

## Case Report: Spotted Fever Group Rickettsioses and Murine Typhus in a Malaysian Teaching Hospital

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**Abstract.** Limited information is available on the etiological agents of rickettsioses in southeast Asia. Herein, we report the molecular investigation of rickettsioses in four patients attending a teaching hospital in Malaysia. DNA of *Rickettsia* sp. RF2125, *Rickettsia typhi*, and a rickettsia closely related to *Rickettsia raoultii* was detected in the blood samples of the patients. Spotted fever group rickettsioses and murine typhus should be considered in the diagnosis of patients with nonspecific febrile illness in this region.

### INTRODUCTION

Spotted fever group (SFG) rickettsioses and murine typhus have been regarded as important vector-borne diseases worldwide.<sup>1,2</sup> Various species of SFG rickettsiae, including *Rickettsia sibirica*, *Rickettsia heilongjiangensis*, *Rickettsia japonica*, *Rickettsia conorii*, *Rickettsia honei*, *Rickettsia tamurae*, and *Rickettsia raoultii* have been implicated in human infections in the Asia-Pacific region.<sup>3</sup> *Rickettsia felis* is an emerging flea-borne pathogen which has been reported in a wide variety of arthropods from more than 20 countries on five continents.<sup>4</sup> *Rickettsia typhi*, the causative agent of murine typhus, is maintained in a biological cycle involving rats (*Rattus* spp.) as reservoirs, and the oriental rat flea (*Xenopsylla cheopis*) as the main arthropod vector.<sup>2</sup> The clinical manifestations of patients with SFG rickettsioses and murine typhus are nonspecific and are difficult to be differentiated from other febrile diseases such as malaria, dengue, leptospirosis etc.<sup>5,6</sup> Despite the seroepidemiologic data, study on the clinical aspects of rickettsioses in Malaysia is limited. This study reports the molecular investigation of rickettsioses in four adult patients attending a Malaysian teaching hospital. The demographic, clinical, and laboratory features of these patients are reviewed in this study. This study was approved by the University Malaya Medical Center Ethics Committee (MEC reference no. 830.6 and no. 944.20).

### CASES

A 15-year-old boy (patient A) was admitted to University Malaya Medical Center in March 2013 with fever, myalgia, arthralgia, mild headache, and loss of appetite for the past 1 week. He had conjunctival suffusion and the presence of petechiae was noted on his limbs. His white blood cell (WBC) count, hemoglobin, urea, creatinine, and bilirubin values were within normal limits (Table 1). A low platelet count, elevated hepatic transaminases and hypoalbuminemia were noted. Examination of peripheral blood smears for malaria and serological tests for dengue and hepatitis C were

negative. His fever persisted despite treatment with ceftriaxone. A rickettsiosis was suspected. He was then started on doxycycline and the temperature subsided within 24 hours. On a follow-up visit after a week, he was well and his platelet count and liver enzymes were normal. Retrospective analysis of rickettsial serology for acute serum sample showed that the patient was positive to two rickettsial antigens available for testing (*R. typhi* [IgM = 1:256, IgG ≥ 1:2048]; *R. rickettsii* [IgM < 1:64, IgG = 1:256]) using an indirect immunofluorescence assay (Focus diagnostic, Cypress, CA). A 4-fold increase in the IgG titer (1:1024) against *R. rickettsii* was noted for the convalescent sample (collected after 12 days) of the patient. High IgM and IgG titers (≥ 2048) against *R. typhi* were detected in the convalescent samples.

Molecular investigation revealed the amplification of rickettsial citrate synthase gene (*gltA*)<sup>7</sup> and the 135-kDa outer membrane protein gene (*ompB*)<sup>8</sup> from the patient's acute blood samples. Sequence analysis of the *gltA* (GenBank accession no.: KU255716, 399/402 nucleotide [nt], 99.3%) and *ompB* (GenBank accession no.: KU255717, 772/772 nt, 100.0%) gene fragments show the closest match with *Rickettsia* sp. RF2125 (GenBank accession no.: AF516333 and JX183538), and next with those of *R. felis* type strain URRWXCa2 (GenBank accession no.: CP000053, 394/402 nt (98.0%) for *gltA* and 764/824 nt (92.7%) for *ompB*).

