

Review Article

Indian J Med Res 136, August 2012, pp 192-204

Paragonimus & paragonimiasis in India

T. Shantikumar Singh, Hiromu Sugiyama* & Achariya Rangsiruji**

*Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India, *Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan & **Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand*

Received December 24, 2010

Ever since the discovery of the first indigenous case in 1981, paragonimiasis has gained recognition as a significant food borne parasitic zoonosis in India. The data available on the occurrence of paragonimiasis, until today, may be just the tip of an iceberg as the study areas covered were restricted to Northeast Indian States. Nevertheless, the results of research on paragonimiasis in India have revealed valuable information in epidemiology, life cycle, pathobiology and speciation of Indian *Paragonimus*. *Potamiscus manipurensis*, *Alcomon superciliosum* and *Maydelliathelphusa lugubris* were identified as the crab hosts of *Paragonimus*. *Paragonimus miyazakii manipurinus* n. sub sp., *P. hueit'ungensis*, *P. skrjabini*, *P. heterotremus*, *P. compactus*, and *P. westermanni* have been described from India. *P. heterotremus* was found as the causative agent of human paragonimiasis. Ingestion of undercooked crabs and raw crab extract was the major mode of infection. Pulmonary paragonimiasis was the commonest clinical manifestation while pleural effusion and subcutaneous nodules were the common extra-pulmonary forms. Clinico-radiological features of pulmonary paragonimiasis simulated pulmonary tuberculosis. Intradermal test, ELISA and Dot-immunogold filtration assay (DIGFA) were used for diagnosis and epidemiological survey of paragonimiasis. Phylogenetically, Indian *Paragonimus* species, although nested within the respective clade were distantly related to others within the clade.

Key words India - lung fluke - paragonimiasis - *Paragonimus heterotremus* - tuberculosis

Introduction

Paragonimiasis, also known as endemic haemoptysis, oriental lung fluke infection, pulmonary distomiasis, parasitical haemoptysis, parasitare haemopte, Gregarinosis pulmonum, etc. is one of the most important food-borne parasitic zoonoses caused by one or more of the trematode species of the genus *Paragonimus*. The disease is endemic in many parts of Asia, Africa and South America¹. There are about 50 species of which 11 are known to cause infections in

humans, *P. westermanni* has been regarded as the most common and widely distributed human pathogen in Asia². In South and Southeast Asia, *P. heterotremus* has been increasingly detected as an important cause of infection in humans³. The natural definitive hosts of the parasite comprise large varieties of wild mammals of the canidae and felidae families and humans. A wide range of fresh water snails, and crabs as well as crayfish served as first and second intermediate hosts, respectively. Humans acquire infection, commonly

by ingestion of uncooked or undercooked crustaceans containing metacercariae, the larval stage of the parasite, and rarely by ingestion of infected uncooked or undercooked meat of pig and wild boar, which serve as paratenic hosts^{4,5}. The parasites primarily infect lung but extra-pulmonary infections are not infrequent. Paragonimiasis is diagnosed in the laboratory by microscopic demonstration of *Paragonimus* ova in the sputum and other clinical specimens such as faeces and pleural fluid or by specific *Paragonimus* serological tests.

In the past, human paragonimiasis was not considered a problem of public health importance in India until recently. Pulmonary paragonimiasis presenting with bloody sputum or recurrent haemoptysis was generally mistaken for sputum smear negative pulmonary tuberculosis or some other serious conditions with similar symptoms. Research on *Paragonimus* and paragonimiasis has revealed that paragonimiasis is endemic in many parts of Northeast States of India and provided valuable information on the epidemiology, *Paragonimus* species, clinico-pathology, diagnosis and treatment. This review is intended to disseminate the research findings and knowledge about *Paragonimus* and paragonimiasis in India from its confinement in the research laboratories to the clinicians who are directly dealing with the patients and more importantly to raise awareness amid health educators and general population.

Historical review of *Paragonimus* research in India

Historically, at least two *Paragonimus* species were first described from India more than a century ago; namely, *P. compactus* from an Indian mongoose (*Herpetes edwardsi*) in 1859⁶ and *P. westermani* from two Bengal tigers in 1878⁷. These mammals were captured in India and died in the zoological gardens in Hamburg in Germany and Amsterdam in Holland, respectively. In 1923, Vevers have described the occurrence of *P. compactus* and *P. westermani* in India⁸. In 1926, Gulati⁹ had described a new lung fluke species which he named as *Paragonimus edwardsi* from a palm civet cat, *Paradoxurus grayi* in Kumaon hills, India, but it was regarded by many as the synonym of *P. westermani*¹⁰. Subsequently, *P. westermani* infection was described in the lungs of two domestic dogs from Malabar and Coimbatore and a panther from Coorg¹¹ and in a cat from Pantnagar¹². About 40 years later Singh and Somvanshi¹³ reported *P. westermani* in the lungs of two tigers (*Panthera tigris*) from the Terai area of the foothills of the Himalayas. The same species was

