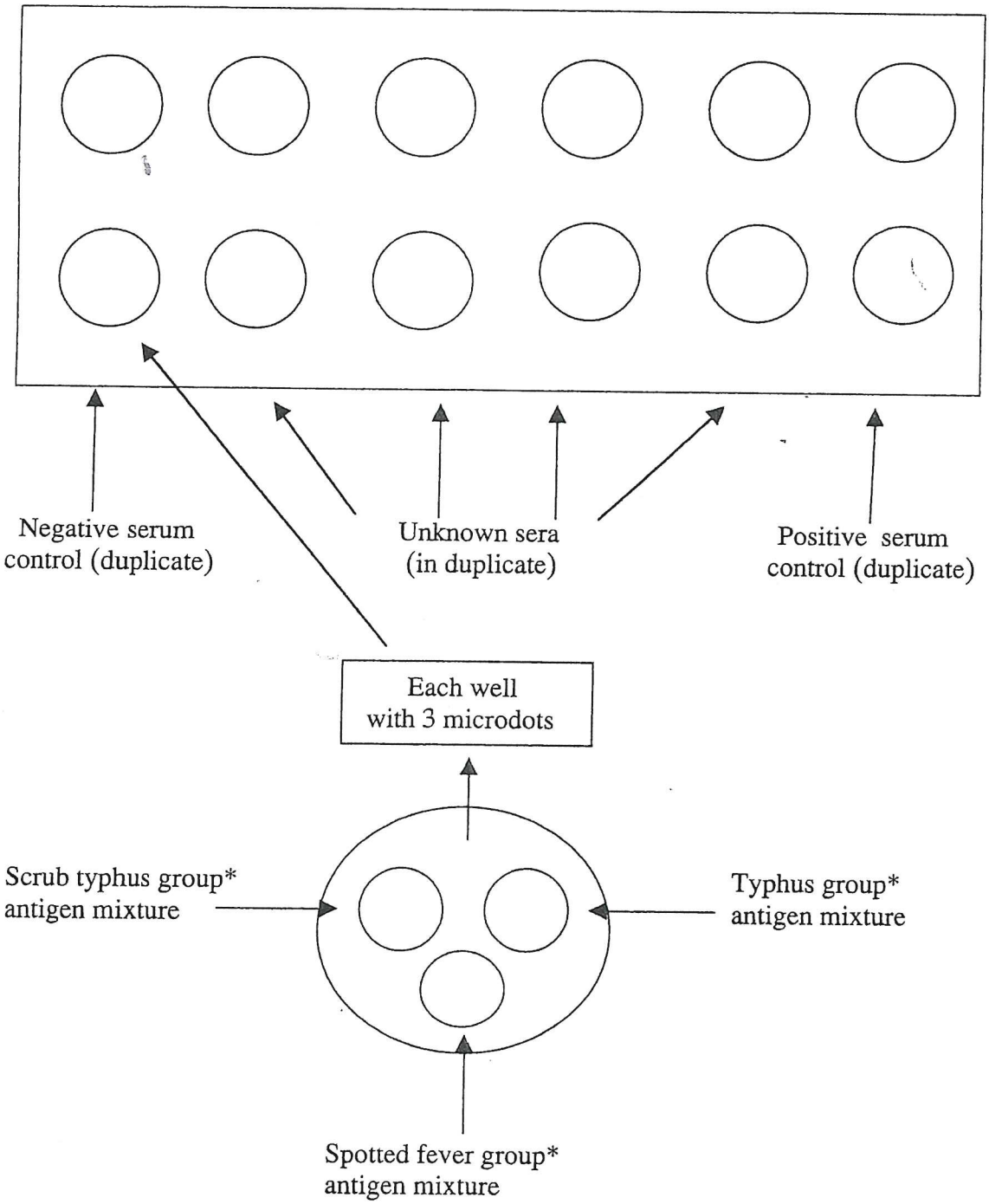
on the coast surrounded by sparse shrubs and tall long grasses known locally as kunai grass.

