Abstract

The present studies incorporate the helminth faunal spectrum of rodent hosts from bamboo growing areas in Mizoram, Northeast India. In the state of Mizoram it is widely believed that rodent outbreaks occur periodically with the gregarious flowering of bamboo that occurs periodically after every 48±1 years and bamboo flowering is a harbinger of famine. The phenomenon was predicted for and has occurred again during the period 2007-2009. Keeping these in mind the main aim of the study is to explore and typify the helminth parasites diversity in rodent population in Mizoram, to study the distribution pattern, species composition of the parasite groups prevalent in the rodent hosts and morphological, ultrastructural and molecular characterization of the parasites species and to identify if any of the helminth species emerges as plausible infectious agents that could serve as a potential tool for biological control of the rodent pests.

I. The parasite spectrum

280 commonly prevalent rodent species in the region, all of which belong to the family Muridae and represented six genera and nine species, namely Rattus rattus, R. nitidus, R. norvegicus; Bandicota bengalensis; Berylmys mackenziei, B. bowersi; Mus musculus; Niviventer fulvescens and Cannomys badius, were collected from 12 different localities in Mizoram.

The survey results revealed that in the parasites spectrum nematode emerged as the predominant group represented by 9 genera and species- Trichuris muris, Capillaria hepatica, Trichosomoides crassicauda, Heterakis spumosa, Rictularia sp., Syphacia obvelata, Aspiculuris (Paraspiculuris) pakistanica, Nippostrongylus brasiliensis and Hepatojarakus bandicoti. Followed by cestodes of the cyclophyllidean group that comprised 4 genera represented by Hymenolepis diminuta, Rodentolepis sp., Raillietina celebensis and the metacestode Cysticercus fasciolaris with Hymenolepis diminuta being the most encountered species. Moniliformis moniliformis was the lone acanthocephalan recovered whereas infection by any
trematode was found to be conspicuously missing. In overall analysis *R. rattus* harboured the widest range of helminth parasites with the presence of 8 nematode and 4 cestode species followed by *R. nitidus*, in which 8 nematode, 3 cestode and one acanthocephalan species were encountered.

All the parasites reported in the present studies constitutes the first report from Mizoram, Northeast India, as studies on the parasite of rodents have never been undertaken in Mizoram. Among the rodent hosts *Rattus nitidus, Berylmys mackenziei, B. bowersi, Niviventer fulvescens* and *Cannomys badius* constitute the new host record.

Sex-wise prevalence of parasite revealed that cestodes showed higher prevalence of infections in female than male excepting *Mus musculus*, so, is the same for nematode infection where prevalence of infection was found to be higher in female hosts excepting *B. bowersi* and *M. musculus*

In analysis of locality-wise infection the prevalence of cestode infection was much higher in case of the hosts collected from urban areas excepting *M. musculus*, whereas, nematode infection was found to be higher in majority of the hosts collected from rural areas except in *B. mackenziei, B. bengalensis* and *M. musculus*.

**II. Molecular characterization of metacestode and *H. diminuta***

During the survey, a metacestode of *Taenia* sp. and the adult tapeworm *Hymenolepis diminuta* were frequently encountered. Owing to their frequent occurrences, molecular characterization of the parasite was done so as to supplement morphological criteria.

Genomic DNA of the parasite was isolated using standard ethanol precipitation technique, the rDNA region spanning the ITS1, ITS2 and mitochondrial COI were amplified by PCR. The primers used were designed based on the conserved sequences of *Schistosoma* spp and are considered to be the universal primers for
trematode species. PCR amplification was done following standard procedure with minor modifications. The PCR product was purified using Genei Quick PCR Purification Kit and sequenced in both directions on an automated sequencer. Sequence analysis was carried out using various bioinformatics tool e.g., BLAST, ClustalW, MEGA etc. ITS2 secondary structures of the cestodes were folded with the help of MFold. The secondary structure in Vienna (dot-bracket-dot) format was used as an input for multiple alignment RNA to calculate sequence structure multiple alignment.

