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A simple and rapid method for detection of *Trypanosoma evansi* in the dromedary camel using a nested polymerase chain reaction

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Abstract

A nested polymerase chain reaction (nPCR)-based assay, was developed and evaluated for rapid detection of *Trypanosoma evansi* in experimentally infected mice and naturally infected camels (*Camelus dromedarius*). Four oligonucleotide primers (TE1, TE2, TE3 and TE4), selected from nuclear repetitive gene of *T. evansi*, were designed and used for PCR amplifications. The first amplification, using a pair of outer primers TE1 and TE2, produced a 821-bp primary PCR product from *T. evansi* DNA. The second amplification, using nested (internal) pair of primers TE3 and TE4, produced a 270-bp PCR product. *T. evansi* DNAs extracted from blood samples of experimentally infected mice and naturally infected Sudanese breed of dromedary camels were detected by this nested PCR-based assay. The nested primers TE3 and TE4 increased the sensitivity of the PCR assay and as little as 10 fg of *T. evansi* DNA (equivalent to a single copy of the putative gene of the parasite) was amplified and visualized onto ethidium bromide-stained agarose gels.

Amplification products were not detected when the PCR-based assay was applied to DNA from other blood parasites including *Thieleria annulata, Babesia bigemina* or nucleic acid free samples. Application of this nPCR-based assay to clinical samples resulted in direct detection of *T. evansi* from a variety of tissue samples collected from experimentally infected mice and blood from naturally infected camels. The described nPCR-based assay provides a valuable tool to study the epidemiology of *T. evansi* infection in camels and other susceptible animal populations.

Background

Trypanosoma evansi (*T. evansi*), the cause of trypanosomiasis (Surra), constitutes one of the major veterinary problems worldwide. The disease causes significant morbidity and mortality in camels in the Sudan, which has a population of over 3 million camels [1]. trypanosomiasis in camels occurs both in chronic and acute forms [2]. The chronic form of the disease is most common and is likely to be associated with secondary infections due to immunosuppression [3]. Clinical signs and pathological lesions caused by *T. evansi* in camels are unreliable for definitive diagnosis [4]. In addition, detection of parasites in the blood is difficult because parasitaemia is intermittent [5]. Serological tests have been developed and evaluated for diagnosis of trypanosomiasis in camels. They include card agglutination test and enzyme-linked immunosorbent assay (Ab-ELISA) [6-8]. In general serological techniques are useful for detection of a past infection but not for