Method of measurement of rickettsial antibodies

We tested for rickettsial antibodies by microimmunofluorescence (IF) using an inhouse test involving a panel of rickettsial antigens (Table 1).

Each rickettsial strain was obtained as a pure culture from the American Type Culture Collection or a recognized rickettsial research laboratory. Antigen was prepared by growing each rickettsial type in Vero cells in RPMI-1640 medium with 10% fetal calf serum (heat-activated) at 35°C (with 5% carbon dioxide in the case of *R. prowazekii* and *R. typhi*). Cultures were examined weekly for appearance of cytopathic effect (CPE) on the monolayer. When a 3+ CPE was present, an aliquot of cells was taken from the flask and stained with Giminez (5) (rickettsiae stain pink and the Vero cells stain green) and immunofluorescence, using human serum from known cases of rickettsial disease. The culture was harvested when most of the stained Vero cells contained a large number of intracellular rickettsiae. Sometimes this would take up to 2 months of incubation, but usually only 3-4 weeks. The rickettsia/Vero cell suspension was rendered nonviable by heating at 56°C for 1 hour. The rickettsia-infected Vero cells were concentrated 10-fold by centrifugation (1500 g x 30 minutes) and resuspended in Hanks balanced salt solution (containing 0.1% sodium azide as preservative) to 1/10 of the original culture volume. This preparation was lightly sonicated (10-20 seconds) to break up large clumps of infected Vero cells so that when the suspension was deposited on to glass slides an even spread of Vero cells was obtained. Some extracellular rickettsiae were present in most preparations but the majority were within the Vero cells. Each rickettsial preparation was titrated before use to determine an optimum dilution for spotting on to glass slides for the microimmunofluorescence test (usually between 1:2 and 1:16).

After the individual rickettsial strain



*See Table 1

Figure 2. Configuration of antigen and serum on slides for rickettsial microimmunofluorescence test.

suspensions had been diluted appropriately, three mixtures were prepared consisting of (i) 6 spotted fever group strains, (ii) both typhus group strains and (iii) 4 scrub typhus strains (Table 1). These mixtures were used for preparing the wells on the screening slides (Figure 2). Three microdots were placed on each well of the slide, allowed to air-dry and fixed by flooding the slide with acetone. Slides were then stored at –20°C, in a sealed plastic bag. Reactivity was retained for several months at –20°C. Serum was diluted 1/128 in phosphate-buffered saline with 0.5% skim milk powder (PBSM, pH 7.4) and added to appropriate wells on the slide (in duplicate). Four specimens were tested per slide and each slide contained a known positive serum (1/128 dilution) and a negative control. The serum arrangements are shown in Figure 2.

After a reaction of 30 minutes at 35°C in a humid atmosphere, the slide was washed for 30 minutes in PBSM, dried at 35°C and anti-human conjugate added. The conjugate was fluorescein-labelled anti-human IgM, IgG and IgA (combined) so that it did not react with uninfected Vero cells at the working dilution. After reaction with the conjugate for 30 minutes, the slides were washed again for 30 minutes in PBS, dried at 35°C and covered with glycerol mounting fluid. The positive control serum (a mixed human serum positive for all three rickettsial groups) and negative control serum (normal human serum) were examined first to ensure that the test had worked. Fluorescing intracellular rickettsiae in all 3 microdrops are present in the positive control wells and absent in the negative control wells. Provided the controls were satisfactory the unknown sera were read in duplicate.

Generally, a titre of 64 is considered to be the lowest positive titre in rickettsial IF (6) but, to reduce the influence of cross-reacting antibodies, a titre of 256 (2 doubling dilutions higher) was the lowest titre accepted as positive in this study. Sera that were positive at 1/128 dilution for any of the rickettsial antigen groups were then retested at 1/256 dilution and, if still positive, at doubling dilutions up to 1/1024. A titre was thus obtained from each serum. All positive results were confirmed by retesting.

