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Project Title: Collection of unengorged larvae of trombiculid mites and establish methods of *Orientia tsutsugamushi* isolation.

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Executing Institute: Centers for Disease Control
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Abstract:

Tsutsugamushi disease is transmitted by unengorged larvae of trombiculid mite, which carrying the pathogen *Oreintia tsutsugamushi*(OT). In the past, trombiculid mites survey have mainly been done by rodents trapping. Although this method was easily preformed and can detect *O. tsutsugamushi* from mites. However we cannot make sure whether the pathogen was from mites or rodents. Therefore rodents trapping was not suitable to confirm vector species. We need to detect unengorged larvae of trombiculid mites on the ground instead. In this study, we spent two years to capture rodents and collect free-living unengorged chiggers in Hualien County, Kinmen County, Taitung County, Lienchiang County and Penghu County. Also, we finished counting chiggers number from rodents, nested-PCR detecting and isolating *O. tsutsugamushi* on chiggers and analyzing phylogeny of our isolated strains based on 56 kDa protein gene. According to field surveillance, chiggers' abundant and harboring *O. tsutsugamushi* were found in grass near a gas station on Jian-guo road, Chian town, Hualien County and Nanlian village, Hushi town, Penghu County. It should alarm residents and tourists to pay attention. Based on four survey index from nine field surveillance, we found significant linear correction between positive rates of antibody against OT on rodents and OT PCR positive rates in chiggers from rodents($r=0.9015$, $p=0.001$), indicated positive rates of antibody against OT on rodents may be an indicator of the scrub typhus infection possibility. Twenty- five *O. tsutsugamushi* strains were found by nested-PCR on chiggers, eight strains were identical with strains on gene bank. Karp was the most abundant strain, except Penghu County, which can find in all survey area. Other strains distributed randomly, including Hualien-12, Hualien-9, Hualien-6 and Taitung-3. In addition, 17 strains were different with gene bank strains, showed they maybe new strains. We have successfully isolated 55 *O. tsutsugamushi* strains from chiggers using shell vial technique. Phylogeny analysis based on 56 kDa protein gene full length sequence, we found high divergence in chigger's isolates and patient's isolates. On the phylogenic tree, most of chiggers isolates and patients isolates co-existence, and sequence of 6 chigger isolates were identical to 3 patient isolates, showed epidemiological meaning,

O. tsutsugamushi transferred from chiggers to patients. Through evaluation, three free-living unengorged chiggers collecting methods: bakelite plate, Suzuki method and Direct method all have merit and drawback. Bakelite plate can collect large number of chiggers; collected from the litter and the soil surface (Direct method) can collect larvae, nymphs and adults; collected using black cloth (Suzuki method) can collect chiggers on tip of grass, so the usage of different methods depended on different habitats. Due to positive results on detecting free-living unengorged chiggers from Taitung County and Penghu County by nested-PCR, indicated *Leptotrombidium delience* can tran-ovarian transmission with *O. tsutsugamushi*, and proved it was vector of scrub typhus in such areas.

Key words: unengorged larvae of trombiculid mites, tsutsugamushi disease, *Orientia tsutsugamushi*, phylogeny, PCR