Murine Typhus and Febrile Illness, Nepal

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Murine typhus was diagnosed by PCR in 50 (7%) of 756 adults with febrile illness seeking treatment at Patan Hospital in Kathmandu, Nepal. Of patients with murine typhus, 64% were women, 86% were residents of Kathmandu, and 90% were unwell during the winter. No characteristics clearly distinguished typhus patients from those with blood culture–positive enteric fever.

In 2001, we found Salmonella enterica serotype Typhi and S. enterica serotype Paratyphi A to be the most common causes of bloodstream infections among adults with febrile illness who sought treatment at Patan Hospital in Kathmandu, Nepal (1). Another important finding was the relatively high percentage of patients (11%) who had immunoglobulin (Ig) M antibodies against Rickettsia typhi in peripheral blood. Because most testing was performed on unpaired acute-phase sera, and a high percentage of seropositive results were found in a group of healthy study participants, we were uncertain whether these participants had acute murine typhus or more distant past infection.

Recent studies have shown the value of PCR for diagnosing scrub typhus (2–4), and a real-time assay for R. typhi has recently been validated (5). In our study, we tested archived blood samples from our febrile adult cohort (1) with this R. typhi PCR to better characterize the incidence of murine typhus and to determine whether clinical features could help distinguish murine typhus from enteric (typhoid) fever.

The Study

We studied consecutive adult patients with fever (axillary temperature >38°C; >13 years of age) who sought treatment at Patan Hospital from January 15 through March 15, 2001 (winter) and July 2 through August 10, 2001 (summer), as detailed elsewhere (1). The study was approved by the Nepal Health Research Council and the Institutional Review Board of the Centers for Disease Control and Prevention.

Blood from each patient was injected into blood culture bottles and serum samples were tested for R. typhi IgM antibodies (INDX Multi-Test Dip-S-Ticks SDLST; Integrated Diagnostics, Inc., Baltimore, MD, USA). In addition, whole blood samples (stored at ~80°C) were tested by real-time PCR for R. typhi at the Naval Medical Research Center, Silver Spring, MD (NMRC) and at Canterbury Health Laboratories, Christchurch, New Zealand (CHL). Details of the assay have been described elsewhere (5). We extracted DNA from 200 μL of whole blood and used primers and probes specific to a portion of the outer membrane protein B (ompB) unique to R. typhi to amplify and detect the target sequence in a SmartCycler (Cepheid, Sunnyvale, CA, USA) at NMRC and in a LightCycler (Roche Diagnostics, Mannheim, Germany) thermocycler at CHL. Thermocycling parameters included an initial denaturation step (2 min at 9°C) followed by 45 cycles of denaturation (94°C for 5 s) and annealing/elongation (60°C for 30 s) steps. Positive samples were defined as those that demonstrated fluorescence above background levels. Template-free controls assayed at the same time and under the same conditions as the experimental and positive control samples consistently showed negative results.

In our study, a diagnosis of murine typhus required a positive R. typhi PCR result; a diagnosis of enteric fever required a positive blood culture for S. Typhi or S. Paratyphi. Data from patients with murine typhus were compared with those from patients with enteric fever by using the χ² test or Fisher exact test for dichotomous and ordinal variables, and 2-sided Wilcoxon rank sum test and the Student t test for continuous variables. We used multivariable logistic regression analysis to further evaluate variables associated with murine typhus. Murine typhus was the outcome variable in the final model; other variables were those associated with the outcome with p<0.1 on bivariable analysis. Data were analyzed by using STATA version 8.2 (StataCorp, College Station, TX, USA).

We enrolled 876 patients, 370 in winter and 506 in summer. In 323 (37%) patients, a putative diagnosis was established; 117 (13%) patients had positive blood cultures for S. Typhi or S. Paratyphi A.