A 72-year-old lady (patient B), living on a farm at the east coast of Malaysia, was admitted to our hospital in June 2010 with febrile illness and underlying interstitial pulmonary fibrosis. During admission, she presented with the complaints of backache, anorexia, diarrhea, abdominal pain, reduced exercise tolerance, and productive cough. She developed atrial fibrillation and was hypotensive. Chest examination revealed bilateral lung crepitation. Her examination was otherwise normal and there was no rash or eschar. The patient was thrombocytopenic with raised plasma levels of urea, creatinine, and aspartate aminotransferase (Table 1). Hypoalbuminemia was also noted. The thorax computed tomography scan showed extensive patchy consolidation, bilateral pleural effusion, and mediastinal lymphadenopathy, suggesting severe pneumonia. Septic workup including blood and stool cultures did not reveal any bacterial growth. Examination of peripheral blood smear for malaria parasites and serological tests for *Leptospira*, *Mycoplasma*, and *Legionella* were all negative. She was treated for septic shock and was started on amoxicillin and clavulanic acid (Co-amoxiclav [IV], KALP, Bangalore, India) and azithromycin. However,

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TABLE 1  
The demographic, hematology, and blood chemistry profiles of patients investigated in this study

Demographic data	Patient A	Patient B	Patient C	Patient D	Reference range
	15 Male Student	72 Female Farmer	33 Female	42 Male	
Age (years)	15	72	33	42	12-15
Gender	Male	Female	Female	Male	4-10
Occupation	Student	Farmer			150-400
Blood profiles					2.5-6.4
Hemoglobin (g/L)	12.6	13.3			0.7-1.3
White blood cells ( $10^9/L$ )	4.8	7.6	1.9	3.5	3-17
Platelets ( $10^9/L$ )	78	50	115	126	35-50
Urea (mmol/L)	4.8	13.8			50-139
Creatinine (mg/dL)	1.2	2.2			12-78
Bilirubin ( $\mu\text{mol/L}$ )	11	15			15-37
Albumin level (g/L)	32	28			
Alkaline phosphatase (U/L)	129	122			
Alanine aminotransferase (U/L)	111	67			
Aspartate aminotransferase (U/L)	141	126			
Tick bite history	No	No	No	No	
Rashes/eschars	Yes (limb)	No	No	No	
Molecular detection of <i>Rickettsia</i>	<i>Rickettsia</i> sp. RF2125	<i>Rickettsia typhi</i>	<i>Rickettsia</i> closely related to <i>R. raoultii</i>	<i>Rickettsia</i> closely related to <i>R. raoultii</i>	

En dashes (–) indicate that data are not available.

due to poor response and worsening symptoms, her antibiotics were changed to piperacillin–tazobactam and doxycycline on day 4 of admission. Her clinical condition improved gradually and the fever subsided. Amplification of *gltA-1* (GenBank accession no.: KU255718, 726/726 nt) and *ompB* (GenBank accession no.: KU255719, 767/767 nt) gene fragments from the patient's blood DNA sample demonstrated 100% sequence similarity to those of *R. typhi* strain Wilmington (GenBank accession no.: AE017197), thus confirming the diagnosis of murine typhus in this patient.

Patient C (33 years old, female) presented to our hospital in May 2010 with 5 days of fever, headache, and presumed upper respiratory tract infection. She had nausea but no jaundice or rash. Blood investigations showed leukopenia and thrombocytopenia. She was advised on hydration and discharged without further treatment when her WBC count reverted back to normal range. Patient D (42 years old, male) presented to our outpatient clinic in May 2010 with a history of fever for 7 days. He had arthralgia, myalgia, rhinorrhoea, and cough with yellowish sputum but no hemoptysis or dyspnea. No rash or eschar was noted. Physical examination and blood investigations were unremarkable except for a slightly lower than normal WBC and platelet counts. He was given antihistamines and antipyretics, and discharged the following day since his symptoms had resolved and his WBC and platelet counts had returned to normal limits. Rickettsial *gltA-1* and *ompB* gene fragments were amplified from the serum DNA sample of both patients. BLAST analyses of the *gltA-1* amplicons from both patients showed the closest match (GenBank accession no.: KU255720 and KU255721, 711/722 nt, 98.5%) to a rickettsia closely related to *R. raoultii* (GenBank accession no.: JQ697956) detected from *Haemaphysalis hystricis* ticks in Japan.<sup>3</sup> The amplified *ompB* gene fragments showed the highest similarity (GenBank accession no.: KU255722 and KU255723, 679/691 nt, 98.3%) to *R. raoultii* strain Khabarovsk (GenBank accession no. DQ365798). On the basis of the sequence analysis, the *Rickettsia* detected from these two patients is tentatively identified as a rickettsia closely related to *R. raoultii*. Both patients recovered without any specific medication for rickettsioses. There was no clinical follow-up after the patients were discharged. Analysis for rickettsial serology for patients B–D was not performed due to the unavailability of patients' sera.