Results

Of the 191 sera examined, 7 (4%) were positive for antibodies to two of the three rickettsial groups (Table 1): 3 were positive to *O. tsutsugamushi* (scrub typhus) and 4 to the spotted fever group of rickettsiae (Table 2). No significant antibody levels (≥256) were demonstrated for the typhus group. Interestingly, all the positive sera were from the residents of Port Moresby and none of the sera from the highland villages had any positive antibodies to the rickettsial species tested.

In the 4 individuals positive to the spotted fever group, 2 had antibodies to all the six strains tested (*R. honei, R. conorii, R. sibirica, R. australis, R. rickettsii and R. akari*) and the other 2 had antibodies to the first three strains only (Table 2). In the 3 individuals who were positive for scrub typhus, all had antibody levels to *Orientia tsutsugamushi* strains Karp and Gilliam only.

Discussion

In this limited survey, we report the first serological evidence (by IF) of specific antibody levels to rickettsial infections in PNG. Positive rickettsial antibodies were present in sera of individuals living in Port Moresby but not in those from the highland villages of Samberigi. Antibodies were positive for STG and SFG but not for TG rickettsioses. The existence of positive antibodies in these individuals suggests previous exposure to rickettsial agents, either due to post-disease or asymptomatic seroconversion. This is not surprising given the past reports of typhus-like illnesses in PNG 60 years ago (3,7-10) and the similarity in climate and vegetation to parts of northern Australia and Southeast Asia where scrub typhus is endemic. So far, isolated cases of scrub typhus infection have been reported in several regions of PNG including Central, Oro Bay, Milne Bay, Morobe, Madang, East and West Sepik, Manus, East New Britain and Eastern Highlands (Figure 1). All except Eastern

TABLE 2

SERA FROM PNG THAT REACTED WITH MORE THAN ONE STRAIN OF THE SCRUB TYPHUS OR SPOTTED FEVER RICKETTSIAL GROUP

(a) Scrub typhus group *O. tsutsugamushi* strains	**Serum number and IF titre**		
	A	**B**	**C**
Gilliam	256	256	256
Karp	256	256	256
Kato	-	-	-
Litchfield	-	-	-

(b) Spotted fever group rickettsial strains	**Serum number and IF titre**			
	D	**E**	**F**	**G**
R. honei	256	512	256	256
R. conorii	512	1024	256	256
R. sibirica	256	256	256	256
R. australis	256	512	-	-
R. rickettsii	256	256	-	-
R. akari	256	256	-	-

IF = microimmunofluorescence

Highlands are coastal provinces whose climatic and environmental conditions are different from that of the highland provinces. It is unclear whether the cases reported in the Benabena area of Eastern Highlands were acquired locally or in neighbouring Madang and Morobe provinces. Considering the active movements of soldiers during the war, the cases could have been acquired in the bordering coastal villages of Madang or Morobe.

This is the first report on positive identification of rickettsia-specific antibodies in PNG. Although no definite cases of rickettsioses in PNG have been published recently, the difficulty of diagnosis where malaria is prevalent would suggest that the illness could be under-diagnosed. In PNG, Sinclair (11) was the first to report a typhus-like illness in a female patient in Rabaul during May of 1930. Subsequently, medical officers employed at the Wau-Bulolo goldfield mines published several cases of rickettsiosis affecting expatriates between 1935 and 1940 (4,12-14). Even though confusions as to the type of rickettsiosis still remain, the disease's resemblance to Japanese river fever or tsutsugamushi disease was clearly recognized.

