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Undifferentiated Febrile Illness in Kathmandu, Nepal

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Abstract. Undifferentiated febrile illnesses (UFIs) are common in low- and middle-income countries. We prospectively investigated the causes of UFIs in 627 patients presenting to a tertiary referral hospital in Kathmandu, Nepal. Patients with microbiologically confirmed enteric fever (218 of 627; 34.8%) randomized to gatifloxacin or ofloxacin treatment were previously reported. We randomly selected 125 of 627 (20%) of these UFI patients, consisting of 96 of 409 (23%) cases with sterile blood cultures and 29 of 218 (13%) cases with enteric fever, for additional diagnostic investigations. We found serological evidence of acute murine typhus in 21 of 125 (17%) patients, with 12 of 21 (57%) patients polymerase chain reaction (PCR)-positive for Rickettsia typhi. Three UFI cases were quantitative PCR-positive for Rickettsia spp., two UFI cases were seropositive for Hantavirus, and one UFI case was seropositive for Q fever. Fever clearance time (FCT) for rickettsial infection was 44.5 hours (interquartile range = 26–66 hours), and there was no difference in FCT between ofloxacin or gatifloxacin. Murine typhus represents an important cause of predominantly urban UFIs in Nepal, and fluoroquinolones seem to be an effective empirical treatment.

Undifferentiated febrile illnesses (UFIs) are a common clinical problem in south Asia.1,2 Defined as a fever without a focus of infection on initial physical examination or in basic laboratory tests, UFIs represent a considerable burden of disease with diagnostic and therapeutic challenges. Empirical broad-spectrum antimicrobials are generally prescribed but with little evidence-based guidance on likely etiologies or potential treatment responses. Previous studies on UFIs, including those originating from our research group in Nepal,3,4,5 have been limited by a lack of molecular testing, little convalescent serological testing, and few data on treatment outcomes.

We sought to address this knowledge gap by expanding investigations to determine further causes and treatment outcomes of Nepalese patients with UFIs. Diagnostic tests were performed for scrub typhus, murine typhus, Spotted Fever Group (SFG) rickettsiosis, Q fever, leptospirosis, Hantavirus, Brucella, and dengue (Table 1). The patients described in this report were previously enrolled into a randomized, controlled trial (RCT) comparing gatifloxacin with ofloxacin for treating enteric fever in Patan Hospital, a 450-bed teaching hospital within the Kathmandu Valley, Nepal. All patients had UFIs at enrollment and came from predominantly urban regions; the methods and results from patients with subsequent blood culture-confirmed enteric fever have been reported previously.3 Briefly, patients were eligible to enter if they were > 2 years old, had an untreated UFI for > 3 days, and could be treated in the community. Each patient was randomly assigned to 7 days of treatment with either gatifloxacin (10 mg/kg per day in a single oral dose) or ofloxacin (20 mg/kg per day in two divided oral doses). All patients were managed as outpatients, with assessment of fever clearance time (FCT) and collection of acute (day 1) and convalescent (day 30) blood samples by trained community medical auxiliaries. Approval for the study was obtained from the Nepal Health Research Council and the Oxford Tropical Research Ethics Committee. The trial was registered as ISRCTN 63006568. Written informed consent was obtained from all study participants.

Between July of 2008 and August of 2011, 627 patients with UFIs were enrolled in the RCT: 311 of 627 (49.6%) patients received gatifloxacin, and 316 of 627 (50.4%) patients received ofloxacin (Figure 1). Salmonella Typhi and Salmonella Paratyphi A were cultured from the blood of 109 of 311 (35%) and 109 of 316 (34%) patients in each treatment arm, respectively. The remaining 409 of 627 (65%) patients had UFIs with negative blood cultures. Although no formal sample size calculation was carried out for this study, we randomly selected 125 of 627 (20%) UFI patients for additional diagnostic testing, consisting of 96 of 409 (23%) UFIs and 29 of 218 (13%) enteric fever patients (Salmonella Typhi, N = 17; Salmonella Paratyphi A, N = 12) (Table 1).

