

Distribution of *Rickettsia tsutsugamushi* Strains in Thailand

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OBJECTIVE : To determine the geographical distribution of various *Rickettsia tsutsugamushi* strains occurring in natural populations of vector chiggers in Thailand.

BACKGROUND : The vectors of scrub typhus feed on mammals only once during their life cycle. Transovarian transmission must therefore occur for the larval mite to act as a vector of the rickettsial organism. Consequently, examination of unfed larvae can establish whether wild-caught mites are infected with the disease organism. Formerly the detection of *Rickettsia tsutsugamushi* in different chigger vectors depended primarily on serological and clinical findings in laboratory animals. Animal isolation procedures are long and laborious, sometimes taking two to three months before the final results are determined. Now a technique using direct immunofluorescence has been developed to detect the rickettsia in naturally infected mites (1). Using this technique nine different strains of *Rickettsia tsutsugamushi* can be screened against, using the internal contents of each unengorged chigger.

METHODS : Unengorged chiggers were collected in Chiangmai, Korat, and Ubon Provinces from various types of habitat such as grassland, scrub and forest. Formica black plates measuring 5" x 5" were used to find chiggers that were resting on leaves or grass. Larval mites seen moving across the plates were picked-up with a moist applicator stick and placed into screwcap vials containing water. Collections of live chiggers were then sent to USAMRU in Malaysia for testing by direct immunofluorescence. Identification of the chiggers was also done at this time. The nine strains of *Rickettsia tsutsugamushi* used in the preparation of conjugates in this study were as follows : Karp, Gilliam, Kato, TA678, TA586, TA686, TA716, TA763, and TH1817. The first three strains are prototype scrub typhus strains and the other six are strains originally isolated from Thailand.

Chiggers were placed individually in five lambda of 0.5% normal yolk sac suspension. The exoskeleton of the chigger was punctured dorsal-posteriorly and the internal contents squeezed out. The contents were spotted in ten predetermined areas on a microscope slide with four mites being tested each time. Slides were fixed with carbon tetrachloride and dried at room temperature. The exoskeleton of the mite was mounted in a drop of Hoyer's mounting media for species identification.

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Fixed slides stored at -20°C were warmed to room temperature before testing by direct immunofluorescence. Each group of spots was ringed with nail polish and the strain-specific conjugates were added to the spots. Slides were read within 24 hours.

RESULTS : Over 2,400 chiggers were collected off black plates at 24 different locations in the three provinces. Most of the mites collected were *Leptotrobium* (L.) *deliense*. Not all chiggers were analysed in USAMRU-Kuala Lumpur due to mortality and loss of chiggers from the collecting vials. The results of the direct immunofluorescence test to detect the presence of *Rickettsia tsutsugamushi* in the different species of mites are not yet complete and will be reported later.

REFERENCES

1. Dohany, A.L., Shirai, A., Robinson, D.M., Ram, S., and Huxsoll, D.L. (1977). Identification of *Rickettsia tsutsugamushi* in naturally infected chiggers (Acarina : Trombiculidae) by direct immunofluorescence. (Submitted for publication).