During World War 2, rickettsial infection caused debilitating outbreaks among the Australian and American troops in combat operations against the Japanese in many parts of PNG (3,7-10). The risk of infection among the soldiers was higher during the first week of residence in an area cleared for camping than 2 to 4 weeks after settlement. This was thought to be due to a decrease in the number of mite vectors and rat hosts as a result of prolonged occupation and disturbance of habitat (3,10). Rickettsiosis often gained priority over malaria for evacuation (15). The case fatality rate was reported as high as 20% (3,9), since this was the period when no

rickettsia-specific antibiotics were available. The infection crippled troops operating in parts of Morobe (Wau, Bulolo, Nadzab, Finschhafen, Salamaua), Milne Bay, Oro (Dobadura, Oro Bay, Buna, Kokoda) and Central (Port Moresby, Sogeri) provinces (3,8,9).

As a result of this endemic, the Typhus Commission of the United States of America funded extensive investigations into the epidemiology, clinical features and aetiology of rickettsioses in 1943 (3,8,10). It was these studies that first confirmed the existence of scrub typhus in PNG and further defined the causative organism including its mite vector, habitats and animal hosts.

The studies revealed that the trombiculid mites of genus *Leptotrombidium (L. fletcheri* and *L. deliensis)* were the vectors responsible for carrying rickettsial organisms in PNG. This confirmed the role of trombiculid mites in transmission of the rickettsial organism in PNG already suspected previously (4,12-15). In Australia, *Leptotrombidium deliensis* species transmits scrub typhus infection (15-17). In PNG, this mite species is known as 'bush mokkas' and this name was familiar to Australian soldiers though the Americans often referred to them as 'mites' or 'itch mites' (3). Often, the terms 'scrub itch' and 'mite bite' are also applied to dermatitis resulting from attacks of leptotrombiculid mites in various regions of PNG. In other countries, it has acquired various common names peculiar to the regions concerned, such as 'chiggers' and 'red-bugs' in the United States, 'harvest mites' in Europe, 'akamushi' and 'kedani' in Japan, and 'tiree itch' and 'scrub itch' in Australia (3).

Only scrub typhus has been fully investigated and reported in PNG, while very little is known about other rickettsial infections. However, previous reports were based on clinical diagnosis and the nonspecific (Weil-Felix) antibody test. Serology using specific microbial antigens (for example, microimmunofluorescence with rickettsial antigens) as used in our survey is much more specific (and usually more sensitive) than serology using heterogeneous antigens (for example, the Weil-Felix test for rickettsial diseases using *Proteus* OXK, OX2 and OX19 strains). However, there is always a risk of cross-reaction.

In this study, the use of a cut-off titre of 256, at two doubling dilutions above the titre of 64 normally accepted as the cut-off between negative and positive sera, reduced this risk of detecting cross-reacting antibody (6). As low-titre, genuinely positive sera will be excluded from the positive subset this will enhance the specificity but decrease the sensitivity of the results. This is not a concern in the current study as the non-random method of serum selection invalidates any attempt to provide data on the seroprevalence of rickettsial infections. All that can be said is that antibodies were detected in individuals living in Port Moresby but not in those residing in the highland villages of Samberigi. It is important to note that serology is not a substitute for the isolation of rickettsial agents. However, until such time as the agents are obtained from patients living in the area or isolated from animals (mammals and invertebrate ectoparasites) caught in the region, the present results are the best evidence, and the first specific antibody evidence, that spotted fever and scrub typhus infections do occur in Port Moresby.

Repeated infection by different strains or cross-reacting antibodies among strains of a group could explain why each individual with a positive serum had multiple strain-specific antibodies. The antigen-antibody test employed to separate the 3 major groups of rickettsiae is more specific than that used to differentiate the various strains within a group. In this survey, individuals who tested positive to STG had antibodies to Karp and Gilliam strains of the four *O. tsutsugamushi* strains tested (Table 2). The Karp strain, first isolated and named after an American soldier infected while living in PNG (3,10), causes scrub typhus and has been used extensively worldwide for laboratory experiments (18). It is also the common strain associated with scrub typhus infection in northern Queensland (17).

