NATURAL INFECTION OF SPARGANUM IN FROGS IN NAGALAND (NORTHEASTERN INDIA) - AN AMPHIBIA-BORNE ZOOONOSIS?

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Abstract

Several species of anuran Amphibia in Nagaland (northeastern India) were found to harbour Plerocercoid (=Sparganum) larvae of Spirurida, species of Cestodes in their muscle tissue the adults of which occur as intestinal parasites in felids and canids. In view of their use in traditional medicine and local cuisine among the native tribes, these frog species may possibly be involved as paratenic hosts in dissemination of sparganosis as a zoonosis among human hosts in the region.

Key words

Sparganum, zoonosis, paratenic hosts, amphibians

Introduction

Associations of amphibians with several zoonotic infections have been frequently reported, particularly in southeast and far eastern countries where eating of frogs and treatment of wounds with raw flesh of frogs is customary (Hoeden, 1964; Suzuki et al., 1982; Shen, 1988; Arora, 1994; Bodri, 1994; Mastura et al., 1995a,b). Amphibians are implicated as one of many paratenic hosts for the pseudophyllidean tapeworm of the genus Spirurida, adults of which are common mammalian parasites, particularly in cats and dogs (Miyazaki, 1991). According to the same authority, only one species of Spirurida is reported in Asia: S. erinacei (= mansoni). This cestode is of medical importance as its larval stage, the plerocercoid (= sparganum), causes sparganosis in humans (Huang & Kirk, 1962; Tansurat, 1966; Kittipongkansa et al., 1988). Spirurida involves two intermediate hosts in its life cycle; the first is a copepod ( Cyclops ) in which the Procercoid larval stage develops and the second may be either frog, snake, bird or mammal in which spargana are found in the muscle tissue (Kobayashi, 1931; Mueller, 1938; Iwata, 1972). Humans become infected by three possible routes -through copepod-contaminated drinking water, by ingestion of raw or partially cooked frog or snake-flesh containing spargana (which migrate through the intestinal wall and encyst in the subcutaneous tissue) and through direct contact by application of frog/snake flesh poultice on open wounds.

Material and Methods

Study area

The study area comprises three major localities in the state of Nagaland, northeastern India, which offer varied climatic conditions which are expected to have an impact on the abundance of both amphibian hosts and their parasitic fauna. Of the localities under survey (Fig. 1), Kohima (which is the state capital, district headquarters and the abode of Angami tribe) lies at the foot hill of Japhu Peak, the second highest peak in Nagaland, covering an altitude range of 1444.12 - 1590 +15 - 5 m., has high rainfall, mild temperature in summer ranging from 12 - 21°C and cold in winter with average temperature ranging from 1 - 6°C. Mokokchung, situated to the northeast of Kohima, is another district headquarters and the abode of the Ao tribe; its altitude ranges from 1000 - 1400 m.; the rainfall is high and the temperature is mild to cold through the year, ranging from 8.6 - 25°C and most of the time it is misty and cloudy; the rainy season starts from the month of May and lasts till October (some times until December). Dimapur, another district headquarters and a fast growing commercial town, is identical to any of the tropical plains with an altitude ranging between 195 - 260 m. and has a hot and humid climate for most part of the year.

Hosts collected/surveyed

The anuran hosts collected and examined for plerocercoid infection included in all 13 species under six genera representing 3 families (identified following Dutta, 1997). These included Euphlyctis cyanophlyctis, Limnonectes limnocharis, L. mawphlangensis, Rana khare, R. liebigii, Rana sp.,...
Hoplatastracus tigerinus, Scutiger(?) sp., Rhacophorus reinwardtii, R. nigropalmatus, Polypedates leucomystax, Hyla annectens and Amolops afghanus. Collections were carried out in different spots in the main localities mostly by day or at nightfall, either with a net or hands following the croaking sounds of the males or by locating the nest constructed by the female in a few species.

Methodology
In captivity, anurans do not feed and tend to get rid of their worm burden (Smyth & Smyth, 1980). Therefore, immediately after capture, they were narcotised, dissected or autopsied after returning to the laboratory. Muscles of the limbs, peritoneal cavity and its wall and abdomen of the host were examined. For whole mount preparation, parasites were first stretched in warm water, flattened and fixed in 70% alcohol, stained with borax carmine, followed by dehydration in ascending grade of alcohol, followed by clearing in methyl benzoate and mounting in canada balsam.

For scanning electron microscopy, live specimens recovered were fixed in 4% cold neutral phosphate buffer formalin, dehydrated in ascending grades of acetone and treated with tetramethylsilane following Roy and Tandon (1991), metal coated in a fine coat ion sputter JFC-1100(JEOL) and observed under a scanning electron microscope JSM 35CF(JEOL) under an electron accelerating voltage ranging between 10 and 20 kV.

