Human paragonimiasis is a major food-borne parasitic disease caused by lung flukes belonging to the genus *Paragonimus* of the family *Paragonimidae*. *Paragonimus* infection in humans is caused by eating undercooked / raw infected crabs and crayfishes and affects at least 22 million people worldwide. The public health and economic impact of paragonimiasis is considerable in terms of morbidity and loss of productivity and this disease is often misdiagnosed as smear negative pulmonary tuberculosis (TB) because of overlapping clinical manifestations including chest pain, cough, haemoptysis and confusing chest radiological findings. Previously, paragonimiasis was known to occur in India only in the state of Manipur and was suspected to be caused by *Paragonimus westermani*. However, the evidence for the presence of *P. westermani* group of lung flukes in India was scant. In particular, unambiguous molecular and morphological evidence showing the presence of *P. westermani* complex in India was lacking. Moreover, the identity of the species of lung fluke causing human infection in India was also not known with certainty and there was no information on the prevalence and clinicoradiological features of paragonimiasis in the community. Keeping the above facts in view studies were initiated to: (i) identify the aetiological agent of human paragonimiasis in north eastern region of India using DNA sequences from ova collected from sputum of infected patients, (ii) describe the prevalence of human infection in the community, (iii) report the clinical profile and radiological features of the pulmonary paragonimiasis cases detected in the population, (iv) detect prevalence of paragonimiasis and tuberculosis among coughers in the remote rural communities, (v) identify medically important lung
fluke species prevalent in study area, (vi) identify crab species acting as intermediate host of lung flukes, (vii) determine prevalence of metacercarial infection in crabs, (viii) develop adult lung fluke in experimental rodent models for determining adult morphology and molecular characterization, (ix) clone and characterize of Phospagen kinase (a potential drug target) of Indian isolate Paragonimus westermani.

**Presence of three distinct genotypes within the Paragonimus westermani complex in northeastern India**

Surveys of the freshwater crabs *Maydelliatelphusa lugubris* in NE India revealed two morphologically distinct types of lung fluke metacercariae. Phylogenetic analyses, using DNA sequences from ITS2, 28S and cox1 gene regions indicate that these lung metacercariae belong to *P. westermani* complex. Type 1 metacercariae have a more basal position within the complex whereas type 2 metacercariae are closely related to the relatively derived forms of *P. westermani* from NE Asia (Japan, Korea, China) and Vietnam. A third type of metacercaria (type 3), detected in another crab host, *Sartoriana spinigera* in Assam, was phylogenetically close to *P. siamensis*, also a member of the *P. westermani* group. Thus molecular evidence has demonstrated the existence of three genotypes of lung flukes within the *Paragonimus westermani* complex in NE India.

**Human pulmonary paragonimiasis due to Paragonimus heterotremus**

Initial parasitological and immunological surveys revealed that paragonimiasis was endemic in Changlang district of Arunachal Pradesh. DNA extracted from eggs from the sputum of patients from Arunachal Pradesh was sequenced. Analyses of the second internal transcribed spacer (ITS2) of nuclear rDNA revealed that the species responsible is *Paragonimus heterotremus*. Chronic cough (97.2%) and haemoptysis (83.3%) were common respiratory symptoms among egg-positive cases. Chest radiography (n = 68) images from egg-positive cases showed that air space consolidation (75%), cavitary lesions (14.7%) and mediastinal adenopathy (11.8%) were very frequent lesions. Less frequent findings were nodular lesions, bronchiectasis, mediastinal adenopathy, pleural thickening and pleural effusion.
Active detection of tuberculosis and paragonimiasis in the remote areas in North-Eastern India using cough as a simple indicator

We also performed a cross-sectional study in 63 remote villages from two states Arunachal Pradesh and Assam to determine prevalence of undiagnosed tuberculosis and paragonimiasis cases using cough as a simple indicator. In Arunachal Pradesh 2961 individuals aged 5 years and above were examined. The prevalence of new smear positive TB in Arunachal Pradesh was 3.7 per 1000 persons. In Assam on the other hand the prevalence of new smear positive TB cases was 7.8 per 1000 population. Sero-positivity of paragonimiasis in coughers of Arunachal Pradesh was 7.6% (n=1091) which was significantly higher (p<0.01) as compared to that in Assam (1.2%, n=321).

Mitochondrial gene sequences of Indian Paragonimus westermani type 1.

In this study complete sequences of 12 genes encoding mitochondrial enzymes (cox3, CYTB, ND4L, ND4, ATP6, ND2, ND1, ND3, cox1, cox2, ND6 & ND5), 2 ribosomal RNA genes (16S & 12S), and 23 tRNA genes of P. westermani were determined. All genes were transcribed in same direction. Only 21 tRNAs had characteristic cloverleaf secondary structure while 2 other tRNAs had smaller than usual D stems and loops. In tRNA-Ser even D-arm was absent and replaced by D-replacement loop. The gene arrangement, direction of transcription and composition was determined by comparison with previously reported sequences. Comparison of P. westermani from India with P. westermani from Korea showed overall identity of only 84%. Maximum likelihood and Bayesian phylogenetic analyses based on all 12 protein coding genes of mitochondrial genome supports an independent species status of Indian P. westermani-like lung flukes. Similar results are generated using 16S and 12S ribosomal genes.

Molecular cloning and characterization of phosphagen kinases from Paragonimus westermani lung fluke of India.

Phosphagen kinases (PKs) are enzymes that play a key role in maintaining energy homeostasis in the cells of various animal species by catalyzing the reversible transfer of high-energy phosphoryl groups of ATP to naturally occurring guanidine compounds. We have completed the entire cDNA sequence of two-domain PK from the Indian origin lung fluke,
Paragonimus westermani Type1, the PK was also cloned in pMAL plasmid and expressed the enzyme in Escherichia coli (TB1). By enzyme kinetic studies it was found that both the domains showed activity for the guanidine substrate taurocyamine. Since TK plays a key role in energy metabolism and is not present in mammals, TK could be possible novel chemotherapeutic target against P. westermani.

Incrimination of crab intermediate hosts of lung flukes

Maydelliathelphusa lugubris, Barytelphusa cunicularis, Indochinamon manipurense and Sartoriana spinigera were found to be important crab hosts of lung flukes in India.

Development of rodent model for pulmonary Paragonimiasis.

Wistar rats were experimentally infected with metacercariae of Paragonimus westermani. Rats were sacrificed at 18, 36, 48, 63 and 77 days post-infection to determine recovery rate of worms from different organs. An overall recovery rate of 55.7%. Most were recovered from the lungs (53.7%) followed by the pleural cavity (27.3%), skeletal muscles (9.0%), peritoneal cavity (8.6%) and liver (1.4%). The proportion of worms recovered from sites other than the lungs decreased dramatically during the course of the experiment. The adult worms recovered from experimental animal rats were used for morphological, immunological and sequencing studies.

Conclusion

The findings of the present study suggest that paragonimiasis and pulmonary tuberculosis are major public health concerns in North-eastern region of India especially in the remote places. Paragonimus heterotremus is the major parasite for causing human pulmonary infection. In addition our studies have confirmed the existence of three members of Paragonimus westermani complex in India. Important fresh water crab hosts acting as intermediate hosts of lung flukes have also been identified in India. Phosphagen kinase gene of P. westermani were successfully cloned in pMAL plasmid and expressed the enzyme in Escherichia coli (TB1). By enzyme kinetic studies it was found that both the domains showed activity for the guanidine substrate taurocyamine. Since TK plays a key role in energy metabolism and is not present in mammals, TK could be possible novel chemotherapeutic target against P. westermani.