Scrub typhus is an endemic mite-borne infec-

tion in parts of Southeast Asia including Japan, Korea, Malaysia and Thailand. In these countries, scrub typhus is also referred to by various names such as Japanese fever, Malay scrub fever and mite fever. The infection is also common in the Solomon Islands and northern Vanuatu (19). Both scrub typhus and spotted fever infections occur in the Northern Territory, the Kimberley region of Western Australia and along he eastern coast of north Queensland in Australia where the environmental and climatic conditions are similar to Port Moresby (17,20). In Australia, the flea-borne murine or endemic typhus (*R. typhi*), which was first described in South Australia in 1922, causes sporadic outbreaks and small epidemics (15).

Rickettsial organisms are about the size of bacteria and are usually seen microscopically as coccobacilli. Each of the rickettsiae pathogenic for humans is capable of multiplying in one or more species of arthropod as well as in humans and animals. The majority of the rickettsiae are maintained in nature by a cycle that involves an arthropod vector and an animal reservoir and the infection of humans is unimportant in the cycle. The organism is only introduced through the bite of the infected arthropod vector. It requires special tissue culture and cannot be grown on routine laboratory culture media.

The larval stage of the mite is the only parasitic form and can feed on a large variety of vertebral hosts. The larva is microscopic and is barely visible with the naked eye. It measures about 0.15 mm by 0.3 mm and is difficult to observe in its natural habitat. The adults are described as measuring 1 to 2 mm in length and are frequently reddish in colour, although some are pale and nearly white. The infective rickettsia is inoculated into the human body during the bite or feeding of an infected larva. The organism can be isolated from the site of the bite and, before the availability of specific antibiotics, it was usual for the lesion to be excised for treatment (13).

Worldwide, mites are vectors for scrub typhus infection and ticks transmit spotted fever infection, although some SFG rickettsiae are not tick-transmitted. These vectors can be seen in nature throughout PNG. Therefore, the risk of acquiring rickettsia-related infections in these regions is high. The exposure of individuals with positive rickettsial antibodies to rickettsial organisms is unlikely to have occurred in locations other than Port Moresby, since no individual in the present study had ever been to other provinces.

The rat has been identified as the intermediate animal host in PNG (3,8,10) although suggestions that other animals such as birds, bandicoots and wild pigs are involved have yet to be confirmed. Kunai grassland is reported as the major environmental feature associated with rickettsial infection in PNG (3,10). Others have described sago swamps (12), abandoned gardens, banana and coconut groves with neglected undergrowth of grass and shrubbery, sparse, coarse growths of native vegetation overlaying coralline ridges, or breaks in the interior of certain islands as typical environmental characteristics. On the contrary, thick tropical rainforest has not been implicated as an associated risk feature of rickettsial infection in PNG. In Port Moresby and surrounding areas, the vegetation is typically kunai grass compared to the Samberigi area, where tropical rainforest is dominant. The average daily temperature and humidity in Port Moresby is also higher than in the highland villages of Samberigi. The differences in the antibody levels and therefore the prevalence of rickettsiosis between Port Moresby and the highland villages are expected given these differences in environmental characteristics. Similarly, the prevalence of rickettsioses will be different in different parts of PNG, because the environment also varies from one region to another. Unlike scrub typhus, the role of the SFG in causing rickettsial disease, including its vector, animal host, human epidemiology and environmental characteristics, requires further studies.

