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Main topic : Other Topics

Ehrlichia canis: Molecular characterization and genetic diversity based on the p28 and trp36 genes

POOLSAWAT N. [1], NOOROONG P. [1], JUNSIRI W. [1], WATTHANADIREK-WIJIDWONG A. [1], SRIONROD N. [1], SANGCHUAI S. [1,2], MINSAKORN S. [1], TAZAWA K. [3], ANURACPREEDA P. [1]

[1] Parasitology Research Laboratory (PRL), Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom 73170, Thailand, Nakhon Pathom, Thailand; [2] Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, Bangkok , Thailand; [3] Worldwide Veterinary Service Thailand, Hang Dong, Chiang Mai 50230, Thailand, Chiang Mai, Thailand

Introduction: *Ehrlichia canis* is a common tick-borne intracellular pathogen causing canine monocytic ehrlichiosis (CME) in dogs worldwide. *E. canis* can affect a dog's monocytes and macrophages, resulting in hematologic disorders and severe clinical signs. The *E. canis* P28 and TRP36 proteins were immunoreactive proteins of *E. canis* that involved with host-pathogen interactions. However, there has not been much information about the genetic diversity of *E. canis* isolates in Thailand when employing *p28* and *trp36* genes. The aims of this study were to investigate the genetic diversity and antigenicity of *E. canis* based on the *p28* and *trp36* genes in dogs in Thailand.

Materials and Methods: A total of 120 dogs blood samples taken from canine shelter or hospital in the northern (Chiang Mai province) and central (Nakhon Nayok province) of Thailand were amplified for *p28* and *trp36* genes of *E. canis* detected by the polymerase chain reaction (PCR). *E. canis* infection status confirmed by PCR assay. Three positive samples of *p28* gene and four positive samples of *trp36* gene of *E. canis* were amplified and cloned into the pGEM®-T Easy vector for sequencing and bioinformatic analyses including phylogenetic trees, haplotype diversity, entropy and epitopes analyses among the isolates discriminated in the present study and those from other countries are also evaluated.

Results: The *E. canis p28* and *trp36* genes were amplified by the polymerase chain reaction (PCR) and cloned for sequencing and bioinformatic analyses. 36% (44/120) of dog blood samples were positive for *E. canis* DNA consisting of p28 (31%, 14/44) and trp36 (69%, 30/44) genes with 792 and 882 bp of PCR products size, respectively. The *E. canis* TRP36 from all Thailand sequences exhibited encoded nine amino acids (TEDSVSAPA) with 11 copies of tandem repeats along the sequences. The phylogenetic trees of *E. canis*, using the p28 and trp36 genes, exhibited that the Thailand isolates fell into two clades and one clade with similarities ranging from 55.95 to 100% and 100%, respectively. The results of diversity analysis revealed 10 and 20 haplotypes of the p28 and *trp* 36 genes, respectively. The entropy analysis of the p28 and trp36 nucleic acid sequences showed 442 and 1321 high entropy peaks respectively, whereas those of the P28 and TRP36 amino acid sequences showed 477 and 388 high entropy peaks, respectively. For B-cell epitopes analysis, the conserved amino acid of P28 and TRP36 sequences has been also demonstrated.

Conclusions: Therefore, the results could be utilized to improve the understanding of phylogenetic relationship, genetic diversity and antigenicity of *E. canis* Thailand isolates.