RESEARCH NOTE

USE OF HEMOLYMPH TEST FOR DETECTION OF RICKETTSIAE IN MALAYSIAN TICKS

ST Tay[1], TM Ho[2] and MY Rohani[1]

[1]Division of Bacteriology; [2]Division of Acarology, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

A number of arthropod-borne rickettsial infections have been reported in Malaysia. These include tick typhus, Q fever, murine typhus and scrub typhus (Marchette, 1966). Although much is known about scrub typhus in this region, there is little information at present, on prevalence of tick typhus and the vector-pathogen-host interrelationships. Most tick-borne rickettsiae belong to the spotted fever group (SFG), *ie R. rickettsii, R. parkeri, R. conorii, R. siberica* and *R. akari*. In Asia, *R. conorii* has been isolated from human cases of SFG rickettsiosis in India and Pakistan but no isolates have been obtained in the Southeast Asian region. A SFG rickettsiae, Thai tick typhus (TT118) was isolated by Elisberg and co-workers (Robertson and Wisseman, 1973 which demonstrated cross-reactivities with human *R. conorii* sera (Raoult and Dasch, 1989). Rickettsiae within the spotted fever group are generally immunologically interrelated with each other (Bozeman *et al,* 1960; Bell and Stoenner, 1960).

Ticks can be collected and screened for the presence of rickettsiae by a hemolymph test. The test, first conducted on laboratory-reared *Dermacentor andersonii* and *Dermacentor variabilis* infected experimentally with strains of the spotted fever rickettsia (*Rickettsia rickettsii*) was also found to be useful for identifying ticks infected with particular disease agents causing tick-borne relapsing fever, bovine babesiosis and anaplasmosis (Burgdorfer, 1970). This paper reports the experience of using the hemolymph test and the findings of the hemolymph test performed on Malaysian ticks.

A relatively undisturbed slope of hill dipterocarp forest behind a government health clinic in Selangau, Sarawak was selected because high prevalence of tick typhus had been reported in the vicinity (unpublished data). Below the dipterocarp crowns is a stratum of trees of many species and families which forms a dense, nearly continuous canopy 15-30 metres above the ground. On the forest floor are shrubs, palms, tree seedlings, ferns and herbs.

Adult ticks were collected by hand from vegetation. These ticks were kept at room temperature in moist glass vials and transported alive to a laboratory in the Institute for Medical Research, Kuala Lumpur, for identification prior to examination by the hemolymph test.

Hemolymph from each tick was obtained as described by Burgdorfer (1970). Each tick was firmly grasped with a pair of fine forceps and a drop of hemolymph was obtained by amputating the distal portion of one or more legs with pointed dissecting scissors. The small drop that exuded from the wound was touched to 2 slides within circles marked on its lower surface. After air-drying, one of the slides was heat-fixed, stained by Gimenez method (Gimenez, 1964) and examined by light microscopy under oil immersion. The other slide was fixed in acetone for 10 minutes and subjected to direct immunofluorescence test using a FITC-labeled antibody against *R. conorii* (provided by Naval Medical Research Institute, Bethesda, MD, USA).

Thirty-nine *Haemaphysalis conigera* were collected. Hemolymph tests revealed that 11 ticks contained hemocyte-associated rickettsia-like organisms. The Gimenez stained rickettsia-like organisms appeared as pink or red, small rod or coccobacilli and often pleomorphic microorganism. Hemocytes and other tissue materials stained blue or pale-green. Generally, rickettsia-like organisms are located within the cytoplasm of the greenish blue hemocytes, although extracellular forms are occasionally found if the degree of infection is intense. A total of 7 hemocyte preparations

were without any bacteria and 6 were contaminated with other bacteria. The rest of the hemolymph preparations were inconclusive as no hemocytes were observed. Only 2 of the hemolymph test positive smears were reactive to specific *R. conorii* FITC-labeled antibody.

Laboratory animals are traditionally used for isolation of rickettsiae from field samples such as ticks; the technique is laborious and time consuming. Moreover, in the case of a strain causing mild infections, the clinical signs and illness are usually not detectable in the animals. The hemolymph test is a rapid, simple and economical technique for detection of rickettsiae in the suspected ticks from endemic area. When large numbers of ticks need to be examined, the hemolymph test can be used first for screening the specimens. Hemolymph test positive specimen can then be examined by DFAT using specific conjugated antibodies. By this combination of screening with first the hemolymph test followed by the DFAT, there should be a higher success rate when the ticks are eventually inoculated into laboratory hosts for rickettsial isolation.

In this study, positive hemolymph smears showed typical rickettsial like organisms; pink coccobacili or pleomorphic forms associated with the hemocytes. Other bacteria were readily differentiated from rickettsiae by their morphological and tinctorial properties. Absence of hemocytes in some preparations was probably due to insufficient hemolymph collected from the ticks. This has been reported in other studies where only little amount of hemolymph could be obtained from small ticks of genera *Ixodes, Hemaphysalis* and *Rhipicephalus* (Burg-dorfer, 1970). Thus, misdiagnosis can occur since the number of hemocytes varies greatly from tick to tick and not all are infected.

For preliminary identification of rickettsiae in wild ticks, direct fluorescent antibody staining was successfully used in combination with hemolymph test. Fluorescence of rickettsiae in hemocytes suggests that the antigen is identical to or closely related to the antigen used for preparation of immunoglobulin. Ideally, hemolymph from each tick should be tested with a range of specific antibodies towards known rickettsiae. However, this is not possible in this study owing to the limited hemolymph obtained from each tick and the unavailability of standard labeled FITC-antibody against other rickettsial species.

In this study, two hemolymph preparations were DFAT positive. These organisms probably belonged to the spotted fever group and serologically similar to *R. conorii*, a tick transmitted causative agent for spotted fever infection. Further isolation attempts are necessary to propagate and characterize the strains.

Tick-borne typhus occurs primarily in the climatic climax forest and jungle rats with their ectoparasitic *Dermacentor, Ixodes* and *Haemaphysalis* ticks, had been reported as the reservoirs (Marchette, 1966). *H. leporispalustris* which parasitizes a wide variety of birds in the ecology of Rocky Mountain Spotted Fever had been postulated as a vector for SFG rickettsiae (Parker, 1923). This study demonstrated that *H. conigera* is also associated with spotted fever rickettsiae. This species has not been linked with a rickettsiae of the SFG previously either by antibody tests or rickettsiae isolation. The role of *H. conigera* in the ecology of SFG rickettsiae needs to be further investigated.

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