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Research Article

MOLECULAR STUDIES ON HEPATOINTESTINAL SCHISTOSOMES IN CATTLE

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ABSTRACT

A molecular study was carried out on hepatointestinal schistosomes in cattle for characterization of species. The worms were recovered from the liver and mesentery from the cattle slaughtered at the abattoir from Bengaluru and Hassan district. Polymerase chain reaction was carried out for Schistosoma worms by targeting genus specific 12S ribosomal RNA and cytochrome oxidase subunit I and species specific mitochondrial gene (SPMit) for *S. spindale* and SI 16S RNA for *Schistosoma indicum*. All the isolates yielded 328 and 1088 bp DNA fragments specific for Schistosoma genus and 330 bp specific to *S. spindale*. No specific amplification was obtained for *S. indicum*. The sequencing and BLAST analysis of 328 bp amplicon showed homology with *S. spindale* isolates deposited at GenBank database. Phylogenetic analysis indicated that *S. spindale* Karnataka isolate(Accession no. MG052937) shared 100 per cent homology with isolate of *S. spindale* from Sri Lanka (EF534282).

Key words: Abattoir, Cattle, Liver, Mesentery, PCR, *S. spindale*

Schistosomosis in bovines is widely reported as a disease entity in many parts of Asia and Africa. Schistosomosis is one of the most prevalent infectious diseases, endemic in more than 70 countries, mainly within the developing world (Hovnanian *et al.*, 2010). About 530 million of cattle live in areas endemic for bovine schistosomosis in Africa and Asia while at least 165 million cattle are infected with schistosomes worldwide (Bont and Vercruysse, 1997). Schistosomosis is now well recognized as the fifth major helminthosis of domestic animals in the Indian sub-continent. Identification of worms based on phenotypic may be difficult due to overlapping of the characters *viz*., length of flukes, intestinal caeca and number of testes. Molecular techniques based on genomes are very useful for the epidemiological diagnosis as well as for research on genetic variation of the parasitic organism. Hence, the present study was undertaken for the molecular characterization of the worms.

MATERIALS AND METHODS

Extraction of genomic DNA: The collected worms from liver and mesenteries were subjected to DNA extraction. The DNA was extracted from the worms using QIA amp DNA extraction mini kit (Qiagen, Germany) as per the protocol described by the manufacturer.

Polymerase chain reaction: A total reaction volume of 25 µl pre mixture of PCR consisting of two micro litre of forward primer, two micro litre of reverse primer, five micro litre of template DNA and 12.5 µl of PCR master mix (2X) (Genei Laboratories Pvt. Ltd.) and 3.5 µl of NFW was constituted.

The PCR was carried out in a gradient thermal cycler (Eppendorf, Germany) programmed for genus specific Schistosoma primers. No template control was maintained without a DNA template. DNA extracted from the blood containing Babesia organisms was used as a negative control. *S. spindale* DNA obtained from Dr. Sreenivasa Murthy, Department of Veterinary Parasitology, Veterinary College, Hyderabad was used as positive control. Primary pairs used and thermal cycles followed are given in Table 1-3.

Sequence alignment and phylogenetic analysis: The PCR products of 12S ribosomal RNA (rrnS) gene of *Schistosoma* spp. were sequenced at M/s Bioserve biotech, Pvt Ltd, Hyderabad. The BLAST analysis for the obtained sequences was performed on GenBank database at National Centre for Biotechnologies Information (NCBI) on the website www.ncbi.nlm.gov/ BLAST. The sequences were aligned with the published sequences deposited in the GenBank database and the phylogenetic tree was constructed by maximum likelihood method with 1000 bootstrap replicates using MEGA 6.0 software (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

PCR was carried out by targeting Schistosoma genus specific 12S rrnS, COI and species specific SPMit and SI16s RNA genes. All the isolates yielded 328 (Fig. 1) and 1088 bp (Fig. 2) DNA fragments specific for Schistosoma genus and 330 bp (Fig. 3) specific to *S. spindale*. No specific amplification was obtained for *S. indicum*. Genus specific 328 bp amplicon of both Bengaluru and Hassan isolates were selected and were subjected for nucleotide sequencing. The sequencing and BLAST analysis of 328 bp amplicon showed homology with *S. spindale* isolates deposited at GenBank database. Phylogenetic analysis showed that *S. spindale* Karnataka isolate (Accession number MG052937) (Fig. 4) shared 100 per cent homology

