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Application of a recombinant protein for the serological diagnosis of canine leishmaniasis

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Abstract

This work reports the results obtained by a new enzyme-linked immunosorbent assay (ELISA) test developed for the serological diagnosis of canine leishmaniasis.

The new ELISA is based on a recombinant protein obtained by joining different antigens of *Leishmania infantum*.

Test performances have been evaluated through the screening 227 sera of dogs, infected and uninfected by *L. infantum*. The new ELISA test has been compared to the indirect immunofluorescent-antibody test (IFAT) as a reference assay of canine leishmaniasis, and to a commercial ELISA.

Excluding from the total number of IFAT positive sera the 27 sera with IFAT titre 1:40 (considered doubtful), the recombinant ELISA showed 97.0% specificity, 93.9% sensitivity and 95.5% agreement with IFAT. The commercial ELISA showed 78.2% specificity, 94.9% sensitivity and 86.5% agreement with IFAT.

The results demonstrate a higher performance of the new recombinant ELISA test for the detection of negative samples, with a greater agreement with the reference test (IFAT).

Keywords: Canine leishmaniasis; Recombinant antigen; ELISA; Diagnosis; Dog

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Résumé

Ce travail rapporte les résultats obtenus avec un nouveau test ELISA développé pour le diagnostic sérologique de la leishmaniose canine.

Ce nouveau test repose sur l'utilisation d'une protéine recombinante issue de l'assemblage de différents antigènes de *Leishmania infantum*.

Les performances du test ont été évaluées sur 227 sérums de chien infectés ou non par *L. infantum*. Le nouveau test ELISA a été comparé à un test d'immunofluorescence indirecte comme référence de leishmaniose canine, ainsi qu'à un test ELISA commercial.

En excluant du nombre total de sérums positifs par immunofluorescence, 27 sérums qui présentaient un titre de 1:40 (considéré suspect), le test ELISA recombinant présentait 97% de spécificité, 93,9% de sensibilité et 95,5% de confiance avec immunofluorescence indirecte. Le test ELISA commercial a montré une spécificité de 78,2%, 94,9% de sensibilité et 86,5% de confiance avec immunofluorescence indirecte.

Ces résultats prouvent que le nouveau test ELISA recombinant s'avère très performant pour la détection d'échantillons négatifs, avec une plus forte confiance dans le test de référence.

Mots clés: Leishmaniose canine; Antigène recombinant; ELISA; Diagnostic; Chien

1. Introduction

Visceral leishmaniasis (VL) is a severe disease that occurs in different mammalian species, including the human population. VL is caused by hemoflagellate protozoa belonging to the *Leishmania* genus. Wild canids and domestic dogs are the main reservoirs of zoonotic visceral leishmaniasis (ZVL) caused by *Leishmania infantum* [1].

The complexity and the variety of symptoms, and the presence of asymptomatic but infective dogs, hamper the clinical diagnosis of leishmaniasis. Parasitological examinations (bone marrow, spleenic or lymph node aspirate, or cutaneous lesion biopsy) are time consuming, and required trained personal. Although parasitological examination is the most specific technique, it can be scarcely sensitive [2], especially for asymptomatic dogs.

Other approaches to direct detection of parasites have been developed, such as PCR amplification of specific nucleotidic sequences of *L. infantum*. In recent years several studies have shown the interest of PCR for the detection of canine leishmaniasis. Although these methods have considerable sensitivity for the detection of symptomatic or parasitologically proven infections [3–5] theoretically being able to detect the DNA of 0.01 parasite in nested PCR [6] their potential use in routine diagnosis is hampered by the complex procedure [7]. The high sensitivity of PCR methods may depend not only on the targeted gene, but also on the type of DNA extraction procedure and source of biopsy material [8]. Additionally, this method detects amplified parasite DNA not the intact parasite and does not reflect the severity of the infection nor the disease stage [9].

Therefore, serological techniques are commonly used to detect circulating anti-*Leishmania* antibodies. Among serological methods, the indirect immunofluorescent-antibody test (IFAT) is currently considered the reference assay for the diagnosis of