Whole blood samples were available for testing from 756 (86%) patients. Of these patients, 85 (11%) had R. typhi IgM antibodies detected in acute-phase serum samples and 50 (7%) had positive R. typhi PCR results; 11
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(13%) of the *R. typhi*-seropositive patients were also PCR positive. Sequencing of amplicons from 5 PCR-positive patients showed 100% similarity with the reference strain of *R. typhi* (GenBank accession no. AE017197). None of the patients with positive PCR results for *R. typhi* had a positive blood culture.

The features of the 50 patients with murine typhus and the 94 patients with enteric fever are presented in the Table; all had negative *R. typhi* PCR results. Sixteen of the murine typhus patients had chest radiographs; 6 (38%) of these patients were reported as having lung infiltrates. Of the 50 patients with murine typhus, 45 were managed as outpatients and 5 were ill enough to be admitted to a hospital. No deaths occurred.

After logistic regression analysis, only 3 variables were significantly associated with murine typhus compared

| Variable                                      | Murine typhus, n = 50 | Enteric fever, n = 94 | p value |
|-----------------------------------------------|-----------------------|-----------------------|---------|
| **Demographics**                              |                       |                       |         |
| Age, y, median (range)                        | 28 (15–85)            | 22 (14–72)            | 0.0001  |
| Male gender                                   | 18 (36)               | 61 (65)               | 0.001   |
| Occupation                                    |                       |                       | <0.001  |
| Housewife                                     | 17 (41)               | 10 (12)               |         |
| Student                                       | 7 (17)                | 36 (42)               |         |
| Business person                               | 2 (5)                 | 7 (8)                 |         |
| Government employee                           | 2 (5)                 | 8 (9)                 |         |
| Other                                         | 13 (32)               | 24 (28)               |         |
| Residence                                     |                       |                       | <0.001  |
| Kathmandu                                     | 31 (86)               | 27 (32)               |         |
| Patan                                         | 0                     | 28 (33)               |         |
| Kathmandu Valley                              | 4 (11)                | 10 (12)               |         |
| Other                                         | 1 (3)                 | 20 (24)               |         |
| Winter season                                 | 45 (90)               | 27 (29)               | <0.001  |
| Admission diagnosis                           |                       |                       | <0.001  |
| Enteric fever                                 | 21 (42)               | 66 (70)               |         |
| Lower respiratory tract infection             | 13 (26)               | 3 (3)                 |         |
| Urinary tract infection                       | 6 (12)                | 3 (3)                 |         |
| Upper respiratory tract infection             | 3 (6)                 | 1 (1)                 |         |
| Other                                         | 7 (14)                | 21 (22)               |         |
| Symptoms                                      |                       |                       | <0.001  |
| Cough                                         | 33 (66)               | 30 (33)               |         |
| Shortness of breath                           | 16 (32)               | 6 (7)                 | <0.001  |
| Nausea                                        | 14 (28)               | 34 (37)               | 0.26    |
| Diarrhea                                      | 5 (10)                | 16 (18)               | 0.24    |
| Abdominal pain                                | 16 (33)               | 25 (28)               | 0.58    |
| Headache                                      | 41 (82)               | 78 (86)               | 0.56    |
| Joint pain                                    | 12 (24)               | 11 (12)               | 0.07    |
| Duration of symptoms, d, median (range)       | 5 (1–10)              | 5 (1–30)              | 0.23    |
| Examination findings                          |                       |                       |         |
| Temperature, °C, mean (SD)                    | 38.9 (0.7)            | 38.8 (0.6)            | 0.54    |
| Respiratory rate, breaths/min, mean (SD)      | 26 (9)                | 21 (5)                | 0.0002  |
| Heart rate, beats/min, mean (SD)              | 112 (17)              | 105 (15)              | 0.02    |
| Systolic blood pressure, mm Hg, mean (SD)     | 109 (17)              | 107 (11)              | 0.52    |
| Diastolic blood pressure, mm Hg, mean (SD)    | 70 (10)               | 70 (8)                | 0.88    |
| Crackles                                      | 13 (26)               | 8 (9)                 | 0.006   |
| Hepatomegaly                                  | 2 (4)                 | 9 (10)                | 0.22    |
| Splenomegaly                                  | 2 (4)                 | 12 (13)               | 0.09    |
| Rash                                          | 0                     | 1 (1)                 | 0.46    |
| Laboratory findings                           |                       |                       |         |
| Hematocrit, %, mean (SD)                      | 39 (6)                | 39 (5)                | 0.70    |
| Leukocyte count, cells × 10^9/L, median (IQR)  | 8.9 (6.2–11.1)        | 5.8 (4.8–7.6)         | <0.001  |
| Neutrophils, %, median (IQR)                  | 83 (74–87)            | 68 (60–73)            | <0.001  |
| Lymphocytes, %, median (IQR)                  | 13 (9–22)             | 27 (20–35)            | <0.001  |
| Monocytes, %, median (IQR)                    | 2.5 (1–5)             | 2 (0–4)               | 0.57    |
| Eosinophils, %, median (IQR)                  | 0.5 (0–2)             | 0 (0–2)               | 0.80    |

