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Occurrence of *Paragonimus* metacercariae in the crustacean host of certain areas of northeast India

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ABSTRACT
Background*: Crustaceans especially freshwater crabs act as the second intermediate host for a number of digenetic trematode parasites including that of the lung fluke (Paragonimus sp.). A wide variety of crab species are available in the northeastern states of India. Moreover, people living these states have the habit of consuming crabs either by way of roasting/cooking or as raw in the form chutney. Owing to this habit, they are exposed to a higher risk of acquiring the disease (paragonimiasis/endemic haemoptysis) and also of other zoonotic infections. Therefore, with a view towards assessing endemicity and occurrence/distribution of larval stages of bio-medically important zoonotic parasites, especially of metacercariae of Paragonimus sp by examining the fresh water crabs, a parasitological survey was carried out in certain selected areas of Manipur (Churachandpur district), Assam (Sonitpur district) and areas of Tezpur town (Assam) adjoining/bordering Arunachal Pradesh.*
Materials and Methods*: Fresh water crabs for the present study were collected from the markets of Churachandpur town (Manipur), Balitika and Balipara of Tezpur town (Sonitpur district, Assam) and Bhalukpong a border area/town in between Tezpur town and Arunachal Pradesh. Examination of the crabs for the detection and presence of larval forms (if any) of trematodes were done by employing a stereoscopic binocular dissecting microscope and a calibrated compound microscope. The standard laboratory protocol (WHO, 1995) was adopted for the present investigative study.*
Results: *Of the 312 crabs examined, 26 (8.33%) were found to have harboured oval shaped metacercariae measuring 476µm to 493 µm in dimension. Since the isolated metacercariae were found to be morphologically similar and conspecific to metacercariae of Paragonimus heterotremus, it has been inferred that the isolated metacercariae most probably belongs to Paragonimus heterotremus.*
Conclusion: *The present paper stresses the need and importance of more in depth study with emphasis on assessing the occurrence/distribution of larval forms of trematodes of biomedical importance associated with fresh water crustacean host (i.e. crabs) prevalent in this region of India on one aspect and establishing the taxonomic status of the parasite in another aspect using the advanced molecular techniques.*
Key Words*: Paragonimus heterotremus, Barythelphusa lugubris, Metacercariae*

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I. INTRODUCTION

It has been estimated that 40-50 million people worldwide are infected with food borne trematodes [1.] These infections are considered to be endemic in various parts of the world and particularly in Southeast Asia [2]. More than 10% of the world population is potentially at risk of food borne trematode infection [1]. Lack of education on the part of the public and poor recognition of parasitic infections by the public health authorities may be some of the factors that may aggravate the problem. Paragonimiasis is a food borne parasitic zoonosis caused by the members of the genus – *Paragonimus*, a digenetic tretmatode. Previously, some workers considered this disease to be endemic in certain areas of the world except in Europe and Australia[3]. However, the disease has been reported recently from almost all parts of the world, because of the increase in number of overseas travellers and the popularization of the ethnic dishes in developed and developing countries [4].

So far, no detailed and authentic study has been done with regard to the prevalence of food borne parasitic zoonoses (mainly paragonimiasis) in the Sonitpur district in particular and its adjoining areas bordering Arunachal Pradesh in general. Therefore, with a view to assess the endemicity of the food borne trematode

parasites and moreover keeping in view the public health importance of these zoonotic parasites, the present work was undertaken.