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Table 1
Primers and their sequences used for generic identification of *Schistosoma* blood flukes

Name of primer	Primer sequences (5´-3´)	Amplicon size	Targeted gene
SrrnS-F	TCGAGATTGTCGGGC GATGTAC	328 bp	12S rRNA
SrrnS-R	TAGATTCGTCCGGGGG AATGTGC		
SCOI-F	GGTGGATTTATAGGTCT TGGGTTAAG	1088 bp	cytochrome oxidase subunit I
SCOI-R	TCACAAATGGCCACCACC AAACCAACATTA (Sreenivasa Murthy, 2011)		

with isolate of *S. spindale* from Sri Lanka (EF534282) and 99 per cent with *S. spindale* isolate from Bangladesh (EF534281) and Nepal (KR607254).

The present findings were in agreement with the immunological and molecular studies on cattle and buffaloes in Bengaluru conducted by Sreenivasa Murthy (2011) who reported *S. spindale*, *S. indicum* and *S. nasalein* Bengaluru based on PCR. Sreenivasa Murthy (2011) detected and differentiated *S. spindale*, *S. indicum*, *S. nasale* by using 12S rrnS and COI genus specific primers which yielded 328 bp and 1088 bp DNA fragments and these products were sequenced and analysed by NCBI database for the species confirmation and phylogenetic tree was constructed. Similar work was carried out by Lakshmanan (2014) from Kerala and Hossain *et al.* (2015) from Bangladesh.

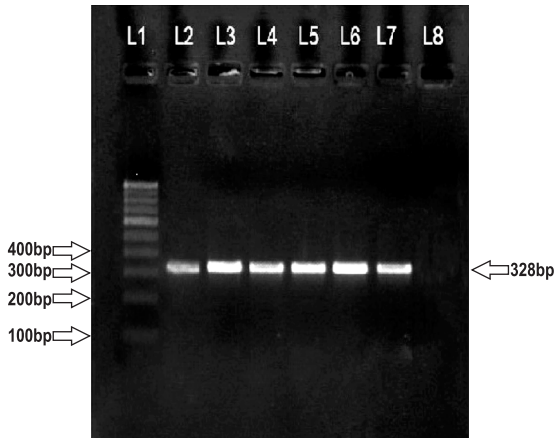

Fig. 1. PCR analysis of 12S ribosomal RNA (rrnS) *S. spindale*

In this study, the phylogenetic analysis 12S rrnS of *S. spindale* Karnataka were in the same clade as *S. spindale* isolate from Sri Lanka deposited in the GenBank (EF534282) and shared 100 per cent identity and also shared 99.99 per cent identity with *S. spindale* from Bangladesh (EF534281) and Nepal (KR607254). Sreenivasa Murthy (2011) from Bengaluru had reported that phylogenetic analysis of 12S ribosomal rRNA showed that *S. spindale* was related to distinct cluster of *S. nasale* from Bangladesh with 660 nucleotide substitutions. Similarly, Lakshmanan (2014) from Kerala reported that phylogenetic analysis of 28S rRNA of *S. spindale* from Kerala shared 100 per cent identity with *S. spindale* from Sri Lanka (AY157257). However, further research work is needed to know the prevalence of *Schistosoma* spp. in other regions of Karnataka and to implement proper control measures.

Table 2
Primers and their sequences used to identify *Schistosoma* species

Name of primer	Primer sequences (5´-3´)	Amplicon size	Targeted gene	Species of *Schistosoma*	Reference
SPMit F	CTTGGAGTCGGGTTGTTTGAG	330 bp	Mitochondrial gene	*S. spindale*	Lakshmanan, 2014
SP Mit R	CAGACCCTCACACCAACAGTG				
SI 6sRNAF	GAGTTTGTAAATGGAGGCTGAG	606 bp	16sRNA	*S. indicum*	
SI 16sRNAR	CCTTATTCAGCCTCTACACCG				Attwood *et al*., 2007

Table 3
PCR cyclical conditions for different genes of *Schistosoma* spp.

