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Project Title: Establishment of differential diagnostic systems for Rickettsial Diseases
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Executing Institute: Center for Disease Control, Department of Health
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Abstract:
Because of the international traffic exchanges become more frequent and greenhouse effect of global climate impact, the spread of vector-borne diseases have expanded rapidly around the world with increased intensity and severity. Similarly, the tendency of these infectious diseases has increased in Taiwan in recent years. Although molecular diagnosis based on polymerase chain reaction (PCR) method and ELISA had been developed for years, laboratory diagnosis of Rickettsial infections was largely relied on serological assay detecting antibody serum conversion between acute and convalescent phase serum samples by immunofluorescence staining. In this study, we reported the development of a real-time SYBR Green I-based quantitative PCR system and ELISA system that can be used to rapidly detect Rickettsial infections in acute-phase blood samples. For scrub typhus fever, two sets of O. tsutsugamushi-specific primer pairs against conserved sequences in the 56kDa outer membrane protein gene and groEL gene were successfully designed and used for routine diagnosis of scrub typhus in Taiwan CDC. The amplification product from real time SYBR Green I-based quantitative PCR were further sequenced to differentiate various serotypes of O. tsutsugamushi. For typhus fever and spotted fever group, primer pairs against conserved sequences in the groEL gene, 17 kDa protein gene and gene D were designed and used to detect epidemic typhus, endemic typhus and spotted fever infections. In addition, primers which targeting 16S rDNA were designed and can be used to detect scrub typhus, typhus group and spotted fever group rickettsia. All these primers had been used for routine diagnosis of rickettsial infections in Taiwan CDC. In the ELISA test, we have been successfully produced 56 kDa recombinant antigens from karp, Gilliam and Kato strains of Orientia tsutsugamushi and Omp B and OmpA recombinant antigens from Rickettsia japonica of spotted fever group. These recombinant antigens can be used to detect rickettsial specific antibodies in the serum samples from patients with rickettsial infection. In the future, real time quantitative PCR and ELISA will replace traditional IFA method for early diagnosis of Rickettsial infection. This improvement will have great impact on the clinical treatment of patients with Rickettsial infections.

Key words: Rickettsia, scrub typhus, typhus group, spotted fever group, Real-Time SYBR Green I-based PCR, ELISA