DIAGNOSIS OF BOVINE TUBERCULOSIS IN WILD ANIMALS BY POLYMERASE CHAIN REACTION

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ABSTRACT

Diagnosis of Mycobacterium bovis is important from the public health perspective since control measures, treatment are very significant, particularly in wild animals. To diagnose M. bovis infection in wild animals polymerase chain reaction had been used, using the primer sequence of pnc A 8 and pncA 11 that are specific for M. bovis pnc A gene. A 744 bp PCR product was generated out of 3 samples that consisted of blood, caseated nodule, and lung tissues collected from hyena, bear and lion maintained at Arignar Anna Zoological Park, Chennai, Tamil Nadu and were identified as M. bovis. A positive control of M. bovis DNA procured from Tuberculosis Research Centre (TRC), Chennai, Tamil Nadu was used and a negative control of a healthy bovine blood was used. The present study demonstrates the use of PCR as a sensitive diagnostic gadget for diagnosing bovine tuberculosis (BTB) among wild animals.

INTRODUCTION

The members of Mycobacterium tuberculosis complex (M. africanum, M. bovis, M. microti and M. tuberculosis) are closely related but differ in Epidemiology and clinical presentation as TB among humans, domestic animals and wild animals (Cosivi et al 1998, Modo et al., 1996 and O. Reilly and Daborn 1995)

In the clinical lab, phenotypic tests such as colony morphology, nitrate and niacin tests are used to identify M. bovis but due to its time consumption for growth and tediousness genotypic identification is being utilized for this purpose. Distinguishing M. bovis infection is very important from the public health point of view. M. bovis is naturally resistant to the antituberculosis drug Pyrazinamide (PZA). Demonstration of this PZA monoresistance is a characteristic finding seen in M. bovis (Niebe et al., 1999 and Hannen et al., 2001). In this present study pncA gene that is for this was selected for the diagnosing of M. bovis in wild animals using the primer sequence pertinent to pnc A gene.

MATERIAL AND METHODS

Mycobacterial strains and genomic DNA isolation

To investigate the incidence of M. bovis infection in wild zoo animals viz., Hyena, lion, bear reared at Arignar Anna Zoological Park, Chennai, Tamil Nadu, samples consisting of citrated blood from live animals and tissue samples consist of caseated nodules of lung, liver and other organs from dead animals were collected. DNA extraction for PCR was performed as per the BIOGENE manufacturer’s protocol.

Polymerase Chain Reaction

The entire pncA open reading frame, as well as 124 bp of upstream and 59 bp of downstream sequence, was PCR amplified. A 744 – bp PCR product was generated using the primers

pncA – 8

(5’-GGTTGGGTGGCCGCCGGTCAG-3’)

pncA – 11

(5’ – GCTTTGCGGCGAGCGCTCCA-3’).

The pnc A open reading frame (561 bp) begins at nucleotide 125 of the 744 -bp PCR product and ends at nucleotide 685. each 50 µl PCR mixture contained 1.0µl of template DNA, 2.5 U of Taq DNA polymerase, deoxynucleotide triphosphates (dNTPs; 200µM, each), add a 0.5µM concentration of
each primer in 1X PCR buffer. Amplification was performed in a Gene Amp PCR system 2400 thermal cycler using a “touchdown” amplification approach in which the primer annealing temperature was decreased 0.5°C per cycle for the first 20 cycles, from 60°C the first cycle to 58°C for 20 to 35 cycles. The amplification profile consisted of an initial 5 min of denaturation at 94°C; 35 cycles of 94°C for 30 sec, annealing for 30 sec, and elongation at 72°C for 30 sec; final 8-min elongation.

RESULTS AND DISCUSSION

Three samples were subjected to PCR for diagnosing bovine tuberculosis (BTB) among wild animals. The samples were suspected to have been infected by BTB. During the study period, the blood samples collected from a live Hyena reacted positive for M. bovis. Using the pnc A gene primer sequences the entire pnc A open reading frame, along with the 124 bp upstream and 59 bp of downstream sequence was PCR amplified which generated a 744 bp PCR product (Fig. 1). The finding of this case with a positive PCR reaction for M. bovis using this pnc A gene primer among the wild animals has not been reported earlier anywhere from India (Hannan et al., 2001).

Figure 1: Polymerase Chain Reaction of M. bovis; Lane 1: 100 bp ladder; Lane 2: Positive control - DNA procured from Tuberculosis Research Center; Lane 3: Lion; Lane 4: Bear; Lane 5: Hyena - 744 bp PCR amplicon.
The results using pnc A gene were reproducible. This pnc A gene is being used for genotypic identification of M. bovis species. This is performed by identifying the characteristic mutations in the pnc A gene that distinguishes it from the other members of the M. tuberculosis (MTB) complex, but it is not generally available in clinical laboratories (Scorpio et al., 1997; Sreevatsan et al., 1996 and Hannan et al., 2001).

M. bovis is essentially a zoonotic disease that affects cattle and many domestic and wild animals. Pyrazinamide (PZA) monoresistance is being used as an indicator of M. bovis species in laboratories (Hannan et al., 2001). PZA monoresistance was taken as the reliable marker of M. bovis to diagnose bovine tuberculosis in wild animals. PZA is an important first line anti-tuberculosis drug and is an important component of WHO directly observed treatment short course chemotherapy strategy (Van Soolingen et al., 1994). A 744 bp PCR product was generated using the pncA 8 and pnc A11 primers which agreed with the other experts (Hannan et al., 2001). Genotypic identification of M. bovis species can be performed by identifying the pncA gene of M. bovis that will distinguish it from other members of Mycobacterium tuberculosis complex (Sreevatsan et al., 1994, Scorpio et al., 1997 and Hannen et al., 2001). As M. bovis is naturally resistant to PZA, this particular primer for pnc A gene were selected to diagnose M. bovis.

The results thus obtained using pnc A gene are in association with other findings (Van Soolingen et al., 1994 and Hannan et al., 2001). The results indicate that the 744 bp pncA amplicon is very characteristic of M. bovis. This study shows that pnc A gene is well suited in diagnostic point of view for bovine tuberculosis diagnosis in wild animals. In addition DNA typing is being planned to be undertaken to study the epidemiology of bovine tuberculosis in wild animals.

Differentiation of M. bovis in wild animals in wild animals from M. tuberculosis species is essential in public health perspective. As M. bovis is resistant to PZA, the finding of PZA monoresistance is considered to be the hallmark of M. bovis species (Nolte and Metchock, 1999) and hence the pnc A gene was selected for its diagnosis. The control did not have any amplification that was performed using the healthy bovine blood. Due to technical difficulty in the diagnosis of BTB in wild animals such as obtaining reliable results from Single Intra Dermal (SID) testing, growth of the organism in the very acidic medium that is required for PZA activity as a result of which PZA susceptibility tests are often unreliable (Davies et al., 2000 and Hewlett et al., 1995).

The results of this study highlight that TB can be caused either by M. tuberculosis or M. bovis species, although clinically they cause very similar disease (Greilly et al., 1995 and Cosivi et al., 1998). As BTB is essentially an important zoonotic disease its differential diagnosis and the monitoring of the spread of the disease to humans is very crucial and an improved veterinary public health is very much needed.

With the aid of PCR analysis, a more rapid, reliable diagnosis is possible and a differential diagnosis from other MTB complex is possible than the usual biochemical tests that are used conventionally.

In the move towards the TB elimination, early, accurate identification of the organism is a must and it is very essential to examine the clinical impact of BTB among wild animals.

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REFERENCES