Indian J Med Res 126, August 2007, pp 101-103

Commentary

Spotted fevers & typhus fever in Tamil Nadu

Rickettsia and *Orientia* represent the genera of causative microorganisms of some of the most covert re-emerging diseases of the present times. These genera are morphologically and biochemically similar to other Gram-negative bacteria. The fastidious bacterial organisms are obligate intracellular parasites. Although rickettsial species are arthropod-associated bacteria, they are also frequently capable of infecting vertebrates, including humans, usually as accidental hosts. The genus *Rickettsia* is classically separated into the typhus group (TG) and the spotted fever group (SFG) based on the presence of distinct group lipopolysaccharide (LPS) antigens. The typhus group includes two human pathogens, *R. prowazekii* and *R. typhi*. SFG contains more than 30 diverse genotypes, which cause at least 13 different diseases or pathological syndromes in humans. *Orientia tsutsugamushi* is the aetiological agent of scrub typhus, also known as tsutsugamushi disease. It is a single species that comprises of many antigenic and genotypic variants[1]. Looking back, we find examples of rickettsial diseases killed more people than the actual war[2].

Rickettsial diseases are widely distributed throughout the world and many recent reports suggest to their continued presence in several parts of the Indian subcontinent, particularly that of scrub typhus[3-8]. Rickettsial diseases are generally incapacitating notoriously difficult to diagnose, and untreated cases can have fatality rates as high as 30-35 per cent. Thus, the reported historical numbers of cases of infections with rickettsiae are probably not very accurate and are known to be severely underreported. When diagnosed properly, they are often easily treated but, lack of definitive diagnostic tools and the hazards of handling these microorganisms aggravate the difficulties of diagnosis and treatment.

The article by Kamarasu and colleagues in this issue[9] provides sufficient information on the existence of rickettsial infections in Tamil Nadu. As there are not many community-based studies on rickettsial infections in our country, the present study is meaningful. It is also true that the specific gold standard techniques like the immunofluorescence antibody test (IFA), the indirect imunoperoxidase (IP) test, ELISA are not available in our country and the isolation of the organisms in animals or cell culture is limited by the lack of containment facility as well as the lack of expertise in handling these high risk group pathogens.

To date, the diagnosis of a rickettsial illness has most often been confirmed by serologic testing. Serologic evidence of infection occurs no earlier than the second week of illness for any of the rickettsial diseases; thus, a specific diagnosis may not be available until after the patient has recovered or died. Higher mortality rates are also correlated with delays in consulting a physician and delays in the administration of appropriate antibiotic therapy. Many of the recent reports of scrub typhus and other rickettsial diseases from the Indian sub-continent are based on clinical findings and the relatively non-specific Weil-Felix test[5] including the study by Kamarasu and co-workers[9]. Using this test, a high occurrence of rickettsial infections was observed in Tamil Nadu. The earlier work undertaken in the laboratory of investigators showed reasonably high specificity but a low sensitivity associated with this Weil-Felix test. The poor sensitivity of the Weil-Felix test is now well demonstrated for the diagnosis of Rocky mountain spotted fever (RMSF)[10-13] MSF[14], murine typhus, epidemic typhus[15] and scrub typhus[16]. Although a good correlation between the results of the Weil-Felix test and detection of IgM antibodies by an IFA is often observed, with the development of techniques that are used to grow rickettsiae, this test should be used only as a first line of testing in rudimentary hospital laboratories. In spite of all the drawbacks associated

with it, the Weil Felix test still serves as a useful and cheapest available tool for the laboratory diagnosis of rickettsial diseases. A four-fold rise in agglutinin titres in paired sera is diagnostic for infection with these febrile agents. However, with a single serum sample available, the test is suggestive of infection only at a high cut-off titre (>1:320) at which the positive predictive value and the specificity is reliable. Kamarasu *et al*[9] used antibody titres of 80 or more from single serum sample to indicate either spotted fever or scrub typhus infection. The positive predictive value and the specificity at this threshold titre may not be very high, even then may indicate possibility of infection.

Different serological assays like plate ELISA, dot blot, lateral flow formats have been attempted by several workers employing immunodominant antigens and have shown good promise in terms of sensitivity and specificity (either IgM or IgG or both) in patients infected with *O. tsutsugamushi*[17-26]. Recently, commercial rapid detection kits like Dip-S-Ticks, Scrub typhus RCT and Scrub typhus IgM and IgG Rapid Immunochromatographic Assay (PanBio, Brisbane, Australia) and Multitest Dip-S-Ticks Scrub Recombinant Assay (Integrated Diagnostics, Baltimore, Maryland, USA) have appeared in the market but are still far from the reach of most of the developing countries due to their high cost. Also, due to antigenic diversity observed in *O. tsutsugamushi* strains, local strains should be included in the antigen pool for effective disease diagnosis.