Observations
Numerous specimens of a plerocercid larval form were recovered during the study period from all the three major localities of Nagaland surveyed. The maximum number of parasites in an infected host was 18. Of the host species surveyed, the plerocercid infection was found to occur only in six. The prevalence and intensity of plerocercid infection is depicted in Table 1. The parasite measured 50.2 - 75.1mm in length. In slightly contracted form, its apical end showed the presence of a frontal pit. Under scanning electron microscope its body surface is revealed to have numerous transverse folds or wrinkles giving it a pseudosegmented appearance, with slight suggestion of the beginning of proglottidization. Under higher resolution the whole tegumental surface is shown to have dense covering of microtriches. The latter did not show any regional differentiation of their shape and density (Figs. 2-6).

Discussion
Plerocercid larvae have earlier been reported from several amphibian hosts, such as, Euphlyctis cyanophlyctis, Hoplatastracus tigerinus, Limnonectes limnocharis and Rhacophorus nigropalmatus from Meghalaya in northeastern India (Diengdoh, 1989). The present report forms a new locality report with Rana sp. from Dimapur, Rhacophorus reinwardtii from Kohima and Polypedatus leucomystax from Mokokchung and Dimapur as new host records. Polypedatus leucomystax was always found to be the most heavily infected of all the host species checked for infection.

The plerocercid larvae, studied herein, showed a similarity in morphology to Spirometra erinacei plerocercoids fixed directly in glutaraldehyde; the latter have a shrunken and wrinkled appearance with a frontal pit at the apical end (Hatsushika & Okino, 1987). The present study recorded considerably high prevalence of plerocercid larvae in as many as six anuran species, all of which have use in traditional medicine or local cuisine among the natives of Nagaland. Highly endemic foci of amphibian borne zoonoses, sparganosis in particular, are known to occur among populations in the neighbouring southeastern and eastern countries where similar practices are prevalent (Mastura et al., 1995a,b). Tropical and oriental forms of spargana occur in frogs (Bonne, 1942; Huang & Kirk, 1962). Dissemination of this zoonotic infection among the native population of Nagaland, therefore, needs to be ascertained.

Acknowledgements
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References
### Table 1. Prevalence and intensity of pleocercoid larvae in anuran hosts in Nagaland

<table>
<thead>
<tr>
<th>Host Species</th>
<th>No. examined (infected)</th>
<th>Prevalence % (M, F)</th>
<th>No. of parasites recovered</th>
<th>Range</th>
<th>Intensity</th>
<th>Mean intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimapur</td>
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<tr>
<td><em>Rana sp.</em></td>
<td>43(7)</td>
<td>16.27(71.4, 28.5)</td>
<td>10</td>
<td>1 - 2</td>
<td>1.42</td>
<td>0.23</td>
</tr>
<tr>
<td><em>Hoplobatrachus tigerinus</em></td>
<td>23(13)</td>
<td>12.03(58.8, 46.1)</td>
<td>20</td>
<td>2 - 6</td>
<td>1.53</td>
<td>0.18</td>
</tr>
<tr>
<td><em>Euphyctis cyanophylctis</em></td>
<td>108(8)</td>
<td>7.40(37.5, 62.5)</td>
<td>11</td>
<td>1 - 3</td>
<td>1.25</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Polypedatus lecomystax</em></td>
<td>25(12)</td>
<td>48(75.25)</td>
<td>19</td>
<td>1 - 7</td>
<td>1.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Kohima</td>
<td></td>
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<tr>
<td><em>Euphyctis cyanophylctis</em></td>
<td>60(5)</td>
<td>8.3(20, 80)</td>
<td>8</td>
<td>1 - 4</td>
<td>1.6</td>
<td>0.13</td>
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<tr>
<td><em>Rhacophorus reinwardtii</em></td>
<td>8(1)</td>
<td>12.5(100, 0)</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>0.12</td>
</tr>
<tr>
<td>Mokokchung</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Euphyctis cyanophylctis</em></td>
<td>50(3)</td>
<td>6(0, 100)</td>
<td>7</td>
<td>2 - 3</td>
<td>2.3</td>
<td>0.04</td>
</tr>
<tr>
<td><em>Rhacophorus nigropalmatus</em></td>
<td>40(3)</td>
<td>7.5(66.6, 33.3)</td>
<td>11</td>
<td>2 - 6</td>
<td>3.66</td>
<td>0.27</td>
</tr>
<tr>
<td><em>Polypedatus lecomystax</em></td>
<td>47(23)</td>
<td>48.93(56.5, 43.4)</td>
<td>89</td>
<td>2 - 18</td>
<td>3.88</td>
<td>1.89</td>
</tr>
</tbody>
</table>

M = Male; F = Female

Intensity = Number of individuals of a parasite species in each infected host in a sample
Mean intensity = Mean number of individuals of a particular parasite species per host examined in a sample.


Figure 2. Plerocercoid larva.  
a. Anterior region  
b. Posterior region.

Figure 3-6. Plerocercoid larva—scanning electron micrographs.
3. Full worm (scale bar = 100 μm).
4. Body tegument under higher resoluation, showing microtriches (scale bar = 10 μm).
5. Magnified view of the apical end, showing the frontal pit and tegumental foldings (scale bar = 100 μm).
6. The posterior blunt end (scale bar = 100 μm).