The clinical features of mite-borne scrub typhus in PNG have been fully described in the early reports (3,4,7,9,12-14,21). The signs and symptoms of all rickettsioses are similar but scrub typhus tends to cause a more severe form of disease than the other rickettsioses. In 1937, Von

Derborch (4) published the first comprehensive description of its clinical features including photographs of the typical lesion of rickettsiosis known as the eschar. He reported that many of his patients presented to him for assistance only after quinine failed to relieve their symptoms of what they initially thought was malaria infection. So far, all cases reported have been predominantly in expatriates with no confirmed case of rickettsial infection in a Papua New Guinean. The typical clinical features of rickettsiosis include progressively increasing temperature, chills, general malaise, headache and anorexia with or without eschar. An eschar at the site of the bite of a mite and local lymphadenitis is followed by a macular skin rash which starts in the trunk and spreads subsequently to the arms, legs and face (3,4,9,14). An eschar is described as a single lesion usually discovered at the ankles, shin, thigh, groin, external genitalia, waistline or axilla. It consists of a central, tough, black scab, 4 to 8 mm in diameter, surrounded by a dull red areola, 2 to 6 mm wide, which in moist areas (scrotum, groin and axilla) appears as a punched-out ulcer because the scab is often lacking in the lesion. Eschar and lymphadenitis were observed in up to 80% of infected military personnel in PNG (3,9). Among Southeast Asian patients, the eschar of scrub typhus infection is rarely seen (22) and may be missed in dark-skinned individuals unless searched for specifically. Multisystem involvement such as atypical pneumonia, hepatitis, septic shock, cardiac rhythm abnormalities and meningeal irritation is less common. Blake et al. (3) observed signs of atypical pneumonia including chest X-ray changes in up to 65% of their patients. In Taiwan, where the infection is endemic, more than 75% of patients present with features of acute hepatitis, and scrub typhus is one of the commonest causes of fever of unknown origin (23). The post-mortem findings of military personnel dying from rickettsial infections in PNG (9) indicated that the underlying pathology was generalized vasculitis and perivasculitis.

The signs and symptoms of rickettsiosis, however, are nonspecific and are not sufficiently distinctive by themselves to be of any diagnostic value. Many of the features resemble malaria, typhoid fever or viral infections that are frequently encountered in PNG. Coexisting malaria and scrub typhus infection has been documented in PNG (9). However, the finding of an eschar with typical rash is an important clue in the diagnosis of rickettsiosis. The diagnosis of typhoid fever by medical officers in PNG in the past was made only after rickettsiosis had been excluded by failure to locate an eschar and by negative rickettsial antibody during the convalescent period (10). Since the early reports, no new patient with rickettsiosis has been reported. Although the reason for this lack of diagnosis is unknown, scrub typhus is often an under-diagnosed disease (4). Since the clinical presentation of a patient with scrub typhus infection is not different from that of malaria and typhoid fever infections, rickettsiosis can easily be overlooked.

The laboratory diagnosis of rickettsiosis is also complex, requiring expertise. The assay is difficult to set up in an ordinary laboratory and in many regions the assay is still performed in reference laboratories. Recently, new tests including ELISA-based kits have been developed and evaluated (24).

Doxycycline is the drug of choice for the treatment of rickettsiosis although chloramphenicol is also effective. The mortality associated with delayed treatment is high. In PNG, chloramphenicol is widely available for the treatment of typhoid fever and is commonly used to treat febrile infections including fever of unknown origin.

The correct diagnosis and treatment of rickettsiosis will continue to be a challenge for clinicians in PNG where malaria and typhoid infections are so common. Our findings thus highlight the need for extra vigilance in investigating patients with acute febrile illnesses in order to avoid missing rickettsial infections. Doxycycline and chloramphenicol are widely available and rickettsiosis can be treated when correctly diagnosed. All clinicians should consider rickettsiosis as a differential diagnosis of typhoid fever or febrile illness in Port Moresby

or when treatment of malaria fails.

Conclusions

There is serological evidence for the existence of STG and SFG (but absence of TG) rickettsioses in Port Moresby. There is no evidence of rickettsioses in the Samberigi area of Southern Highlands Province. Until further research on rickettsial diseases in PNG is conducted, clinicians should be aware that the disease does occur in PNG and should be considered where appropriate as part of the differential diagnosis of an acute febrile illness.

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