Annals of Tropical Medicine & Parasitology, Vol. 95, No. 4, 395–398 (2001)

Serological evidence for the continued presence of human rickettsioses in southern India

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Received 28 July 2000, Revised and accepted 30 April 2001

Rickettsiosis is generally believed to have disappeared from many parts of India. However, the serological testing of 37 residents of southern India who presented with fever of unknown aetiology in 1996–1998 confirmed that spotted fever, epidemic/endemic typhus and scrub typhus continue to occur in southern India. The epidemiology and magnitude of the problem need to be evaluated.

In the past, rickettsial infections of several types occurred in the human residents of India. Epidemic typhus (caused by *Rickettsia prowazekii*), for example, was largely restricted to hilly terrain in the north–west of the sub-continent whereas endemic or murine typhus (*R. typhi*) and Indian tick typhus (*R. conorii*) used to occur in many states, and there were epidemics of scrub typhus (*Orientia tsutsugamushi*) among the troops stationed in northern and eastern areas (Varma and Mahadevan, 1972; Padbidri and Gupta, 1978; Shanmugam *et al.*, 1978; Padbidri *et al.*, 1980; Kumar *et al.*, 1982; Maniya *et al.*, 1988; Jayaseelan *et al.*, 1991). However, the availability of effective treatments for these diseases and the killing of many of the vectors (as a side-effect of the widespread use of insecticides to control other vector-borne infections) has recently led to massive reductions in the incidence of rickettsioses in many areas (Padbidri and Gupta, 1978). Although sporadic cases continue to be reported, a general lack of awareness and of specific tests to identify the rickettsial species involved has meant that very little information is available on the current status of these infections in India. The Weil–Felix (WF) agglutination test, popularized by the Indian army in the 1930s, continues to be the only test available in most medical institutions in India, although a positive result in this test only indicates a rickettsial infection and not

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ISSN 0003-4983 (print) ISSN 1364-8594 (online)/01/040395-04
Carfax Publishing

DOI: 1080/00034980120065804

the causative organism involved (Padbidri and Gupta, 1978). In the present study, samples from Indians found to be WF-positive were subjected to other, more specific tests, in the U.K.

MATERIALS AND METHODS

Samples of blood, collected from patients with fever of 'unknown aetiology' who presented at the Christian Medical College Hospital at Vellore in southern India, between 1996 and 1998, were tested using the WF test with OX-19, OX-2 and OX-K antigens (Myers and Koshi, 1982). Eleven negative samples and the 26 samples which each gave a titre of at least 1:80 with at least one of the antigens were then sent to the U.K.'s national Centre for Applied Microbiological Research, at Porton Down. There, they were subjected to more specific tests for the antibodies indicative of endemic or epidemic typhus (ET), spotted fever (SF) or scrub typhus (ST). Indirect haemagglutination assays (IHA; Shirai *et al.*, 1975) based on glutaraldehyde-stabilized erythrocytes sensitized with antigens from *R. typhi* or *R. rickettsii* were used to test for ET and SF, respectively, whereas a commercial, dot-blot immuno-assay (Dip-S-Ticks®; Integrated Diagnostics, Baltimore, MD) was used to detect the antibodies against *O. tsutsugamushi* that are indicative of ST (Weddle *et al.*, 1995). Samples giving IHA titres of at least 1:160 were considered positive.

RESULTS

During the years 1996, 1997 and 1998, respectively, six (5%) of the 132, four (3%) of the 132 and 21 (10%) of the 211 serum samples investigated using the WF test gave titres of at least 1:160. In all three of these years, most WF-positives occurred during the cooler months (i.e. the first few and last few months of the year).

Of the 37 samples transferred to the U.K., 18 (69%) of the WF-positives and two (18%) of the WF-negatives were considered to have given a true-positive result in at least one of the specific tests. Six samples appeared seropositive for *R. rickettsia*, seven for *R. typhi* and nine for *O. tsutsugamushi*, but these numbers include two samples which each gave a positive result in two tests (the IHA for antibodies to *R. rickettsii* and either the IHA for *R. typhi* or the dot-blots for *O. tsutsugamushi*). On review, these two samples were considered true-positives for *R. rickettsii* (and false-positives for *R. typhi* or *O. tsutsugamushi*), since the signs and symptoms of their donors resembled those of SF rather than of ET or ST.

