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Scrub Typhus: Prevalence and Diagnostic Issues in Rural Southern India

SIR—Recent reports from India and other neighboring countries suggest that there is a resurgence of scrub typhus infection caused by *Orientia tsutsugamushi* in these parts of the world and that the resurgence is associated with considerable morbidity and mortality [1–3]. At present, scrub typhus is rarely diagnosed because of its nonspecific clinical presentation that includes fever, rigors, headache, and occasionally rash, and because of a low index of suspicion and the lack of diagnostic facilities in India. We wish to draw the attention of readers to the magnitude of this disease in rural South India and to the validity of the widely used Weil-Felix test.

Using the Weil-Felix test and ELISA, we conducted a study to determine the prevalence of scrub typhus in the community at one of the block (administrative unit of a district) level community health centers of Christian Medical College and Hospital, Vellore district, Tamil Nadu, South India. This health center is the only hospital with inpatient treatment facilities in that block, which has 100,000 inhabitants. An average of 150 patients are seen daily in the outpatient department. During this study period at the tertiary care hospital, we also evaluated the validity of the Weil-Felix test for a range of titers using a panel of 125 serum samples collected by the microbiology department from the infected and control patients (according to diagnoses made using ELISA results). The institutional review board approved this study.

From mid-October 2002 to January 2003, all patients who reported to the outpatient department of the community health center with confirmed fever of 7–30 days duration were admitted and evaluated. Patients' histories were obtained and clinical examinations and routine investigations were done, which included a total WBC count, a differential count, microscopy of a urine sample, a Widal test, and chest x-ray when indicated. We found that findings of primary evaluations could yield a diagnosis of the most common causes of febrile illnesses (with durations between 7–30 days), such as enteric fever, respiratory tract infection, and urinary tract infection. Blood samples were collected from all patients who had undiagnosed febrile illness to test for rickettsial antibodies with the Weil-Felix test and ELISA. The Weil-Felix test was performed according to standard procedures with whole-cell antigens prepared from the Ox-19, Ox-2, and Ox-K strains of *Proteus vulgaris* [4]. For ELISA, we used the Scrub Typhus Group ELISA Kit (PanBio) to detect IgG and IgM antibodies. These kits use a 56-kDa recombinant antigen and have specificities and sensitivities of ~90% for detecting specific antibodies [5]. To determine whether the disease is endemic in the study area, a serosurvey was conducted among 100 apparently healthy individuals who did not report experiencing fever during the previous month and who resided in 5 different villages where cases of scrub typhus had occurred.

Thirty-three febrile patients were evaluated, and a specific diagnosis could not be established at that time for 18 of these patients clinically or by the routine investigations described earlier. Later, 8 (47%) of these patients received the diagnosis of scrub typhus based on ELISA results. One (12.5%) of these 8 patients tested positive for *P. vulgaris* Ox-K antigens with a titer of 1:80, two patients (25%) with a titer of 1:40, and two patients (25%) with a titer of 1:20. None of the 8 patients had eschars that would suggest a diagnosis of scrub typhus clinically. Two patients also had IgG antibodies for scrub typhus. Of the control subjects in the community, 4 (4%) had IgG antibodies, and 1 (1%) had IgM antibodies.

We attempted to validate the Weil-Felix test in the population using a panel of 125 serum samples. The sensitivity for Ox-K was 30% at a titer breakpoint of 1:80, but the specificity and positive predictive value were 100%. At a breakpoint of 1:20, the sensitivity was 61%, the specificity was 94%, and positive predictive value was 84%. At a breakpoint of 1:40, the sensitivity was 49%, the specificity was 96%, and positive predictive value was 88%.

This study highlights the finding that ~50% of the undiagnosed prolonged fevers that occur during the cooler months of the year in the rural areas of Tamil Nadu, South India, could be due to scrub typhus. Although the disease occurrence was high during the study period, the prevalence of IgG antibodies in the community was low, which suggests that the disease was probably relatively new to the area. Many studies done in the 1960s and 1970s have demonstrated the endemic nature of this disease in many parts of India [6]. However, in later years, the disease virtually disappeared, probably because of widespread use of insecticides to control other vectorborne diseases, empiric treatment of febrile illnesses with tetracyclines and chloramphenicol by practitioners, and changes in lifestyle. There seems to be a resurgence of the disease now [3].