*Data are no. (%) unless otherwise stated. SD, standard deviation; IQR, interquartile range.
with enteric fever. These variables were age (for each increase by 1 year) (odds ratio [OR] 1.07, 95% confidence interval [CI] 1.00–1.16, p = 0.05); Kathmandu residence (OR 14.37, 95% CI 1.07–193.39, p = 0.05); and winter season (OR 28.93, 95% CI 2.47–338.93, p = 0.007).

Conclusions

We detected R. typhi DNA in blood from 7% of the febrile adult study population from urban Nepal. This finding is likely to be an underestimate of the actual extent of rickettsial disease because of the small volume of blood tested in each PCR and possible sample deterioration during transport and storage (between sample collection and testing). Although PCR has yet to be extensively evaluated for the diagnosis of murine typhus (5–8), a sizeable body of evidence supports the high sensitivity and specificity of PCR for the diagnosis of rickettsial diseases (2,4,9,10). The real-time PCR used in our study has a high analytical sensitivity and specificity for R. typhi (5), and sequencing of amplicons from our patients further supports the specificity of the assay. In addition, none of our patients with murine typhus had positive blood cultures.

The results of this and other (11,12) studies indicate that murine typhus is an important endemic infection in Nepal. Although our study did not extend throughout the full year, murine typhus was more common in winter than in summer. This finding contrasts with the summer–autumn predominance reported in other regions (13,14). We also noted a clear predominance of cases from Kathmandu and none from the Patan side of the city, despite the fact that the latter is the main catchment area for Patan Hospital. It is possible that an outbreak of murine typhus occurred in Kathmandu during the winter of 2001, and epidemiologic studies are needed to clarify whether there is a focus of murine typhus activity in Kathmandu. In Nepal, enteric fever is one of the most common causes of febrile illness (1,15). We identified no reliable clinical marker to distinguish murine typhus from enteric fever, and the classic clinical triad of rickettsial diseases (headache, fever, and rash) was not detected in any of our patients. Murine typhus should be considered as an alternative diagnosis in patients with suspected enteric fever in Nepal. This diagnosis is especially important given that first-line antimicrobial drug therapy is different for the 2 diseases.

Our study highlights the importance of rickettsial infections as a cause of febrile illness in Kathmandu. Further epidemiologic and ecologic studies are needed to better clarify the features of murine typhus in Nepal.

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Dr Zimmerman is executive director of the Nick Simons Institute, an organization working in Nepal with a mission to train and support skilled, compassionate, rural healthcare workers. He was previously the medical director of Patan Hospital, Kathmandu, Nepal.

