

Failure to progress  
Fetal distress

### Postnatal score

Two points for each of the following:

Apgar score at 1 min <3                      Pneumonia  
Apgar score at 5 min <7/resuscitated

Artificial ventilation B each week of respiratory distress

Oxygen therapy B each week of high oxygen concentration >70%  
Exchange transfusion  
Apnoea, requiring ventilation  
Apnoea, requiring theophylline  
Convulsions  
Symptomatic hypocalcaemic  
Birthweight not regained within two weeks

## Evaluation of tests for serological diagnosis of scrub typhus

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**SUMMARY** Two specific serological tests, a Dot enzyme immunoassay (EIA) and an immunoglobulin (Ig)M enzyme-linked immunosorbent assay (ELISA) using the 56 kDa antigen and the Weil-Felix test were evaluated for diagnosis of scrub typhus. Sensitivity of 100, 86.5 and 43.5% were observed with Dot EIA, IgM ELISA and Weil-Felix test, respectively. False-positive reactions were observed in patients with falciparum malaria, pulmonary tuberculosis, *S. viridans* septicemia and typhoid fever using Dot EIA and IgM ELISA. Therefore, although Dot EIA and IgM ELISA are useful in the serodiagnosis of scrub typhus, efforts should be made to rule out other febrile illnesses.

### Introduction

The incidence of scrub typhus caused by *Orientia tsutsugamushi* has increased in recent years in Asia, but is still under-diagnosed in India.<sup>1-3</sup> As mortality can be as high as 35%<sup>4</sup> and tetracyclines and chloramphenicol can be very effective against *O. tsutsugamushi*, precise diagnosis becomes necessary.

The Weil-Felix test forms the mainstay in serodiagnosis in India, though the recommended test is the indirect immunofluorescence assay.<sup>2</sup> In doubtful cases, a dramatic response to doxycycline therapy can also be used in establishing diagnosis.<sup>5</sup>

Sera from >95% patients with scrub typhus have antibodies to the 56 kDa, major surface protein antigen of

*O. tsutsugamushi*.<sup>6</sup> Enzyme-linked immunosorbent assay (ELISA) and dot-blot immunoassays<sup>4</sup> using this antigen have been found useful for the diagnosis of scrub typhus.<sup>5</sup> Therefore, we evaluated Dot enzyme immunoassay (EIA), immunoglobulin (Ig)M ELISA and the Weil-Felix test in the diagnosis of scrub typhus in our area, where other febrile infections like tuberculosis and malaria are endemic.

### Materials and methods

Serum samples from patients presenting to Christian Medical College, Vellore, during the period from November 2003 to August 2004, in whom scrub typhus was suspected clinically, were included in the study. Weil-Felix test using antigen prepared in our laboratory was performed on 174 samples,<sup>7</sup> and was considered positive if the titre for OX-K antigen alone was  $\geq 80$ . Rickettsia Screen (PanBio, USA), a Dot enzyme immunoassay that detects both IgG and IgM antibodies to *O. tsutsugamushi* and the Rickettsia Scrub Typhus Group IgM ELISA (PanBio, Australia), were performed as per the manufacturer's instructions. Both these tests use the r56 kDa protein as antigen. The Dot EIA was considered as positive if a distinct spot was seen either in the well coated with the Karp strain antigen or in that with antigens from Kato and Gilliam strains. Sera with  $\geq 16$  Panbio units of IgM antibodies were considered positive in the ELISA test. A final clinical diagnosis of scrub typhus was made if any one of the aforementioned specific serological tests was positive and there was immediate clinical response to Doxycycline or Chloramphenicol, and specific investigation for other illnesses were negative.

### Results

Using specific serology and clinical criteria, 46 patients were diagnosed to have scrub typhus. Results of comparison of the tests are shown in Table 1. Forty-one of the 42 Dot EIA-positive sera reacted with the antigen of the Karp strain. The remaining one was reactive in both wells and also was sputum positive for acid-fast bacilli. There were six false-positive results by this test. Two patients each had pulmonary tuberculosis and falciparum malaria, respectively. The fifth patient had culture proven *Streptococcus viridans* septicemia and responded to treatment with penicillin. The sixth patient was IgM typhidot positive and expired within 24 h of admission and was IgM ELISA negative for scrub typhus. Both patients with tuberculosis tested negative by ELISA test. One of the two patients with malaria and the patient with *S. viridans* septicemia were positive by IgM ELISA.

Sera from six patients considered to have scrub typhus cross-reacted with *Rickettsia typhi* antigen in Dot EIA. Of these, dramatic response to doxycycline therapy was seen in five patients. One patient succumbed due to multi-organ failure following typhus fever in spite of doxycycline therapy.

Twenty-two of the 32 patients diagnosed as scrub typhus by IgM ELISA were also Dot EIA positive. Dot EIA could not be done on the remaining 10 scrub typhus cases. All five patients with scrub typhus but negative by ELISA were positive in Dot EIA. Three of these responded well to doxycycline therapy, whereas another responded to chloramphenicol. The fifth patient developed renal failure, myasthenic crises and succumbed in spite of appropriate therapy.

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**Table 1** Performance of the serologic assays in the diagnosis of scrub typhus

		<i>Dot EIA</i>		<i>IgM ELISA</i>		<i>Weil-Felix test</i>	
		<i>(n=129)</i>		<i>(n=117)</i>		<i>(n=174)</i>	
		<i>Positive</i>	<i>Negative</i>	<i>Positive</i>	<i>Negative</i>	<i>Positive</i>	<i>Negative</i>
Scrub Typhus	Present	36	0	32	5	20	26
	Absent	6	87	2	78	2	126
Sensitivity		100%		86.5%		43.5%	
Specificity		93.5%		97.5%		98.4%	
PPV		85.7%		94%		90.9%	
NPV		100%		94.6%		82.9%	

PPV, positive predictive value; NPV, negative predictive value

The sensitivity of the Weil-Felix test was very low as compared with the Dot EIA and IgM ELISA for diagnosis of scrub typhus.

One feature common to the six patients with scrub typhus who succumbed to their illness was that they presented to our centre with multi-systemic involvement and three of them died within 24 h of admission. Hence the overall case fatality was 13% (6/46) which is similar to that observed in previous years at our centre.

## Discussion

Dot EIA and IgM ELISA were found useful for the serodiagnosis of scrub typhus. However, one has to be cautious about the possibility of false-positive results with both these tests. The predominant serotype responsible for scrub typhus in Tamil Nadu appears to be of the Karp strain.

Ching *et al.*<sup>6</sup> also reported two cases of malaria being falsely positive by the rapid flow assay utilizing the r56 kDa antigen. Serum from a patient with typhoid fever tested positive with PanBio dipstick assay.<sup>5</sup>

As the Weil-Felix test becomes positive only in the second week of illness, diagnosis can be missed early in the illness leading to a delay in specific treatment. Survival was lower in those presenting late with multi-system involvement. Improving diagnostic methods can help in reducing such mortality.

## References

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