All data were analyzed using Stata v13 (College Station, TX). Kruskal–Wallis tests were used to compare clinical parameters between the enteric fever and rickettsial groups. FCTs were summarized by Kaplan–Meier estimates and compared between groups using a Cox regression model with only one covariate. All tests were performed using two-sided 5% significance.

In total, 21 of 125 (17%) patients were identified with acute murine typhus infection on the basis of at least a fourfold antibody titer rise from day 1 to day 30 (Figure 1); 10 of these cases were confirmed by quantitative polymerase chain reaction (PCR; ompB gene target), and 2 cases were confirmed by conventional PCR/sequencing of the 17-kDa and/or gltA genes. In total, 12 of 21 (57%) PCR-positive murine typhus cases were confirmed. Three cases with a Rickettsia spp.-positive quantitative PCR result could not be further differentiated because of limited sample specimen. However, these specimens have a high probability of being murine typhus cases because of their positive R. typhi serology. The possibility remains that SFG Rickettsia could be responsible for these cases. None of the patients with rickettsial infections were coinfected with Salmonella Typhi or Salmonella Paratyphi A.
Additionally, two cases were serologically positive for Hanta-
virus, and one case was serologically positive for Q fever.

Although the study design allowed for limited comparison, the clinical presentations and basic laboratory values, such as complete blood count, liver function test, and creatinine, of 21 rickettsial patients and 29 enteric fever patients were, in general, similar. However, the FCT was significantly prolonged in the enteric fever patients, with a median of 88 hours (interquartile range [IQR] = 54–116), for both drugs compared with the FCT in those with rickettsial infections, with a median of 44.5 hours (IQR = 26–66; hazard ratio = 3.71; P < 0.001).

Our study has a number of limitations. First, we were unable to test the whole study population for alternative causes of UFI, and the 20% proportion of patients selected may not have been truly representative of the whole population. Second, serological testing for *Rickettsia* may lack specificity, although we defined acute infection as a greater than or equal to a fourfold rise in reciprocal antibody titers between admission and convalescence sera.

Despite these limitations, our study highlights that *Rickettsia* spp. are an important cause of UFIs in Nepal and that these patients present with similar clinical characteristics to enteric fever. Although the original study was designed to enroll tephys patients and represents more of an urban population, we detected a 17% murine typhus case rate and a possible 2% *Rickettsia* spp. infection rate in a random subselection of the study. Notably, we have evidence suggesting that Hantavirus and Q fever contribute to UFIs. The absence of scrub typhus is likely because of the predominantly urban patients enrolled in this study.

The recommended therapy for murine typhus is doxycycline, although fluoroquinolones are known to be an effective alternative for the treatment of SFG rickettsioses. Without control groups of untreated or doxycycline-treated patients, only tentative conclusions can be drawn, but despite previous reports of poor responses to ciprofloxacin in murine typhus, our findings suggest that gatifloxacin and ofloxacin may be effective empirical treatment choices in Nepalese patients with UFIs.

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### Table 1

<table>
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<tr>
<th>Organism/diagnostic tests</th>
<th>Supplier</th>
<th>Catalog number</th>
<th>Diagnostic criteria</th>
<th>Methodological reference or validation study</th>
<th>Purpose</th>
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<td>NMRC</td>
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<tr>
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<td>IgM IFA</td>
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<tr>
<td></td>
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<td>47-kDa gene amplification</td>
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<tr>
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<td>11EKS0</td>
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</table>

**Notes:**
- IgM = immunoglobulin; IIFT = indirect immunofluorescence test; MORU = Mahidol Oxford Research Unit; nett OD = net optical density (net stands for the difference from baseline to measured values); NIAH = National Institute of Animal Health-Thailand; NMRC = Naval Medical Research Centre; OSHL = Queensland State Health Laboratory; SD NS1 Ag = standard diagnostics non-structural protein number one (refers to Dengue virus protein) antigen.
- *Leptospiro* serovars tested: pomona, hardjo, tarassovi, grippotyphosa, celledoni, copenhageni, australis, pyrogenes, canicola, hebdomadis, sari, sarmin, autumnalis, cynopteri, ballum, bataviae, dianisam, javanica, panama, shermani, and pohnpei.
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