The development of PCR based diagnostic techniques have offered increased flexibility in choices available for detection of a specific pathogen with the added advantage that it directly detects the DNA of the pathogen rather than detecting antibodies to it[27-31]. Detection of the organism or its DNA seems more logical to antibody detection because it indicates active disease. This is particularly valid when used in the endemic area of infection, where the background antibodies often interfere with the interpretation of antibody assays. The gene sequence of the 56 kDa major outer membrane protein has been recently used to develop a nested PCR for the detection of *O. tsutsugamushi* from infected clinical materials and has also been extensively used for the specific diagnosis of scrub typhus. A quantitative real time PCR assay has been described recently by Jiang and co-workers[28], in which primers based on conserved regions of the 47 kDa outer membrane protein (OMP) antigen gene detected *O. tsutsugamushi* DNA from patients' blood and sera samples at a concentration as low as three to ten copies per reaction and has been reported to be even more sensitive in detecting the presence of *O. tsutsugamushi* than mouse inoculation[32].

The varied presentation of clinical manifestations warrants an increased awareness among doctors and general public to consider these clinical features for suspecting rickettsial infections. Although much advancement regarding research on this organism, its epidemiology, pathogenesis and the development of novel detection systems *etc*., is being carried out by countries like Japan, China, Korea, Thailand, Malaysia, *etc*., the pace at which our country has progressed in addressing the problems related to this infectious agent has been grossly inadequate. We should aim towards identifying specific genes and proteins, which would aid in better diagnosis, and perhaps, candidate vaccine.

H.V. Batra
Defence Food Research Laboratory
Siddharth Nagar
Mysore 570011, India
e-mail: h_v_batra@rediffmail.com

References

1. Eremeeva ME, Dasch GA. Rickettsia and orientia. In: Sussman M, editor. *Molecular medical microbiology,* vol. II. London: Academic Press; 2001 p. 2177-2217.
2. Kelly DJ, Richards A, Temenak J, Strickman D, Dasch GA. The past and present threat of rickettsial diseases to military medicine and international public health. *Clin Infect Dis* 2002; *34* (Suppl 4) : S145-S69.
3. Mahajan SK, Bakshi D. Acute reversible hearing loss in scrub typhus. *J Assoc Physicians India* 2007; *55* : 512-4.
4. Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Parasher BS, *et al*. Scrub Typhus in Himalayas. *Emerg Infect Dis* 2006; *12* : 1590-92.
5. Sharma A, Mahajan S, Gupta ML, Kanga A, Sharma V. Investigation of an outbreak of scrub typhus in the Himalayan region of India. *Jpn J Infect Dis* 2005; *58* : 208-10.
6. Sundhindra BK, Vijaykumar S, Kutti AK, Tholpadi SR, Rajan AS, Mathai E, *et al*. Rickettsial spotted fever in Kerala. *Natl Med J India* 2004; *17* : 51-2.
7. Mathai E, Lloyd G, Cherian T, Abraham OC, Cherian OC. Serological evidence of the continued presence of human rickettsioses in southern India. *Ann Trop Med Parasitol* 2001; *95*: 395-8.
8. Mathai E, Rolain JM, Verghese GM, Abraham OC, Mathai D, Mathai M. *et al*. Outbreak of scrub typhus in southern India during cooler months. *Ann N Y Acad Sci* 2003; *990* : 359-64.
9. Kamarasu K, Malathi M, Rajagopal V, Subramani K, Jagadeeshramasamy D, Mathai E. Serological evidence for wide distribution of spotted fevers and typhus fever in Tamil Nadu. *Indian J Med Res* 2007; *126* : 128-30.

10. Hechemy KE, Stevens RW, Sasowski S, Michaelson EE, Casper, EA, Philip RN. Discrepancies in Weil-Felix and microimmunofluorescence test results for Rocky Mountain spotted fever. *J Clin Microbiol* 1979; *9* : 292-3.

11. Marx RS. Rocky mountain spotted fever. *Compr Ther* 1983; *9* : 43-8.

12. Walker DH, Burday MS, Folds JD. Laboratory diagnosis of Rocky Mountain spotted fever. *South Med J* 1980; *73* : 1443-9.

13. Kaplan JE, Schonberger LB. The sensitivity of various serologic tests in the diagnosis of Rocky Mountain spotted fever. *Am J Trop Med Hyg* 1986; *35* : 840-4.

14. Raoult D, Rousseau S, Toga B, Tamalet C, Gallais H, de Micco P, *et al*. Serological diagnosis of Mediterranean boutonneuse fever. *Pathol Biol* 1984; *32* : 791-4.