This left six cases of SF, six of ET and eight of ST. However, the identification of one of the ST cases was considered dubious because the patient had concurrent seropositivity to HIV and several other infectious agents. The number of cases identified was therefore reduced to six each of SF and ET and seven of ST. Two of these 19 cases (one positive for ST and one for ET) were WF-negatives. Information on the age, gender, state of residence and symptoms of 17 of these cases is presented in the Table (such information was not available for two of the probable cases of ST). Fever was the only common manifestation, present in all the cases except one asymptomatic subject who was positive in the WF test and the IHA for ET. There was no record of a '*tache noir*' in any of the patients, and only one, a boy with SF, gave a definite history of tick bite. All but one of the WF-positive cases were treated with doxycycline or chloramphenicol and all showed clinical improvement. The two WF-negative cases, who were not treated, were lost to follow-up but are presumed well. One subject, although eventually found WF-positive, was not suspected as a case of rickettsial infection until his disease (ET) was well progressed, and he died before specific therapy could be initiated. He was a diabetic who presented not only with fever and generalised 'itching' but also with an abnormal sensorium, seizures and the features of hepatitis; he developed acute renal failure within 2 days of presentation.

TABLE

*Features of the cases found positive in specific serological tests (indirect haemagglutination assays or dot-blot immuno-assays) for rickettsial infection**

	Spotted fever	*Endemic or epidemic typhus*	*Scrub typhus*
NO. AND (%) OF CASES:			
With fever	6 (100)	5 (83)	5 (100)
With rash	6 (100)	3 (50)	2 (40)
Aged < 12 years	4 (67)	1 (17)	0 (0)
Female	2 (33)	3 (50)	1 (20)
Male	4 (67)	3 (50)	4 (80)
Resident in Vellore	4 (67)	4 (67)	0 (0)
Resident in Tamil Nadu	1 (17)	1 (17)	2 (40)
Resident in another state	1 (17)	1 (17)	3 (60)
Other features	Arthralgia, oedema, haematuria, headache, seizures	Headache, oedema, seizures, arthralgia, hepatitis, hepatomegaly, renal failure	Hepatitis, splenomegaly, headache, cough, arthritis, haematuria (two cases were diabetic)

* The information here refers to the six cases of spotted fever, six of endemic/epidemic typhus and five of scrub typhus for whom the relevant data were available.

DISCUSSION

The data presented here indicate that several types of human rickettsial infection remain prevalent in southern India and that such infection may result in death if not recognized early and treated adequately. Accurate, early diagnosis of human rickettsioses remains a challenge in India, where most clinicians seem unaware that such diseases persist in the country and are probably often confused by the varied clinical manifestations of rickettsial infection.

Although all the present cases of SF had a rash—the major sign of rickettsial infection—only 60% of the ET cases had this symptom. It is clear that a rash is not always present in rickettsial infection (Dumler and Walker, 1995; Walker and Raoult, 1995) and that the other clinical features and the systems and organs affected can vary considerably (Dumler and Walker, 1995; Walker and Raoult, 1995). This can make diagnosis difficult. In India, rickettsial infection should still be considered when attempting to diagnose a patient with fever with or without other features. Specific diagnosis depends on a high index of suspicion. Although culture- and PCR-based methods exist for the identification of rickettsial infections, specific serology remains the usual method of confirmation (Dumler and Walker, 1995; Walker and Raoult, 1995). However, serological tests may give negative results in the early stages of the disease, and antigen-sharing between the rickettsia may cause cross-reactivity, false-positive results, and subsequent confusion in diagnosis (Dumler and Walker, 1995; Walker and Raoult, 1995), as seen in the present study.

In the present study, SF was seen mostly in children. The predominance of the young among cases of SF has been observed in other areas and is believed to be related to the vector involved (Walker and Raoult, 1995).

It seems that, after a period of rarity, human rickettsial infections may be re-emerging in southern India. There is a need for prospective studies to determine the burden and epidemiology of these infections in this area, so that effective control measures can be implemented.

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