Undifferentiated Febrile Illness in Kathmandu, Nepal

Corinne N. Thompson, Stuart D. Blacksell, Daniel H. Paris, Amit Arjyal, Abhilasha Karkey, Sabina Dongol, Abhishek Giri, Christiane Dolecek, Nick Day, Stephen Baker, Guy Thwaites, Jeremy Farrar, and Buddha Basnyat

Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom; Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam; The London School of Hygiene and Tropical Medicine, London, United Kingdom; Wellcome Trust, London, United Kingdom; Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Oxford University Clinical Research Unit–Nepal, Patan Academy of Health Sciences, Lalitpur, Nepal

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.
Additionally, two cases were serologically positive for Hantavirus, 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 (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 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 typhoid 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.

Table 1
Diagnostic tests used for the study

| Organism/diagnostic tests          | Supplier       | Catalog number | Diagnostic criteria                                                                 | Methodological reference or validation study | Purpose                                      |
|-----------------------------------|----------------|----------------|-------------------------------------------------------------------------------------|----------------------------------------------|----------------------------------------------|
| *Orientia tsutsugamushi* IgM ELISA| NMRC           | In house       | ≥ 0.2 nett OD                                                                       | 11                                           | Serological screening                        |
| *Orientia tsutsugamushi* IgG ELISA| NMRC           | In house       | ≥ 0.2 nett OD                                                                       | 11                                           | Serological screening                        |
| *Orientia tsutsugamushi* IgM IFA  | ARRL           | RT-001         | ≥ Fourfold rising titer in paired samples                                           | 12                                           | Quantitative serological confirmation       |
| *Orientia tsutsugamushi* IgG IFA  | ARRL           | RT-001         | ≥ Fourfold rising titer in paired samples                                           | 12                                           | Quantitative serological confirmation       |
| Real-time PCR                     | MORU           | In house       | 47-kDa gene amplification                                                           | 13                                           |                                             |
| *R. typhi* IgM ELISA              | NMRC           | In house       | ≥ 0.2 nett OD                                                                       | 11                                           | Serological screening                        |
| *R. typhi* IgG ELISA              | NMRC           | In house       | ≥ 0.2 nett OD                                                                       | 11                                           | Serological screening                        |
| *R. typhi* IgM IFA                | ARRL           | RT-001         | ≥ Fourfold rising titer in paired samples                                           | 12                                           | Quantitative serological confirmation       |
| *R. typhi* IgG IFA                | ARRL           | RT-001         | ≥ Fourfold rising titer in paired samples                                           | 12                                           | Quantitative serological confirmation       |
| Real-time PCR                     | MORU           | In house       | ompB gene amplification                                                             | 14                                           | Confirmation of infection                    |
| *Coxiella burnetii* Phase II IgM ELISA| Serion   | ESR132M        | Manufacturer’s criteria                                                             | 14                                           | Serological screening                        |
| *Coxiella burnetii* Phase I/II IFA | Fuller |                | Product insert                                                                      |                                               | Quantitative serological confirmation       |
| Hantavirus Puamala                | Serion         | ESR145M        | Manufacturer’s criteria                                                             | 11                                           | Serological screening                        |
| Anti-Hantavirus IIFT              | Euroimmun      |                | Product insert                                                                      |                                               | Quantitative serological confirmation       |
| Leptospirosis Mosaic II Test       | Serion         | ESR125M        | Manufacturer’s criteria                                                             | 15                                           | Serological screening                        |
| *Leptospiro* Microscopic agglutination test* | OSHL | ESR125M        | ≥ Fourfold rising titer in paired samples                                           |                                               | Quantitative serological confirmation       |
| *Brucella* spp. Rose–Bengal       | NIAH           |                | Positive agglutination reaction                                                     | 16                                           | Serological screening                        |
| Dengue                            | Alere          | 1IEK50         | Manufacturer’s criteria                                                             | 17                                           | Serological screening                        |

ARRL = Australian Rickettsial Reference Laboratory; ELISA = enzyme-linked immunosorbent assay; IFA = indirect immunofluorescence assay; Ig = 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.
Financial support: This work was funded by the Wellcome Trust of Great Britain.

Authors’ addresses: Corinne N. Thompson, Christiane Dolecek, Stephen Baker, and Guy Thwaites, Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit–Vietnam, Ho Chi Minh City, Vietnam, E-mails: cthompson@oucru.org, cdolecek@gmail.com, sbaker@oucru.org, and gthwaites@oucru.org. Stuart D. Blacksell, Daniel H. Paris, and Nick Day, Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, E-mails: stuart@tropmedres.ac, parigi@tropmedres.ac, and nickd@tropmedres.ac. Amit Arjyal, Abhilasha Karkey, Sabina Dongol, Abhishek Giri, and Buddha Basnyat, Oxford Clinical Research Unit–Nepal, Patan Academy of Health Sciences, Patan Hospital, Lalitpur, Nepal, E-mails: amitarjyal@yahoo.com, abhilashakarkey@hotmail.com, dongolsabina@yahoo.com, giriabhishek@hotmail.com, and buddhabasnyat@gmail.com. Jeremy Farrar, Wellcome Trust, London, United Kingdom, E-mail: JeremyFarrar@gmail.com.