## DISCUSSION

The impact of rickettsioses as the leading causes of treatable fever of unknown origin has been documented in south-east Asian countries.<sup>9</sup> However, as most studies were based on serological diagnosis,<sup>10–12</sup> information is lacking on the genetics and biology of pathogenic rickettsiae in this region. Although rickettsioses have been known to occur in Malaysia for many years, data is scarce on *Rickettsia* spp. associated with human infections.<sup>13</sup> Since the first report of *R. felis* infection amongst rural residents of the central Thai–Myanmar border,<sup>10</sup> the rickettsiae have been identified from febrile patients in several Asian countries, including Korea,<sup>14</sup> Thailand,<sup>15</sup> and Laos.<sup>16</sup> This study reports for the first time the detection of *Rickettsia* sp. RF2125, a *R. felis*-like organism (RFLO) in the blood sample of a Malaysian febrile patient by molecular method. However, specific

identification of rickettsiae is not possible merely by IFA alone due to serological cross-reactivity, especially when high-endpoint titers were noted for more than one rickettsial antigen. The diagnosis of spotted fever was confirmed based on the observation of a 4-fold rise in the IgG antibody titers against *R. rickettsii*. The high IgG titers against *R. rickettsii* and *R. typhi* in the convalescent sample suggest cross-reactivity between both rickettsial antigens. In addition, mixed infections due to spotted fever and typhus group rickettsiae could also complicate the interpretation of serological results, as reported in some studies in southeast Asia.<sup>17,18</sup> Recent zoonotic surveillance studies showed the detection of *Rickettsia* sp. RF2125 in cat fleas and cynomolgus monkeys in Malaysia.<sup>13,19</sup> It will be interesting to investigate whether an enzootic cycle of RFLO involving fleas and monkeys exists here.

The rickettsia detected from patients C and D is closely related to a newly reported SFG rickettsia associated with scalp eschars and neck lymphadenopathy following tick bites in patients from France,<sup>20</sup> Slovakia,<sup>21</sup> and Poland.<sup>22</sup> The clinical entity of the rickettsial infection, first named as tick-borne lymphadenopathy in a female patient in France in 1997,<sup>23</sup> can be due to *R. slovacica* or *R. raoultii*.<sup>20</sup> *Rickettsia raoultii* infection has been reported in two individuals from China who had painful rashes around the site of tick bites, but no lymphadenopathy.<sup>24</sup> The presence of *R. raoultii* in *Dermacentor*, *Haemaphysalis*, and *Amblyomma* ticks has been reported in China, Japan, Thailand, and Malaysia.<sup>3,25</sup> In this study, the infections caused by *R. raoultii* were considered mild as both patients recovered without any specific medication for rickettsioses. The typical features such as eschar and neck lymphadenopathy were not noted.

Although the rash is a typical feature of rickettsioses, only one patient (A) presented with petechial rash. Rash can be difficult to see especially in patients with darker complexion.<sup>26</sup> Rash (mostly maculopapular) has been reported in *R. felis* infection; however, a lack of cutaneous rash amongst Senegalese patients has been reported.<sup>27</sup> The rash associated with murine typhus is variable (nonpruritic, macular, or maculopapular) and has been reported in 20–80% of infected patients.<sup>5</sup> For *R. raoultii* infection, localized rashes around sites of tick bites has been described in two (100%) patients in China,<sup>24</sup> but only one (20%) of the five patients in France diagnosed with *R. raoultii* infection developed rash.<sup>20</sup>

The most severe presentation noted in this study was pneumonia and septic shock in the patient diagnosed with murine typhus. However, as the patient also had underlying interstitial pulmonary fibrosis and precipitated by the existing lung pathology, it is difficult to conclude that her respiratory problems were solely related to murine typhus. Severe pulmonary manifestations of murine typhus are rare,<sup>28</sup> but has been reported from travelers returning from Thailand<sup>29</sup> and Indonesia.<sup>30</sup> It has been reported that elderly patients have more severe clinical manifestations, as evidenced by a higher complication rate and longer duration of fever.<sup>31</sup>

In conclusion, the molecular investigations in this study suggest *Rickettsia* sp. RF2125, *R. typhi*, and a rickettsia closely related to *R. raoultii* as the etiological agents for rickettsioses in four Malaysian patients. The finding of human cases and surveillance of possible vectors and animal reser-

voirs will improve our knowledge on the transmission of the newly identified rickettsiae.

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