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Cloning and expression of Toxocara canis arginine kinase recombinant protein for serodetection of toxocarosis in dogs

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Abstract

Toxocarosis is a widespread zoonosis caused by the ascarid Toxocara canis. Serodiagnosis of toxocarosis is carried out using Toxocara excretory-secretory and recombinant antigens. In the present study T. canis arginine kinase gene was amplified by polymerase chain reaction, cloned in a prokaryotic expression vector and expressed in Escherichia coli. The recombinant protein was used in IgG-ELISA for detection of T. canis infection in the adult dogs. Further evaluation of this recombinant antigen in the diagnosis of T. canis infection in dogs is under way.

Keywords: Toxocara canis, Dog, Arginine kinase, Recombinant antigen, ELISA, Serodiagnosis.

Introduction

Toxocara canis is the etiological agent for toxocarosis in dogs and other canids. Humans become infected with T. canis by ingestion of infective eggs in contaminated environments, leading to larval toxocarosis. Direct demonstration of the adult parasite, larvae or eggs is the only way to make a definite diagnosis but in T. canis infection in dogs, infective second-stage larvae migrate through the organs where detection is based on serological methods. Visceral larva migrans (VLM) caused by T. canis in animals and man is an occult infection and can be accurately detected by serological assays. The most widely used method for diagnosis of VLM is ELISA, which is based on larval excretory-secretory antigens (Glickman et al., 1978; De Savigny et al., 1979; Radman et al., 2000; Iddawela et al., 2007). Although the sensitivity (78–91%) and specificity (86–92%), (Glickman et al., 1978; Radman et al., 2000; Iddawela et al., 2007) of larval ES antigens are fairly high, cross-reactions to other parasites, especially nematode parasites have been identified (De Andrade Lima et al., 2005). The use of recombinant antigens offer significant benefits for toxocarosis detection because of their increased sensitivity and specificity compared to the assays using native TES antigens (Yamasaki et al., 1998; 2000; Fong et al., 2003; Fong and Lau, 2004; Norhaida, et al., 2008) but more validation studies are needed to establish their specificity and sensitivity for use as diagnostic reagents.

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Arginine kinase is a member of phosphagen kinases widely distributed among invertebrates. It catalyzes the reversible transfer of phosphate from MgATP to arginine yielding phosphoarginine and MgADP (Ellington, 2001, Wickramasinghe et al., 2007, 2008). Arginine kinase in T. canis was characterized by Wickramasinghe et al. (2007), who developed an arginine kinase based antigen capture ELISA in a mouse model for its future use in the diagnosis of toxocarosis (Wickramasinghe et al., 2008). In the present study, arginine kinase gene of T. canis, isolate Izatnagar was cloned and expressed as recombinant protein in Escherichia coli. The protein demonstrated the diagnostic potential in the detection of T. canis larval infection in dogs using IgG-ELISA.

Materials and Methods

Isolation of total RNA: Adult T. canis worms were retrieved from two street pups (~ two months old) naturally infected with T. canis, after treatment with piperazine citrate @ 200 mg/ kg body weight. Total RNA was isolated from the live worms by their lysis in Trizol (Invitrogen, USA), following standard protocols. Total RNA was isolated from 50-100 mg tissue of the adult worm by trituration in 1 ml of Trizol reagent using pestle and mortar. Worm lysate was incubated for 5 min at room temperature to permit the complete dissociation of nucleoprotein complex. Chloroform