Application of LAMP Assay for the Diagnosis of *Ehrlichia canis* in Dogs

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**Abstract**  
Canine monocytic ehrlichiosis (CME) is caused by the rickettsial parasite *Ehrlichia canis* transmitted by *Rhipicephalus sanguineus*. Conventional microscopic examination of blood smear for the presence of morulae in acute infection has been the mainstay in the diagnosis of *E. canis*. Molecular technique such as the Loop mediated isothermal amplification (LAMP) which is gaining momentum recently to detect sub-clinical form of infection. The LAMP technique has been employed in the current study to assess its efficacy in the diagnosis of *E. canis*. A positive result was obtained in 87 (61.70%) out of 141 samples in contrast to 23 (16.31%) in blood smear examination.

**Key words:** LAMP, *Ehrlichia canis*, dogs

Dogs may develop canine monocytic ehrlichiosis (CME) 8 - 20 days after infection. The disease can appear in its acute, subclinical, or chronic form (Harrus and Waner, 2011). Acute phase of the disease is characterized by clinical signs such as pyrexia, depression, lethargy, anorexia, splenomegaly, conjunctivitis, and ocular discharge. Thrombocytopenia and leukopenia are common laboratory findings of this phase. Subclinical phase is characterised by the absence of evident clinical signs other than mild thrombocytopenia, which can last 40 - 120 days to years. A matter of concern is the progression of CME to the chronic form, which is characterised by the presence of physical and hematological signs similar to those seen during the acute phase (Faggion et al., 2013). CME is a multisystemic disease with clinical signs overlapping other infections; therefore its diagnosis can be improved by using specific diagnostic tests combined with physical and hematological evaluation (Harrus and Waner, *loc. cit*). Morulae can be identified only in the acute phase of the disease while it is not present in other phases. Serological tests are unreliable as the antibody titres remain high long after the disease has subsided. More recently, a molecular technique termed loop mediated isothermal amplification (LAMP) has gained significance due to its cost effectiveness as it disposes the use of a thermal cycler and possibility of being used as a pen-side assay. Hence, in the current study LAMP technique has been employed for the diagnosis of ehrlichiosis in dogs.

**Materials and Methods**  
Whole blood samples in an anticoagulant were collected from 141 dogs with clinical signs such as pyrexia with lymphadenitis, tick infestation and epistaxis, from cases presented at the Small Animal Clinics of Madras Veterinary College Teaching Hospital and private clinics in Chennai. Blood smears collected from suspected animals were subjected to Giemsa’s staining technique and examined.

DNA was isolated using QIAMP DNA mini kit (Qiagen, Germany). The step by step protocol as given by the manufacturer was followed. The isolated genomic DNA was subjected to quantification and purity assessment by nanodrop technique. The procedure for diagnosis of canine ehrlichiosis using LAMP was based on the protocol of Faggion *et al.* (*loc. cit*) with slight modifications. The sequence of primers used in LAMP assay and the composition of reaction mixture are as mentioned below.

Each LAMP reaction mixture contained 1 μl extracted DNA, 20 pmol of each FIP and BIP primer, 5 pmol of each F3 and B3 primer,

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0.5 mM of each dNTP, and 1X ThermoPol reaction buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄, 0.1% Triton X-100, pH 8.8). After the addition of 8 units Bst DNA polymerase large fragment (New England Biolabs, UK), the reactions were incubated at 60°C for 60 min, and subsequently at 80°C for 10 min, to terminate the reaction in a water bath. Since the typical ladder pattern was not obtained, the reaction was then standardised by addition of 3 μl 0.8mM MgSO₄ and 3 μl betaine. A 10 μl aliquot of each reaction was used for electrophoresis on 2.5% agarose gel in Tris-acetic acid-EDTA (TAE) buffer and visualised under UV light after staining with ethidium bromide.

**Results and Discussion**

Of the 141 Giemsa stained blood smears, morulae of *Ehrlichia canis* was detected in 23 (16.31%) blood smears. In the LAMP assay, 87 (61.70%) samples were diagnosed to be positive for *E.canis* due to the presence of typical ladder pattern.

Based on the results obtained it can be inferred that LAMP assay is more sensitive in the diagnosis of *E.canis* compare to conventional blood smear examination. Also, results can be obtained within one hour (Faggion et al., *loc. cit*) and with further development, i.e. using additional loop primers, the reaction incubation period can be further reduced (Nagamine et al., 2002). By using SYBR dye, the use of gel electrophoresis can be eliminated to read the results of a LAMP reaction as the presence of fluorescence indicates a positive reaction. In a LAMP assay, even without using a dye, the results can be directly read due to the formation of visual turbidity in the positive samples.

**References**