II. MATERIALS AND METHODS

A total of 312 crabs (*Barythelphusa lugubris*) were collected from three different areas namely, Balitika, Balipara of Sonitpur district, Assam and Bhalukpong area of Arunachal Pradesh, India, during 2002 – 2003. The crabs were then brought to the Parasitology Section of the Defence Research Laboatory, Tezpur, Assam. Crabs examined ranged in size from 33 mm to 56 mm carapace width. The carapace was removed, the haemocoel, digestive glands, gonads, the surface of cardiac stomach and buccal musculature were inspected for parasites. The examination of the crabs was done as per the standard procedure adopted by WHO [5]. At a time, 5 crabs of identical size were crushed with the help of a mortar and pestle and are then treated with artificial gastric juice. Then, the mixture was poured into a 50 ml beaker and was kept in a hot water bath for 1-2 hours at 37°C. Then, the coarse particles were removed by filtering them with the help of a sieve/ mesh. The sediments were then pipetted out from the bottom of the beaker using a Pasteur pipette in a cavity block, made of glass and were examined for the presence of metacercariae using a stereoscopic dissecting binocular microscope (in the Parasitology Section of the Defence Research Laboratory, Tezpur, Assam). The identification and measurement of the dimensions of the metacercariae were done using a previously calibrated compound microscope (Olympus) in the Parasitology Section of the Defence Research laboratory (DRL), Tezpur, Assam. Further Identification/confirmation of the metacercariae was done in the Parasitology laboratory of the Department of Life Sciences, Manipur University using a calibrated compound microscope (Olympus) and a high resolution Olympus compound Microscope.

III. RESULTS AND OBSERVATIONS

Out of 312 crabs examined, only 26 (8.33%), 18 from Bhalukpong and 8 from Balipara were found to have harboured the metacercariae. None of the crabs collected from Balitika were infected with the larval stage of any parasite. However, sub oval to oval shaped metacercariae measuring 476µm to 493µm in longitudinal axis were isolated from the crabs collected from Bhalukpong and Balipara. The metacercaria has a thin outer cyst wall and a thick inner cyst wall. The outer thin layer helps in adhering to the host tissue while the inner thicker cyst wall encloses the developing larvae. The inner cyst wall has a dimension of 193 µm in the longitudinal axis and 167 µm in the transverse axis. The inner cyst wall is thickened at both the poles. The average number of metacercariae isolated from each crab ranges from 15 to 20. The oval shaped metacercariae are the characteristics of *Paragonimus heterotremus*. Since the presently isolated metacercariae were found to be morphologically similar and conspecific to the typical *P. heterotremus* metacercariae, it has been inferred that the isolated metacercariae probably belongs to *P. heterotremus* type.

IV. DISCUSSION

Paragonimiasis is an important cause of pulmonary disease worldwide [6]. The pulmonary paragonimiasis, also known as endemic haemoptysis is an important public health problem in many countries of the world [7]. Although the lungs and pleural cavity are the principal sites affected by this parasite, ectopic infection can also occur in unexpected sites such as skin, brain, liver, spleen, intestinal wall, eye, muscles, gall bladder and kidneys [8-10]. There are also reports of omental paragonimiasis [11]. The occurrence in such a wide range of habitats suggests that man may not be the natural hosts. The lung cysts in which the worms most commonly occur usually contain 1-3 flukes. The eggs are freed into the bronchial tubes and pass out with sputum, but they may also appear in the faeces in large numbers, as a result of being swallowed. The number of eggs produced varies greatly with species.

Of the more than 40 paragonimus species described /reported so far, 9 species affect humans in over 39 countries in Asia and America. It is estimated that paragonimiasis affects at least 22 million people throughout the world [5]. It has also been calculated that 195 million people are at risk of being infected [12]. The infection in humans occurs as a result of a complex transmission cycle that includes two obligate intermediate hosts, a snail (first intermediate host) and a crustacean or a crayfish (second intermediate host) and a definitive mammalian host. Men acquire infection after eating undercooked freshwater crabs and crayfishes infected with the metacercariae of paragonimus. The encysted metacercariae are digested in the duodenum and the freed metacercariae bore through the intestinal wall into the body cavity to reach pleural cavity, in about four days and the lungs in about fourteen to twenty days. In the lungs, the host forms a fibrous capsule around the parasite; after about six weeks the worms mature and produce eggs, which characteristically appear in the sputum. The illness is usually caused once the parasite settled in the lung of the mammalian host. The main clinical symptoms include cough, thoracic pain and haemoptysis.