described in the lungs of a beer cat from the zoological park in Chandigarh¹⁴. *P. westermani* was also isolated from the lungs of a mongoose and two tigers which had died of unknown aetiology at the National Corbett Park, India^{15,16} and tigers and cats at Kanha National Park, Mandla (India)^{17,18}. In spite of the fact, that paragonimiasis was common among wild mammals and wide spread in India; no record of scientific research on *Paragonimus* and paragonimiasis in India was available until late 1900s. The earlier reports were mainly focused on autopsy findings of mammalian paragonimiasis. The first epidemiological survey of paragonimiasis in India was conducted in Imphal east district, Manipur¹⁹ during the period from 1986 and 1987. In November 1990, the first Indo-Japan joint research on *Paragonimus* and paragonimiasis was conducted in Manipur to study the life cycle, pathobiology, and morphological characterization of *Paragonimus* species, and resulted in the discovery of endemic areas of paragonimiasis, identification of crustacean second intermediate hosts and morphological characterization of *Paragonimus* species occurring in Manipur. Two fresh water crab species namely, *Potamiscus manipurensis* (Alcock, 1909) and *Maydelliathelphusa lugubris* (Wood-Mason, 1871)²⁰, formerly known as *Barytelphusa lugubris* which habitate in most of the mountain streams in Manipur were investigated for *Paragonimus* metacercarial infection. *P. manipurensis* was identified as the natural crab hosts harbouring at least three types of *Paragonimus* metacercariae such as *P. heterotremus*, *P. westermani* like metacercariae (large and small types) and easily excysted thin cyst walled metacercariae²¹. We have described *Paragonimus* infection in the lungs of a civet cat from Tamenglong district in Manipur²². The morphological features (Carmin stained) of the adult worm isolated from the worm cyst in the lungs were distinct from other species; therefore, it was named as *P. miyazakii manipurinus* n. sub spp (as a new subspecies) due to close morphological similarity with *P. miyazakii*. Subsequently *P. hueitu'ngensis*, *P. heterotremus*, and *P. skrjabini* were described from the experimental laboratory animals infected with metacercariae isolated from the crab hosts; *Potamiscus manipurensis* collected from mountain streams in Manipur²³⁻²⁵. The occurrence of *P. heterotremus* infection in crab host, *B. lugubris* (now known as *M. lugubris*) and human was reported from Arunachal Pradesh²⁶. Recently, an endemic focus of paragonimiasis has been located in Nagaland due to *P. heterotremus*²⁷. The crab host in Nagaland was found to be *P. manipurensis*. Tandon *et al*²⁸ had described the molecular characteristics and ultrastructure of *P. westermani* metacercariae isolated from *M. lugubris*

in Arunachal Pradesh. Devi *et al*²⁹ reported the morphological and molecular characterization of *P. westermani* adult worm isolated from the experimental Wistar rats infected with *P. westermani* metacercariae harvested from *M. lugubris* collected from Arunachal Pradesh and Meghalaya States of India and showed that Indian *P. westermani* complex represented a distinct lineage. In June 2011, we found that fresh water crab species, *Alcomon superciliosum* (Kemp, 1913) collected from the mountain streams in Moreh town in Manipur (located on the Indo-Myanmar border) were infected with at least two types of *Paragonimus* metacercariae *viz.* *P. heterotremus* and *P. westermani* (unpublished data).

Paragonimiasis in humans

In India, *Paragonimus* ova were detected from the sputum and faecal samples from a Chinese patient in Bombay (now Mumbai), Maharashtra in 1919, but believed the infection might have acquired from outside India³⁰. Therefore, this case was not considered as an autochthonous case of paragonimiasis in India. The first indigenous case of human paragonimiasis in India was described by Singh *et al*³¹ in 1981 in Manipur. The *Paragonimus* ova were demonstrated in the sputum specimens and identified as ova of *P. westermani* based on morphological features only. The next case of human paragonimiasis was reported in 1984 from Maharashtra, India³². In 1986, Singh *et al*³³ have described 39 cases of pulmonary paragonimiasis in Manipur. Subsequently, several cases of paragonimiasis were reported from Manipur^{34,35}. In an epidemiological survey of paragonimiasis conducted in Imphal east district, Singh *et al*¹⁹ had discovered endemic areas with an estimated prevalence rate of 6.7 per cent in Manipur. Paragonimiasis cases have been detected almost every year in Manipur but only a few have been published^{36,37}. In the Northeast States of India, *P. heterotremus* has been identified as the causative agent of paragonimiasis in human^{38,39} contrary to the earliest concept that *P. westermani* was the agent in Manipur.