The sequences obtained were deposited in GenBank with Accession numbers:

**Sequences deposited in GenBank:**

i) **FJ939132- Hymenolepis diminuta** adult 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

ii) **FJ939133- Taenia taeniaeformis** 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

iii) **FJ939134- Taenia taeniaeformis** adult internal transcribed spacer, partial sequence.

iv) **FJ939135- Taenia taeniaeformis** cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

**Metacestode:** PCR amplification of the three region showed a single band of size 744bp for ITS2, 443bp for ITS1 and 373bp for COI respectively. The BLAST hits result shows that the sequences of the metacestode are closer to those of species of *Taenia* sp. with maximum similarity to *Taenia taeniaeformis*. In pairwise alignment of the ITS sequences and flanking regions of the query sequences with
sequences of *Taenia taeniaeformis* (Hyderabad India isolate) showed the presence of 6.4% mismatches for ITS2 and for ITS1 14.3% mismatches. However, multiple alignment of CO1 of the query sequence with those of the three different geographical isolates showed the presence of 2.1% mismatches with no gap. Phylogenetic trees obtained through both the methods (i.e. NJ and MP) emerged to be quite similar placing both the *Taenia taeniaeformis* and the query sequences in the same clade; the bootstrap values of (90% and above) of the nodes within the same species were large in all the three regions. Morphological similarities supplemented by molecular characterization confirms the metacestode to be *Taenia taeniaeformis*.

**Hymenolepis diminuta:** PCR amplification of ITS2 region showed a single band of size 455bp. The BLAST hits result shows that the sequences are closer to those of *H. diminuta*. Multiple sequence alignment of the query sequence with the sequences of *H. diminuta* Japan isolate shows maximum similarity, though with 8.1% mismatches. In phylogenetic analysis the topology of the trees obtained for ITS2 turned out to be quiet similar placing the *H. diminuta* isolates from Japan, India, USA and Australia in the same clade, with high bootstrap value of 99% for both NJ and MP tree. Thus, morphological similarities supplemented by close matching of the ITS2 region confirms that the cestode that commonly inhabits the intestine of the rodent hosts is indeed *H. diminuta*.

**ITS2 Secondary structure:** ITS2 of *T. taeniaeformis* and *H. diminuta* showed distinct hallmark of a core secondary structure with four helices, helix III being the longest, an UGGU motif in the 5’ end and U-U mismatch in the second helix.

**GC content:** GC richness contributes to physical attributes of RNA secondary structures as there is a correlation between GC content and optimal growth temperatures. The GC content in the ITS2 region was calculated using oligo calculator, the GC content of the metacestode (*Taenia taeniaeformis* Accession no. FJ939133.1) was found to be 61.6%, whereas for *H. diminuta* (FJ939132.1) it was as low as 43.7%.
III. Hepatic histopathological studies in metacestode and *Capillaria* - infected rodents

During the survey *Capillaria hepatica* and the metacestode of *Taenia* sp. showed a considerably high prevalence even up to 50% in several of the hosts species. In view of the frequent occurrence of the two parasites in the liver tissue of the rodents collected, the present studies aimed to find out the effect of these two parasites on the liver of the rodent hosts so to ascertain their potential as a tool for biological control of rodents, for which a histopathological approach was adopted.

Histopathological studies revealed that the presence of these two parasites altered the normal morphology of the liver. The cells appeared spindle shaped with abnormal nuclei around the area where the metacestode occurs. With Masson’s trichrome stain, numerous neoplastic cells were observed with abundant collagen sheath.

In *C. hepatica* infected liver lipid content was found to be more than the uninfected liver, partially calcified worm debris were found in the area where the worms disintegrate. Granulomatous lesions surround the eggs of *C. hepatica* and septal formation was evident within the infected liver.

Abundant eosinophilic cytoplasm was observed in the region where both the parasites occurs adjacently.

Considering the damaging effects of both the parasites on the liver tissue it would be worthwhile to investigate further and ascertain the possibility of using these parasites as a potential biological control tool for the rodent pests.