DETECTION OF *EHRlichia canis* IN CANINE BLOOD SAMPLES BY REAL-TIME FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) PCR AND MELTING CURVE ANALYSIS

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**Abstract.** *Ehrlichia canis* is a small pleomorphic gram-negative, coccoid, obligatory intracellular bacterium and the cause of canine monocytic ehrlichiosis. A real-time fluorescence resonance energy transfer polymerase chain reaction (real-time FRET PCR) coupled with melting curve analysis was established for detection of *E. canis* infection in canine blood samples. The *VirB9* gene was amplified using one pair of primers and the melting curve analysis was generated by heating the hybridizing probes and amplified products. Eight *E. canis*-infected dog blood samples were initially identified using the Giemsa staining/microscopic method followed by conventional PCR (cPCR)/Sanger sequencing for confirmation. The sensitivity and specificity of the real-time FRET PCR detection were 87.5% and 100%, respectively and the limit of detection was $6.6 \times 10^3$ copies of positive *E. canis* control plasmids. The real-time FRET PCR with melting curve analysis reported here is better than microscopic visualization or cPCR because the method is not affected by the false bias inherent in the microscopic method. Furthermore, many samples can be processed rapidly at the same time. This convenient tool is beneficial as an alternative assay for the epidemiologic study of canine ehrlichiosis as well as for eradication of these organisms in prevention and control programs in endemic areas.

**Keywords:** *Ehrlichia canis*, detection, real-time FRET PCR, melting curve analysis