MMS5 e-Conference

Grant number: MRG 6080110

Molecular detection of multiple tick-borne pathogens in canine blood using realtime PCR with high resolution melting (HRM) analysis

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Abstract:

Currently, the prevalence of parasites infected dog has increased. The limitation of the parasite examination takes time-consuming and can detect a single species per a reaction. This research aimed to develop a multiplex real-time PCR HRM technique. This technique was used to simultaneously detect four blood canine parasites including *Babesia vogeli*, *Hepatozoon canis*, *Ehrlichia canis* and *Anaplasma platys*. Our study was divided into four parts: (1) the primer design, (2) the specificity, (3) the sensitivity and (4) the detection of canine blood whether is infected. The results indicated that Protz6 and Bact3 primer were the appropriate primer to detect these agents because their melting temperature (Tm) value derived from two primers can clearly discriminate four parasites. The highest Tm value with 83.10°C was observed in *A. platys* followed by *B. vogeli*, *E. canis* and *H. canis* with Tm value of 82.41, 80.37 and 78.56°C, respectively. For sensitivity, it was found that *B. vogeli*, *E. canis* and *A. platys* have the limit of detection at 10^3 copies/µl while the limit of detection for *H. canis* was 10^4 copies/µl. Of the 68 dogs tested, 34 dogs were infected (50%). We found that *E. canis* showed the highest prevalence with 28 samples while *B. vogeli*, *A. platys* and *H. canis* was detected in 6, 5 and 4 samples, respectively. These results exhibited that a multiplex real-time PCR HRM technique served as the effective tool for the detection of the blood canine parasites.

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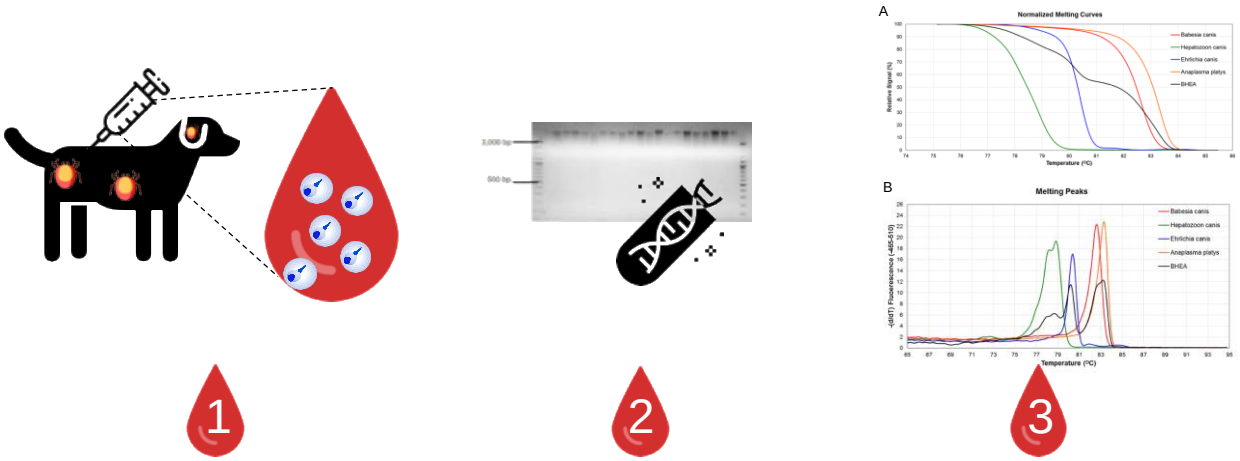
Graphical abstract



The research scope of the MRG 6080110 project