RESEARCH ARTICLE

DNA based Survey of Protozoan Parasites in some common herpatofauna of Amravati-Maharashtra (India)

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ABSTRACT

The study of parasite diversity not only helps to estimate the diversity of organisms but also help to address question related to host specificity and coevolution. Hepatozoonosis, is an enzootic disease caused by several species of Hepatozoon, a genus in the phylum Apicomplexa. Hepatozoon infect wide variety of domestic and wild animals. The present pilot study was aimed to screen the common herpatofauna namely garden and wall lizard for the presence of Hepatozoon species using DNA based markers specific for 18S rRNA region. The findings of this study confirms the occurrence of Hepatozoon in garden and wall lizard using HepF300 and HepR900 primers targeted for ~800 bp of 18S rRNA gene. The study also highlights the need to screen more taxa for the occurrence of this parasite in both domestic and wild species to understand parasetemia caused by Apicomplexan species.

Key words : Apicomplexan, Hepatozoon, herpatofauna, 18S rRNA.

INTRODUCTION

Parasites, one of the potential cluster of organism contribute to 50% of diversity of living fauna on the earth planet, comprising different species of bacteria, viruses and eukaryotes (Brooks and Hoberg, 2007). Among all the parasites, apicomplexan are the most dominant parasitic protists with huge vicinity of host and habitat. Some of them like gregarines are generally found in mollusca, annelids and arthropods, while coccidians are the parasite of vertebrates and haemogregarines generally found in blood stream of vertebrates as well as in arthropods. Hepatozoon are well known to parasitize the wide range of vertebrates from herpatofauna to mammals. Some comprehensive studies suggest the existence of 300 different species of hepatozoon with their diverse morphological features and life cycles (Smith 1996, Smith and Desser, 1997). They are the member of haemogregarinidae family; conjoin with erythrocytes and blood cell producing organs like spleen, bone marrow, lymph nodes of host animals (Baneth *et al.,* 2003, Golen, 2004*).* Hepatozoons are categorised in 6 major genera and shows significant

similarity in morphological traits as well as life cycle patterns with most prevalent host like tetrapods and reptiles (Telford, 2009). Hepatozoons are the host specific parasites inhabiting wide range of vertebrates including some domestic canides (Forlano *et al*., 2005) and responsible for the Canine hepatozoonosis (James, 1905). Development of molecular techniques and availability of whole genome information of wide range of protists facilitates the emergence of some specialise disciplines like molecular parasitology and vector biology. Most of the parasites are widely used as molecular markers to resolve the questions related to the diversity of vast range of animal species (Gardner and Campbell, 1992) as well as host specificity and co-evolution (Poulin and Mouillot, 2005, Paterson and Piertney 2011).

The present study was undertaken to identify some common protozoan parasites in herpatofauna of Amravati using DNA based markers.

MATERIALS AND METHODS

DNA was extracted from road killed garden and wall lizard using DNA sure mini prep kit™ of Genetix Biotech Asia Pvt. Ltd. 800 bp of 18S rRNA region was amplified using Hepatozoon specific primers, *HepF300* and *HepR900*. The PCR amplification were carried out in 50 ul of reaction mixture containing 2.5 mM MgCl2, 200 µM dNTP, 1 µM of each primer and 0.50 U Taq polymerase. Initial denaturation of 3 minutes at 94°C was followed by 35 cycles cycle was consist of denaturation of 30 seconds at 94°C, primer hybridization of 30 seconds at 60°C and extension of 1 minutes at 72°C. Products were finally extended for 10 minutes at 72°C. After the completion of PCR, the amplicons were separated by loading 5 µl of PCR product onto 2% agarose gel. The sizes of unknown bands were estimated by comparison with 1 kb DNA ladder. Photographic images of agarose gel were taken to observe the presence ~800 bp of hepatozoon specific stretch.

RESULTS AND DISCUSSION

Using long range molecular markers, we investigate the presence of hepatozoon species in some commonly found herpatofauna including garden lizard and wall lizard Figure 1. (S. No-1 and S. No-2). Presence of band of with ~800 bp reveals the existence of hepatozoon in the blood stream of above experimental animals.

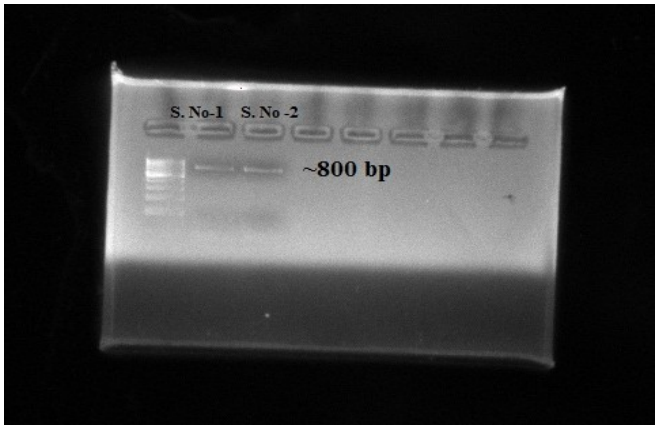

Fig1- 800 bp amplification of 18SrRNA region in garden and wall lizard using Hepatozoon specific *HepF300* and *HepR900*

Screening of protozoan parasites in economically important animals like chicken, cattle etc will help in the management and epidemiology of protozoan diseases using DNA based marker.

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