# Introductionleishmaniasis, are available for the diagnosis of leishmaniasis(schal...

Business, Management

IntroductionLeishmaniasis, caused by protozoan parasites of theLeishmania genus (Kinetoplastida order, Trypanosomatidaefamily) represents an important health problem in manyregions of the world. Parasites of the Leishmania (Leish-mania) donovani complex, are the most pathogenic causingvisceral leishmaniasis (VL) which is fatal if untreated(Desjeux, 2001). Canine infection with Leishmania has two effects, firstlyas being a reservoir for human leishmaniasis and secondlyas a cause of a severe disease in dogs (Chargui et al., 2009). Infected dogs have an important role in the transmissionof the disease; they were clinically categorized as symp-tomatic, when they presented one or more clinical signs, and as asymptomatic, when no such signs were present(Rioux et al.

, 1979; Alvar et al., 1994; Dye, 1994). In Tunisia, canine leishmaniasis (CanL) is caused by Leishmania infan-tum, a species transmitted by the bite of vector femalesof the Phlebotomus (Larroussius) subgenus. In spite of itsimportance, few studies have investigated CanL in Tunisia(Dedet et al.

, 1973; Ben Said et al., 1992; Chargui et al., 2007, 2009; Aoun et al., 2009). Accurate and rapid diagnosis of CanL is essential toinitiate an early management of infected cases and to pre-vent transmission.

Several direct methods, i. e. culture ofparasites, microscopy and PCR, and indirect tools, such asserology, are available for the diagnosis of leishmaniasis(Schallig and Oskam, 2002). PCR assays have been used inthe diagnosis of human and canine leishmaniasis in Tunisia(Chargui et al., 2005, 2009). However, there is currently nogold standard for the diagnosis of this disease (Rodriguez-Cortes et al., 2010) and in-house made protocol was

usedby many laboratories for the detection of Leishmania infec-tion (da Silva et al., 2006; Reithinger and Dujardin, 2007; de Lima et al.

, 2011; Costa et al., 2012). Control and surveil-lance programs and accurate diagnosis of canine infectionswould greatly benefit from a sensitive, specific and easyto use diagnostic tool (or combination of tools) (Reithingeret al., 2002).

A recent advance in molecular diagnostics has beendeveloped with the introduction of the loop-mediatedisothermal reaction (LAMP) (Notomi et al., 2000). Thismethod has the potential to combine the high sensitivity ofa molecular diagnostic test with the possibility of perform-ing the test under field conditions with limited technicalresources. The assay uses four to six primers that recog-nize six to eight regions of the target DNA, respectively, inconjunction with the enzyme Bst polymerase, which hasstrand displacement activity. The simultaneous initiationof DNA synthesis by multiple primers makes the techniquehighly specific (Notomi et al.

, 2000). LAMP has emerged asa powerful tool for diagnostics and has been successfullydeveloped for other protozoan parasitic diseases such asthe Plasmodium (Poon et al., 2006; Han et al., 2007); theTrypanosoma (Njiru et al., 2008a, b) and Leishmania (Adamset al., 2010). We have developed in the present study a species-specific L.

infantum LAMP assay for the diagnosis of clinicalCanL using the cysteine protease B (cpb) multi-copy gene astarget. The polymorphic and multi-copy nature of the cpbgenes allows major structural change between species

andstrains of Leishmania (Rogers et al., 2011) and is an excel-lent target gene for the development of species specific andsensitive LAMP assay (Kuru et al., 2011). The sensitivity and specificity of the LAMP assay hasbeen also compared to IFAT, PCR and microscopic diagnosisof L. infantum which has been used as a gold standard