Diversity of helminth parasites in selected fishes of Tripura

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By

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Parasitism is one of the common mode of lifestyles prevailed among eukaryotes. Presence of parasites becomes evident only after causing disease and sometimes leading to the mass mortality of infested hosts. Parasites belong to different phylogenetically distinct taxa and display a variety of body forms and life histories. Due to complex life cycles of these parasites, they are indicative of many different aspects of their host’s biology, like host’s diet, migration, recruitment, population distinctness and phylogeny. Many parasite species are host specific to some degree and are capable of infecting one or only a limited number of host species.

The hilly regions of north-east India are considered to be one of the hot spots of freshwater fish biodiversity in the world. But, sporadic information is available on the spectrum of helminth parasites in the fishes of northeast India. Except for a few preliminary records, no literature is available about the helminths diversity among different edible and economically important fishes of Tripura. Therefore, the present study was aimed to explore the helminth parasitic spectrum in some slected freshwater fishes of Tripura. A detailed survey of fish-borne helminths was undertaken to determine the parasite diversity, their prevalence, intensity and seasonal variations. For carrying out the study, fishes like *Anabas testudineus*, *Clarias batrachus*, *Channa striatus*, *Heteropneustes fossilis* and *Monopterus cuchia* were selected which have a higher consumer preference in the rural areas of the state. The work components are distributed in three chapters.
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Chapter 1 deals with the light and electron microscopic studies of the parasites recovered from different fishes. With a view to identify the parasites, morphological criteria based on light microscopy, electron microscopy and standard literature references were used. Two different types of trematode parasites namely, Tylodelphys sp. (metacercaria) and Astiotrema reniferum were recovered from H. fossilis and C. batrachus, respectively, during the study period. A total of 8 different species of cestode were identified during the study. Of which 7 caryophyllidean cestodes belonging to the genus Lytocestus (namely, Lytocestus indicus, Lytocestus birmanicus, Lytocestus longicollis, Lytocestus attenuatus, Lytocestus clariae, Lytocestus filiformes) and Djombangia (namely Djombangia penetrans) were recovered from the catfish Clarias batrachus, and one bothriocephalid cestode (Senga sp.) was recovered from Monopterus cuchia. Four different types of nematode species namely Anisakis simplex, Contracaecum osculatum, Camallanus anabantis and Pallisentis ophiocephali were recovered from Clarias batrachus, Heteropneustes fossilis, Anabas testudineus and Channa striatus, respectively.

Chapter 2- out of fourteen helminth species collected, two zoonotically important parasites, which could not be identified morphologically, were further processed for molecular characterization using DNA-based molecular techniques involving two aspects: sequencing and phylogenetic analysis to identify the parasites up to species level. The ITS2 genes were successfully amplified from 2 isolates representing 2 species. BLAST results showed that the sequences of the Anisakis and
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_Contracaecum_ species closely matched those of the other anisakid nematodes belonging to the genus _Anisakis_ and _Contracaecum_, respectively.

Phylogenetic trees were reconstructed using Neighbour-Joining and Bayesian Inference methods. From the analysis, it was found that ITS2 genes proved to be effective marker as shown by the tree reconstructed, which showed that the Anisakidae family to be a monophyletic group with high bootstrap value. This results demonstrate the utility of this gene region at higher taxonomic levels.

Chapter 3- deals with the prevalence, abundance, mean intensity and seasonality of the frequently occurring helminth parasites of the fishes under study. Recovered parasites showed seasonal changes in the freshwater fishes throughout the study period.

The seasonal occurrence of parasites in the freshwater fishes of Tripura revealed that the prevalence of infection was highest during the pre-monsoon in _A. testudineus, C. striatus_ and _M. cuchia_ whereas, in _C. batrachus_ and _H. fossilis_ helminth infection was highest during post-monsoon and monsoon seasons, respectively. The abundance of helminth infection was highest during pre-monsoon in _A. testudineus, H. fossilis_ and _M. cuchia_ and during post-monsoon in _C. batrachus_ and _C. striatus_. However, mean intensity of infection was highest during monsoon season in _A. testudineus, C. batrachus_ and _C. striatus_ and during pre-monsoon season in _H. fossilis_ and _M. cuchia_. The prevalence of helminth infections in all fish hosts showed significant differences among different season, however abundance
and mean intensity values showed significant difference among different seasons only in two fishes namely *H. fossilis* and *M. cuchia*.

The correlation analysis revealed that the prevalence and abundance of helminth parasites in all the hosts (except *H. fossilis*) showed negative correlation with temperature, humidity and rainfall. However, the mean intensity of the helminth parasites in *A. testudineus*, *C. batrachus* and *H. fossilis* showed positive and in *C. striatus* and *M. cuchia* showed a negative correlation with temperature. A negative correlation between the mean intensity of the helminth parasites with humidity was recorded in all the hosts except *M. cuchia*. Whereas, in *A. testudineus* and *C. striatus* a negative correlation and in *C. batrachus*, *H. fossilis* and *M. cuchia* a positive correlation with rainfall was observed.

The cestodes recorded in the study namely, *Lytocestus indicus*, *Lytocestus birmanicus*, *Lytocestus longicollis*, *Lytocestus attenuatus* and *Senga* sp. showed highest prevalence during the post-monsoon season. Whereas, the larval *Anisakis* sp. and *Camallanus anabantis* showed a peak in the monsoon season, and *Contracaecum osculatum* and *Pallisentis ophiocephali* showed peak in the pre-monsoon season. All of the parasites recovered showed significant differences in the prevalence of infection among different seasons. However, *Lytocestus indicus*, *Lytocestus birmanicus*, *Lytocestus longicollis*, *Lytocestus attenuatus*, *Lytocestus clariae*, *Senga* sp. and *Contracaecum osculatum* showed significant difference in abundance while only *Lytocestus indicus*, *Lytocestus birmanicus*, *Lytocestus clariae*,
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*Senega* sp. and *Contracaecum osculatum* showed significant difference in mean intensity in different seasons. The prevalence, abundance and mean intensity of the helminth infections also showed significant correlation with all the meteorological factors like temperature, humidity and rainfall.

Of the samples collected from six different sampling sites in Tripura, the prevalence of helminth infection was found to be highest in Udaipur during Pre-monsoon season, in Pecharthal during monsoon and in Damcherra during Post-monsoon season. The abundance of helminth parasites was found to be highest in the hosts collected from Agartala in pre-monsoon and monsoon season and from Pecharthal in the post-monsoon season. The mean intensity of helminth infection however, was found to be highest in the hosts collected from Damcherra in the pre-monsoon season, from Agartala in the monsoon season and from Pecharthal in the post-monsoon season. Of the different collection sites, the prevalence, abundance and mean intensity of helminth infection in the hosts collected from Pecharthal, Damcherra, Agartala and Udaipur showed significant difference among different seasons whereas, helminths collected from Dharmanagar and Kumarghat showed significant difference in the prevalence of infection only. From among the six different sampling sites in Tripura, namely Dharmanagar, Pecharthal, Damcherra, Kumarghat, Agartala and Udaipur, the prevalence, abundance and mean intensity of helminth infections showed significant correlation with all the meteorological factors like temperature, humidity and rainfall.
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In the present study, the rate of helminth infection in all the hosts increased with increase in temperature except for *C. batrachus*. Therefore, a temperature range of 19-26°C was found to be favorable for the propagation of parasites infecting the host. The results obtained from our research will help the scientific community and also the pisciculturists to know about the parasite species prevailing among different fish hosts in the study area.

Keywords: Diversity, Helminth, Zoonotic, Freshwater, Tripura