To date, four Paragonimus species have been reported from the northeastern states of India. They are *Paragonimus westermani, P. heterotremus, P. skrjabini and P. hueitungensis*. All the four species have been

reported from Manipur [13-17], while only one species, *P. heterotremus* has been reported from Arunachal Pradesh and Nagaland recently [18,19]. In fact, in India, Singh & Singh [13] reported the first case of human paragonimiasis in Manipur. Afterwards, 39 more cases of human paragonimiasis due to *P. westermani* were reported from Manipur [14]. Later, 276 cases of human paragonimiasis have been reported from Manipur [16]. Narain *et al* [18] reported occurrence of *P. heterotremus* in crab host *Barythelphusa lugubris* in Changlang district of Arunachal Pradesh. Moreover, he also reported the prevalence of paragonimus ova in the sputum of the local populace. In India, the occurrence of *P heterotremus* was first reported in Manipur, in freshwater crabs belonging to the species *Potamiscus manipurensis* [15]. Recently, there are reports of crabs belonging to the species *Potamiscus manipurensis* acting as the intermediate hosts for *P. heterotremus* in Nagaland, a north eastern state of India, adjoining the state of Manipur [19]. As far as Sonitpur district is concerned, so far no report of human paragonimiasis has been recorded, although Traub *et al.* [20] reported 5% prevalence of paragonimus ova in the faecal samples of dogs at Balipara, Phulbariae and addabariae areas of Tezpur, Assam. The present work is the first report of occurrence of oval shaped metacercariae, characteristic of *Paragonimus heterotremus* in the crab host of *Barythelphusa lugubris*, in the rural areas of Tezpur, Assam and Bhalukpong area of Arunachal Pradesh. Although Traub *et al.* [20] did not carry out any further investigation to ascertain the species of *Paragonimus* ova, he detected in the faecal samples of the local stray and domesticated dogs, it can be presumed that as the crabs for the present investigation were also collected from the same area/habitat as studied by Traub *et al.* [20], moreover, as these crabs were also found to be infected with the oval shaped metacercariae, characteristic of *P.heterotremus*, the ova detected by Traub *et a*l. [20] might be that of *P.heterotremus* (or probably *P. pulmonis*). Most probably, the dogs might have consumed these infected raw crabs. Singh & Singh [15] mentioned that morphological features especially oval shaped metacercarial feature is the characteristic of *P. heterotremus* only. However, further study is required to confirm the present findings by using animal model and by incorporating findings from molecular biological techniques.

So far, human infection with *P. heterotremus* is reported from South China, Laos, Vietnam and Thailand only, until human infection with this parasite was reported recently from Arunachal Pradesh and Nagaland [18-19]. Moreover, Singh *et al* [21] recently reported a rare case of cerebral paragonimiasis in an eight year old child from Nagaland. It is considered that the pathogenecity of *P. heterotremus* infection is comparatively milder than *P. westermani*.

Of all the Paragonimus species, *P. westermani* is considered as the most pathogenic species to human. De *et al.* [22] reported 76.4% infection rate of human paragonimiasis in Vietnam and based on adult parasite specimen recovered from dogs in the field and laboratory cats, he identified the parasite as *P. heterotremus*.

The local tribal populace working in the tea gardens and other ethnic population in Assam has the habit of eating crabs by roasting it in the oil or by putting it in the fire for few minutes (10-20) and then preparing it in the form of chutney by mixing the grounded roasted crabs with that of the juices of mint, ginger, chillies and salt. The way they roasted the crab is not in the proper way. In spite of being a preliminary study, the present finding of crabs being infected with the metacercariae of most probably *P. heterotremus* (even though it needs further taxonomic confirmation) as well as based on the earlier report of prevalence of *Paragonimus* ova in the stool of dogs [20], immediate attention needs to be given towards conducting a detailed survey and epidemiological work for identifying the possible intermediate hosts. Moreover, an in-depth study by adopting molecular parasitological techniques and animal model towards unravelling the systematic/taxonomic position of the parasite(s) and assessing the occurrence/endemicity, distributional pattern and identifying the risk factors of this zoonotic disease is the need of the hour.

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