Accurate and early diagnosis of scrub typhus remains a challenge in India because of its nonspecific presentation and the paucity of confirmatory diagnostic resources. We found that the Weil-Felix agglutination test had only 30% sensitivity at a titer of 1:80. However, it is worth noting that the specificity of this test was

high even at lower titers, so patients with low titers should also be evaluated for scrub typhus. It is known that Weil-Felix test results may be negative during the early stages of the disease because agglutinating antibodies are detectable only during the second week of illness [7]. ELISA, however, when performed with 56 KDa antigen, has 90% sensitivity and specificity, allows detection of IgG and IgM antibodies, and provides positive results within 3-4 days after the onset of illness. However, the availability and the cost of ELISA are major problems in India.

This study report emphasizes the need for increased awareness of rickettsial infections in rural Southern India. Because of current circumstances in India, we suggest that the diagnosis of scrub typhus should be largely based on a high index of suspicion and careful clinical, laboratory, and epidemiological evaluation. Use of empiric treatment should also be considered to reduce the high mortality observed with the disease. Introduction of improved diagnostic methods would allow greater appreciation for the prevalence of the disease.

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Positive Predictive Value of Epstein-Barr Virus DNA Detection in HIV-Related Primary Central Nervous System Lymphoma

Sir—Epstein-Barr virus (EBV) DNA PCR detection in CSF has been proven to be sensitive and specific for the diagnosis of HIV-related primary CNS lymphoma (PCNSL) [1–6]. In clinical practice, this test has been shown to be useful to achieve a "minimally invasive" diagnosis of PCNSL, in the presence of clinicoradiological findings and thallium 201 single-photon emission tomography findings consistent with PCNSL and no response to antitoxoplasmic treatment [7, 8].

In their recent article, Ivers et al. [9] reported the EBV DNA PCR results for CSF samples obtained from 26 HIV-infected patients with neurological problems. EBV DNA was found in samples from 7 of these 26 patients. Because only 2 of the patients received a diagnosis of PCNSL, the resulting low positive predictive value (PPV) led the authors to conclude that improved standardization may be required for the use of this test in clinical practice.

We would like to discuss 2 important issues that, in our opinion, have not been adequately addressed by Ivers et al. [9]. First, the diagnostic value of a test depends on a number of variables, including the diagnostic standard and the analytical sensitivity of the assay [10]. Nucleic acid amplification protocols may vary significantly between laboratories, because of the use of different nucleic acid extraction techniques, primers, and amplification technology. Assays with high analytical sensitivity may be associated with increased rates of false-positive results—although not necessarily with increased CSF lymphocyte counts—and, thus, with poor diagnostic specificity and a low PPV. It is unfortunate that methodological information was not provided by Ivers et al. [9], making it difficult to compare their results with the results obtained in previous studies.

Second, the positive and negative predictive values of a diagnostic test vary substantially, depending on the prevalence of

Table 1. Diagnoses given to 22 HIV-infected patients whose CSF samples were positive for Epstein-Barr virus DNA.

Diagnosis	No. of patients
PCNSL	
Histologically proven[a]	5
Probable[b]	7
Possible[c]	5
Lymphomatous meningitis	2
Other CNS disorder[d]	3

NOTE. PCNSL, primary CNS lymphoma.

[a] Determined by brain biopsy (3 cases) or at postmortem examination (2 cases).

[b] Defined by abnormalities in brain CT or MRI results that are consistent with PCNSL, lack of response to antitoxoplasmic treatment, and a positive result of thallium 201 single-photon emission tomography (SPECT) examination.

[c] Defined by abnormalities in CT or MRI results that are consistent with PCNSL, lack of response to antitoxoplasmic treatment, and either a negative result of SPECT examination or no performance of SPECT.

[d] CNS tuberculosis, HIV encephalitis, or CNS toxoplasmosis (1 case each).