First case of *Paragonimus westermani* infection in a female patient in India

*TS Singh, S Hiromu, KR Devi, WA Singh*

**Abstract**

Paragonimiasis is a foodborne parasitic zoonosis caused by lung fluke species of the genus *Paragonimus*. The *Paragonimus westermani* is the most common human pathogen in Asian countries. In northeast India, *Paragonimus heterotremus* has been documented as the only human pathogen in the earlier literature. In India, *P. westermani* infection in humans remained undetermined. Herein, we report a case of pulmonary paragonimiasis due to *P. westermani* in an adult female in Manipur. The diagnosis was made by morphological and molecular characterisation of the eggs in the sputum. This is the first confirmed case of paragonimiasis due to *P. westermani* in India.

**Key words:** *India, northeast India, Paragonimus westermani, pulmonary paragonimiasis*
Paragonimiasis or lung fluke infection is one of the neglected tropical foodborne parasitic zoonosis distributed worldwide. About 50 Paragonimus species including synonyms have been described from East and Southeast Asia, Africa and America. Paragonimus westermani, the most common species in Asia, was first described by Kerbert from the lungs of a Bengal tiger, which was captured in India and died at a zoo in Amsterdam more than a century ago. However, very little attention has been paid to this parasite because paragonimiasis was never considered to be a public health problem in India until 39 cases were reported from Manipur, in 1986. Thereafter, several endemic foci were discovered in Manipur, Arunachal Pradesh and Nagaland. Most interestingly, P. heterotremus has been identified as the causative agent of human paragonimiasis in this part of India against the widely believed P. westermani, which was reported from many mammals in India and recently the metacercariae were isolated from the second intermediate crab hosts in northeast India. Herein, we present a case of pulmonary paragonimiasis, which was initially diagnosed as smear negative pulmonary tuberculosis and was under anti-tubercular drugs. The diagnosis was based on the clinical features and laboratory findings of the patient, and molecular results demonstrated the causative species as P. westermani. This is the first case of P. westermani infection in humans in India confirmed by the morphological and molecular characterisation of Paragonimus eggs in the sputum specimen.

Case Report

A Manipuri tribal female, 50 years of age, was attended to the Regional Institute of Medical Sciences (RIMS), Imphal, with complaints of cough and recurrent hemoptysis for 2 years. She was initially diagnosed as smear negative pulmonary tuberculosis, at a primary health center and was under anti-tubercular therapy for a period of one and half years. As there was no clinical improvement, she was referred to RIMS. History of her dietary habits revealed frequent consumption of smoked and improperly cooked crabs collected from the mountain streams and bought from the market in Moreh, Manipur. She denied of associated fever, breathlessness, chest pain, and weight loss. Laboratory investigations of three consecutive sputum samples were negative for acid-fast bacilli, pyogenic bacteria, fungi, and malignant cells, but positive for Paragonimus eggs. The chest roentgenogram showed multiple nodular opacities of varying sizes, some with breakdown (cavity) in the upper and middle zones of both lungs and in the right basal and para cardiac regions. Both apices and costophrenic angles were clear. There was no evidence of calcification. Her complete blood count (CBC) revealed a total leucocyte count of 7200/µl with 16% eosinophils and erythrocyte sedimentation rate (ESR) of 80 mm/1st hour (Westergren’s).

The case was diagnosed as a chronic pulmonary paragonimiasis and was treated with praziquantel 25 mg/kg body wt., three times a day for 3 days. Sputum became free from blood and Paragonimus eggs on the second day following the chemotherapy. The clinical and laboratory data including radiological findings were critically reviewed, and identified P. westermani like eggs in the sputum smear, at the Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim. Molecular characterisation of a Paragonimus egg sample from the patient was performed by DNA isolation, amplification of the internal transcribed spacer 2 (ITS2) regions of the ribosomal DNA and sequencing as described by Sugiyama et al. (2002, 2013). Sequence analysis of the polymerase chain reaction (PCR) products revealed that the alignments of the rDNA region spanning the ITS2 were 436 bp in length. An asterisk indicates the presumed beginning of the actual ITS2. The 5’ end of the sequence is of 5.8S rDNA origin. The numbers refer to the alignment positions. A dot in the alignment of the P. westermani sequence with accession No. JN656208 indicates a nucleotide identical to that of our sample: The lengths of 76 bp and 360 bp were estimated for the posterior portion of 5.8S rDNA and almost entire portion of the actual ITS2, respectively, in comparison with the sequence reported for the adult P. westermani. The nucleotide sequence of Paragonimus eggs from the patient determined in this study has been deposited in the DDBJ/EMBL/GenBank database under the accession number AB123456. Searches of the nucleotide databases revealed that the sequences were almost identical to that deposited in the GenBank/EMBL/DDBJ nucleotide database with the accession number JN656208, which was for P. westermani isolated from Arunachal Pradesh as a metacercaria. Pairwise comparison of the sequences over an alignment length of 436 bp revealed just 1 (0.23%) base difference, which was probably due to the intraspecific sequence variation. We, therefore, identified the eggs discharged in a sputum sample of the patient as P. westermani.

Discussion

To date, five Paragonimus species viz. P. heterotremus, P. skrjabini, P. huei'tu'ngensis, P. miyazaki manipurinus n. sub spp and P. westermani have been reported to infect

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hosts in Manipur. The crabs, *P. manipurensis* are the most widely distributed as the host of *P. heterotremus* and other *Paragonimus* species occurring in Manipur and Nagaland. Recently, *P. westermani* metacercariae were isolated from the fresh water crabs, *Alcomon* collected at mountain streams in Moreh, Manipur. The *P. westermani* infection was also described in the two species of fresh water crab hosts, *Maydellia* and *Sartoriana* in Assam. Although, *P. westermani* infection was found in many wild and domestic mammals, infection in humans remained undetermined in India. In the past *P. westermani* was presumed to be the causative agent of paragonimiasis in Manipur only on the observation of *Paragonimus* eggs in the clinical specimens even without describing the morphological characteristics of the eggs. Nevertheless, some morphological features of eggs such as shape, size and shell character can be used to discriminate *Paragonimus* species, for example the eggs of *P. heterotremus* have an almost uniform shell thickness and discernible at the operculated end in contrast to the *P. westermani* eggs that are asymmetrically ovoid, shell thickened mostly at the apopercular end and opercular end as prominent shoulders. However, these features are inconsistent as the eggs of many *Paragonimus* species have overlapping morphological features that make species identification impracticable. Therefore, the earlier reports that implicated *P. westermani* as the causative agent of human infection cannot be considered as valid. Recent advances in the molecular technology such as PCR and DNA sequencing and hybridisation have enabled to identify and discriminate *Paragonimus* species at various stages of development in the life cycle. Subsequently, morphological and molecular characterisations of eggs in the sputum specimens of several patients have revealed *P. heterotremus* as the common human pathogen in the northeast India. In the present case, we confirmed *P. westermani* as the causative agent of the infection based on the morphological features and molecular characterisation of the eggs in the sputum specimen. This is the first record of *P. westermani* infection in human, in India. The low incidence of *P. westermani* infection in this region may be due to the restricted areas of distribution of the parasite, low host specificity, and paucity of suitable intermediate hosts.

Further study will be needed to find some characteristic features of the eggs that can help identify and discriminate different *Paragonimus* species in the clinical specimens. Presently PCR and DNA sequencing of the eggs in the clinical specimens is the only option for species determination.

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