References

1. Murdoch DR, Woods CW, Zimmerman MD, Dull PM, Belbase RH, Keenan AJ, et al. The etiology of febrile illness in adults presenting to Patan Hospital in Kathmandu, Nepal. Am J Trop Med Hyg. 2004;70:670–5.
2. Singhilalark T, Leowattana W, Looareesuwan S, Wongchotigul V, Jiang J, Richards AL, et al. Detection of Orientia tsutsugamushi in clinical samples by a quantitative real-time polymerase chain reaction. Am J Trop Med Hyg. 2005;72:640–1.
3. Saisongkhon W, Chenchittikul M, Silpapojakul K. Evaluation of nested PCR for the diagnosis of scrub typhus among patients with acute pyrexia of unknown origin. Trans R Soc Trop Med Hyg. 2004;98:360–6. DOI: 10.1016/j.trstmh.2003.10.012
4. Kim D-M, Yun NR, Yang TY, Lee JH, Yang JT, Shim S-K, et al. Usefulness of nested PCR for the diagnosis of scrub typhus in clinical practice: a prospective study. Am J Trop Med Hyg. 2006;75:542–5.
5. Henry KM, Jiang J, Rozmajzl PJ, Azad AF, Macaluso KR, Richards AL. Development of quantitative real-time PCR assays to detect Rickettsia typhi and Rickettsia felis, the causative agents of murine typhus and flea-borne spotted fever. Mol Cell Probes. 2007;21:17–23. DOI: 10.1016/j.mcp.2006.06.002
6. Zhang L-J, Li X-M, Zhang D-R, Zhang J-S, Di Y, Luan M-C, et al. Molecular epidemic survey on co-prevalence of scrub typhus and marine (sic) typhus in Yuxi city, Yunnan province of China. Chin Med J (Engl). 2007;120:1314–8.
7. Schriever ME, Sacci JB, Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. J Clin Microbiol. 1994;32:949–54.
8. Williams SG, Sacci JB, Schriever ME, Andersen EM, Fujioka KK, Sorvillo FJ, et al. Typhus and typhuslike rickettsiae associated with opossum and their fleas in Los Angeles County, California. J Clin Microbiol. 1992;30:1758–62.
9. Kim D-M, Kim HL, Park CY, Tyang TY, Lee JH, Yang JT, et al. Clinical usefulness of eschar polymerase chain reaction for the diagnosis of scrub typhus: a prospective study. Clin Infect Dis. 2006;43:1296–300. DOI: 10.1086/508464
10. Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group rickettsiae. Am J Trop Med Hyg. 2005;73:1083–5.
11. Blacksell SD, Sharma NP, Phumratanaprapin W, Jenjaroen K, Peacock SJ, White NJ, et al. Serological and blood culture investigations of Nepalese fever patients. Trans R Soc Trop Med Hyg. 2007;101:686–90. DOI: 10.1016/j.trstmh.2007.02.015
12. Brown GW, Shirai A, Gan E, Berenthal P. Antibodies to typhus in Eastern Nepal. Trans R Soc Trop Med Hyg. 1981;55:586–7. DOI: 10.1016/S0035-9203(81)80210-8
13. Bernabeu-Wittel M, Pachón J, Alarcón A, López-Cortés LF, Viciana P, Jiménez-Mejías ME, et al. Murine typhus as a common cause of fever of intermediate duration. A 17-year study in the south of Spain. Arch Intern Med. 1999;159:872–6. DOI: 10.1001/archinte.159.8.872
14. Whiteford SF, Taylor JP, Dumler JS. Clinical, laboratory, and epidemiologic features of murine typhus in 97 Texas children. Arch Pediatr Adolesc Med. 2001;155:396–400.

15. Maskey AP, Day JN, Tuan PQ, Thwaites GE, Campbell JI, Zimmerman M, et al. *Salmonella enterica* serovar Paratyphi A and *S. enterica* serovar Typhi cause indistinguishable clinical syndromes in Kathmandu, Nepal. Clin Infect Dis. 2006;42:1247–53. DOI: 10.1086/503033