15. Ormsbee R, Peacock M, Philip R, Casper E, Plorde J, Gabre-Kidan T, *et al*. Serologic diagnosis of epidemic typhus fever. *Am J Epidemiol* 1977; *105* : 261-71.

16. Brown GW, Shirai A, Rogers C, Groves MG. Diagnostic criteria for scrub typhus: probability values for immunofluorescent antibody and *Proteus* OXK agglutinin titers. *Am J Trop Med Hyg* 1983; *32* : 1101-7.

17. Chen WJ, Niu DS, Zhang XY, Chen ML, Cui H, Wei WJ, *et al*. Recombinant 56-kilodalton major outer membrane protein antigen of *Orientia tsutsugamushi* Shanxi and its antigenicity. *Infect Immun* 2003; *71* : 4772-9.

18. Jang WJ, Huh MS, Park KH, Choi MS, Kim IS. Evaluation of an IgM capture ELISA assay for diagnosis of *Orientia tsutsugamushi* infection. *Clin Diag Lab Immunol* 2003; *10* : 394-8.

19. Jiang J, Marienau KJ, May LA, Beecham HJ III, Wilkinson R, Ching WM, *et al*. Laboratory diagnosis of two scrub typhus outbreaks at Camp Fuji in Japan in 2000 and 2001 by enzyme linked immunosorbent assay, rapid flow assay and Western blot assay using outer membrane 56-kilodalton recombinant proteins. *Am J Trop Med Hyg* 2003; *69* : 60-6.

20. Coleman R, Sangkasuwan V, Suwanabun N, Eamsila C, Mungviriya S, Devine P, *et al*. Comparative evaluation of selected diagnostic assays for the detection of IgG and IgM antibody to *Orientia tsutsugamushi* in Thailand. *Am J Trop Med Hyg* 2002; *67* : 497-503.

21. Tay ST, Rohani MY, Ho TM, Devi S. Expression of recombinant proteins of *Orientia tsutsugamushi* and their applications in the serodiagnosis of scrub typhus. *Diagn Microbiol Infect Dis* 2002; *44* :137-42.

22. Ching WM, Wang H, Eamsila C, Kelly DJ, Dasch GA. Expression and refolding of truncated recombinant major outer membrane protein antigen (r56) of *Orientia tsutsugamushi* and its use in enzyme linked immunosorbent assays. *Clin Diagn Lab Immunol* 1998; *5* : 519-26.

23. Pradutkanchana J, Silpapojakul K, Paxton H, Pradutkanchana S, Kelly DJ, Strickman D. Comparative evaluation of four serodiagnostic tests for scrub typhus in Thailand. *Trans R Soc Trop Med Hyg* 1997; *91* : 425-8.

24. Seong SY, Huh MS, Jang WJ, Park SG, Kim JG, Woo SG, *et al*. Induction of homologous immune response to *Rickettsia tsutsugamushi* Boryong with a partial 56-kilodalton recombinant antigen fused with maltose-binding protein MBP-Bor56. *Infect Immun* 1997; *65* : 1541-5.

25. Seong SY, Park SG, Huh MS, Jang WJ, Kim HR, Han TH, *et al*. Mapping of antigenic determinant regions of the Bor56 protein of *Orientia tsutsugamushi*. *Infect Immun* 1997; *65* : 5250-6.

26. Kelly DJ, Wong PW, Gan E, Chye CT, Cowan D, Lewis GE. Multi-laboratory evaluation of a scrub typhus diagnostic kit. *Am J Trop Med Hyg* 1990; *43* : 301-7.

27. Furuya Y, Yoshida Y, Katayama T, Yamamoto S, Kawamura A. Serotype specific amplification of *Rickettsia tsutsugamushi* DNA by nested polymerase chain reaction. *J Clin Microbiol* 1993; *31* : 1637-40.

28. Jiang J, Chan TC, Temenak JJ, Dasch GA, Ching WM, Richards A. Development of a quantitative real time polymerase chain reaction assay specific for *Orientia tsutsugamushi*. *Am J Trop Med Hyg* 2004; *70* : 351-6.

29. Kodama K, Senba T, Yamaguchi H, Chikahira Y, Katayama T, Furuya Y, *et al* . Fulminant Japanese spotted fever definitely diagnosed by the polymerase chain reaction method. *J Infect Chemother* 2002; *8* : 266-8.

30. Ono A, Nakamura K, Higuchi S, Miwa Y, Nakamura K, Tsunoda T, *et al*. Successful diagnosis using scab for PCR specimen in Tsutsugamushi disease. *Intern Med* 2002; *41* : 408-11.

31. Saisongkorh 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.

32. Singhsilarak 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.