ABSTRACT

MOLECULAR DIAGNOSIS OF HAEMOPATHOGEN INFECTIONS OF COMPANION ANIMALS USING THE REVERSE LINE BLOT HYBRIDIZATION ASSAY

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Arthropod- transmitted haemopathogens of dogs, cats and horses exist in Trinidad and infections caused by these pathogens can lead to severe clinical disease. A definitive diagnosis of these diseases is difficult because of non-specific clinical signs in the host and difficulty in finding organisms in blood smears. Molecular methods are now being explored for detection and differentiation of various arthropod transmitted haemopathogens of animals and to assist in a rapid and accurate diagnosis.

This thesis describes the simultaneous molecular detection of arthropod transmitted haemopathogens of dogs, cats and horses using polymerase chain reactions (PCR) of DNA extracted from blood, followed by a reverse line blot hybridization assay (RLB) of amplified products. A combination of previously published oligonucleotide probes and new probes designed in this study were used for detecting haemopathogens (Chapters 3, 4, 5, 6 and 7), in addition, newly developed oligonucleotide probes for the feline haemoplasmas were validated in a comparison study with a quantitative real-time PCR assay (qPCR) (Chapter 8).

Members of the *Anaplasma/Ehrlichia* genera (*Anaplasma platys*, *Anaplasma phagocytophilum* and *Ehrlichia canis*), *Babesia/Theileria* genera (*Babesia canis vogeli*, *Babesia caballi* and *Theileria equi*) and the feline haemoplasmas (C*andidatus* Mycoplasma haemominutum (CMhm) and *Mycoplasma haemofelis* (Mhf)) were simultaneously detected using the RLB. The agreement between the qPCR and the RLB assays for an overall positive feline haemoplasma result as well as a positive result for CMhm was strong: kappa = 0.662, and 0.872 respectively with specificity and positive predictive values of 100%. *E. canis* (49/348, 14.1%) and *B. caballi* (8/94, 8.3%) were most frequently detected in dogs and horses, respectively and CMhm most frequently detected in cats (26.3 %; 40/152 by RLB and 31.5%; 48/152 by real time PCR). Mixed

infections of *Anaplasma/Ehrlichia* and *Babesia/Theileria* DNA were observed in only 5/88 positive dogs (5.7%).

The RLB assay is a less expensive option for improving the diagnostic capacity for veterinary laboratories in developing nations by providing molecular diagnostic technology for detecting commonly occurring arthropod- borne haemopathogens and can be developed further and applied to detect other agents of economic and public health importance.

Keywords: PCR, Reverse line blot, arthropod transmitted haemopathogens, dogs, cats, horses