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

1. Chrispal A, Boorugu H, Gopinath K, Chandy S, Prakash J, Thomas E, Abraham A, Abraham O, Thomas K. 2010. Acute undifferentiated febrile illness in adult hospitalized patients: the disease spectrum and diagnostic predictors—an experience from a tertiary care hospital in South India. *Trop Doct* 40: 230–234.

2. Murdoch DR, Woods CW, Zimmerman MD, Dull PM, Belbase RH, Keenan AJ, Scott RM, Basnyat B, Archibald LK, Reller LB. 2004. The etiology of febrile illness in adults presenting to Patan hospital in Kathmandu, Nepal. *Am J Trop Med Hyg* 70: 670–675.

3. Koirala S, Basnyat B, Arjyal A, Shilpakar O, Shrestha K, Shrestha R, Shrestha UM, Agrawal K, Koirala KD, Thapa SD, Karkey A, Dongol S, Giri A, Shukla M, Pathak KR, Campbell J, Baker S, Farrar J, Wolbers M, Dolecek C. 2013. Gatifloxacin versus ofloxacin for the treatment of uncomplicated enteric fever in Nepal: an open-label, randomized, controlled trial. *PLoS Negl Trop Dis* 7: e2523.

4. Arjyal A, Basnyat B, Koirala S, Karkey A, Dongol S, Agrawal KK, Shukla N, Shrestha K, Sharma M, Sama S, Shrestha K, Khati NS, Shrestha U, Campbell JJ, Baker S, Farrar J, Wolbers M, Dolecek C. 2011. Gatifloxacin versus chloramphenicol for uncomplicated enteric fever: an open-label, randomised, controlled trial. *Lancet Infect Dis* 11: 445–454.

5. Pandit A, Arjyal A, Day JN, Paudyal B, Dongol S, Zimmerman MD, Yadav B, Stepniewska K, Campbell JJ, Dolecek C, Farrar JJ, Basnyat B. 2007. An open randomized comparison of gatifloxacin versus cefixime for the treatment of uncomplicated enteric fever. *PLoS ONE* 2: e542.

6. Zimmerman MD, Murdoch DR, Rozmajzl PJ, Basnyat B, Woods CW, Richards AL, Belbase RH, Hammer DA, Anderson TP, Reller LB. 2008. Murine typhus and febrile illness, Nepal. *Emerg Infect Dis* 14: 1656–1659.

7. Mandell GL, Bennett JE, Dolin R. 2010. *Principles and Practice of Infectious Diseases*. 7th Ed. Philadelphia, PA: Churchill Livingstone Elsevier, 2525–2527.

8. Raoult D, Drancourt M. 1991. Antimicrobial therapy of rickettsial diseases. *Antimicrob Agents Chemother* 35: 2457–2462.
9. Laferl H, Fournier P, Seiberl G, Pichler H, Raoult D, 2002. Murine typhus poorly responsive to ciprofloxacin: a case report. *J Travel Med* 9: 103–104.

10. Gikas A, Doukakis S, Pediaditis J, Kastanakis S, Manios A, Tselentis Y, 2004. Comparison of the effectiveness of five different antibiotic regimens on infection with *Rickettsia typhi*; therapeutic data from 87 cases. *Am J Trop Med Hyg* 70: 576–579.

11. Richards A, Rahardjo E, Rusjdi A, Kelly D, Dasch G, Church C, Bangs M, 2002. Evidence of *Rickettsia typhi* and the potential for murine typhus in Jayapura, Irian Jaya, Indonesia. *Am J Trop Med Hyg* 66: 431–434.

12. Phetsouvanh R, Thoaikong T, Phoumin P, Sibounheuang B, Phommasone K, Chansamouth V, Lee S, Newton P, Blacksell S, 2013. Inter- and intra-operator variability in the reading of indirect immunofluorescence assays for the serological diagnosis of scrub typhus and murine typhus. *Am J Trop Med Hyg* 88: 932–936.

13. Jiang J, Maina AN, Knobel DL, Cleaveland S, Laudisoit A, Wamburu K, Ogola E, Parola P, Breiman RF, Njenga MK, Richards AL, 2013. Molecular detection of *Rickettsia felis* and *Candidatus rickettsia asemboensis* in fleas from human habitats, Asembo, Kenya. *Vector Borne Zoonotic Dis* 13: 550–558.

14. Henry K, Jiang J, Rozmajzl P, Azad A, Macaluso K, Richards A, 2007. 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* 21: 17–23.

15. Cole J, Sulzer C, Pursell A, 1973. Improved microtechnique for the leptospiral microscopic agglutination test. *Appl Microbiol* 25: 976–980.

16. OIE, 2009. *OIE Terrestrial Manual: Bovine Brucellosis*. Paris, France: OIE.

17. Blacksell S, Jarman R, Gibbons R, Tanqaunchitcharnehai A, Mammen M, Nisalak A, Kalayanarooj S, Bailey M, Premaratna R, de Silva HJ, Day NP, Lalloo DG, 2012. Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. *Clin Vaccine Immunol* 19: 804–810.