Target gene	Initial denaturation	Denat-uration	Annealing	Extension	No. of cycles	Final Extension	Reference
Schistosoma genus	94 ºC/5 min	94 ºC/30 sec	50 ºC/1 min	72 ºC/1 min	39	72 ºC/10 min	Sreenivasa Murthy, 2011
Schistosoma spindale mitochondrium (SPMit)	95 ºC/5 min	95 ºC/1 min	54 ºC/1 min	72 ºC/1 min	35	72 ºC/10 min	Hossain *et al.*, 2015
Schistosoma indicum 16S rRna (SI 16SrRna)	95 ºC/5 min	95 ºC/1 min	52 ºC/1 min	72 ºC/1 min	35	72 ºC/10 min	Hossain *et al.*, 2015

L1: 100 bp ladder; L2: positive control; L3, L4, L5, L6 and L7: Samples of S. spindale; L8: negative control.

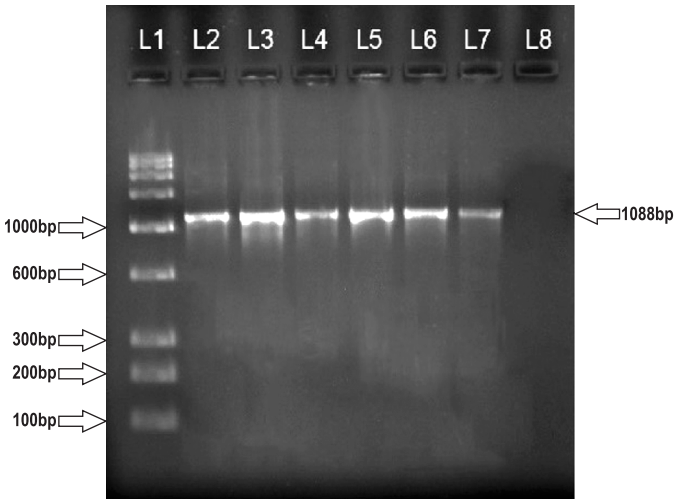

Fig. 2. PCR analysis of COI gene of *S. spindale*

L1: 100 bp ladder; L2: positive control; L3, L4, L5, L6 and L7: Samples of *S. spindale*; L8: negative control.

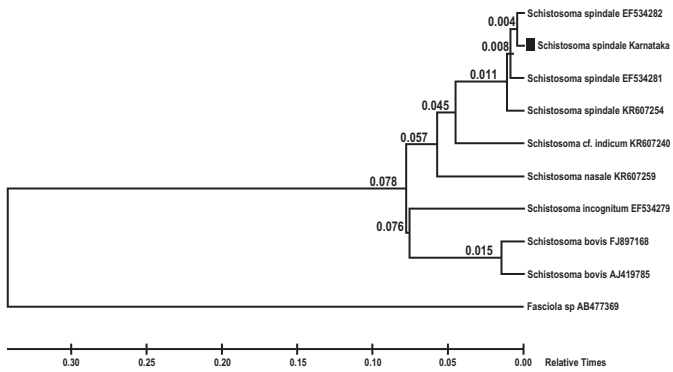

Fig. 4. Phylogenetic analysis of 12S ribosomal RNA (rrnS) gene nucleotide sequence of *Schistosoma spindale*

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L1: 3.5 kb ladder; L2: positive control; L3, L4, L5, L6 and L7: Samples of S. spindale; L8: negative control.

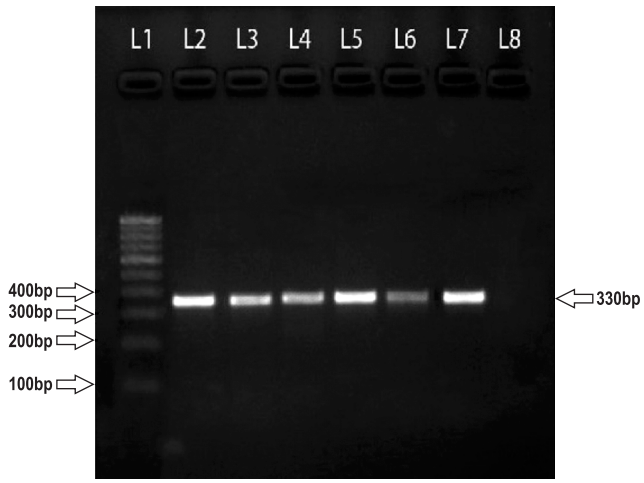

Fig. 3. PCR analysis of mitochondrial gene (SPMit) *S. spindale*

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