Life cycle

The parasites utilize two intermediate hosts and complete the life cycle in wild mammals and humans as final definitive hosts.

First intermediate hosts: Fresh water molluscan species of the genera *Semisulcospira*, *Oncomelania*, *Brotia*, and *Thiara* serve as the first intermediate hosts of *Paragonimus* species in China, Japan and Thailand². In India, the molluscan host has not yet

been determined. *Paragonimus* ova discharged either in the faeces and/or sputum of definitive hosts reach water and hatch ciliated miracidia, which swim about to find a suitable snail host. The ova required at least two weeks to complete embryonation and hatching in water⁴⁰. In the snail host, the miracidium develops into a mother sporocyst which produces asexually first and second generations of rediae, which finally develop into cercariae. The development from miracidium to cercariae in the snail takes about 9 to 13 wk^{41,42}. Further development of the cercariae takes place in a suitable crustacean host.

Second intermediate hosts: Fresh water crab species of the genera *Potamiscus*, *Potamon*, *Paratelphusa*, *Eriocheir*, *Geothelphusa*, *Barytelphusa*, and crayfish species of the genus *Camberoides* and shrimps such as *Acrohthalmium* and *Caridina* serve as common second intermediate hosts². In Northeast India, *M. lugubris*, *P. manipurensis* and *A. superciliosum* were found to serve as the crab hosts of *Paragonimus*. In China a frog species, *Rana boulengeri* has been described to contain metacercariae of *P. skrjabini* and served as paratenic host⁴³. The crab hosts are infected with cercariae either by ingestion of the snail hosts or free cercariae directly entering through the soft tissue of the crab host⁴⁴. A single crab may harbour metacercariae of more than one *Paragonimus* species simultaneously. The cercariae develop to metacercariae which remained encysted in the liver, gills, intestine, skeleton muscles and sometimes, in the heart of the crab host. For further continuation of the life cycle, the metacercariae are to be taken up by a suitable definitive mammalian host.

Definitive hosts: Many mammals of Canidae and Felidae family and humans serve as definitive hosts. Tigers, civet cats, toddy cats, dogs, mongooses, *etc.* were found to serve as definitive hosts of *Paragonimus* species in India. Raccoon dogs, in Japan, served as natural reservoir hosts of *P. westermani* and *P. ohirai*^{45,46}. In the Philippines and Malaysia, rats serve as natural definitive host as well as paratenic hosts^{47,48}. The metacercariae ingested by the definitive hosts excyst larvae in the small intestine, penetrate through the intestinal wall and reach the abdominal cavity in 3 to 6 h⁴⁹. The larvae migrate into the peritoneal cavity leaving behind haemorrhagic and rusty brown inflammatory exudates and then enter through the diaphragm into the thoracic cavity. Thereafter, the worms wander to find their suitable partners and usually in pairs invade the lung parenchyma to form a worm cyst where they mature to adult worms and start

laying ova. A few worms may remain in the pleural cavity or settle in organs and tissues other than the lungs. The time taken by the larval worms to become mature adults varies from one to three months or even longer in some species. Generally, the time taken from infection to laying eggs has been estimated to be from 65 to 90 days⁵⁰. In their final hosts, the parasites may survive from 1 to 20 years despite the absence of specific therapy.

Species differentiation

Phenotypic characteristics: Traditionally, the study of the morphological characteristics of the adult worms, ova and metacercariae forms the basis of taxonomy of *Paragonimus*. The important morphological features of adult worms for species differentiation include: (i) shape and size of the whole mount of adult worms, (ii) arrangement of cuticular spines, (iii) relative size of transverse diameters of suckers, (iv) sizes, numbers and branching patterns of ovary and testes, and (v) shape, size and shell character of ova². The shape, size, number of cysts and presence of pink granules in the body of encysted larvae are important characters for species differentiation of *Paragonimus*. Most species possess outer and inner cysts whereas some are without cyst, *e.g.* *P. mexicanus*. Other useful characters at the metacercarial stage include body proportions of the excysted larvae, relative size of the suckers, the anterior extent of the excretory bladder, the presence and length

of oral stylet, the distribution of spines and the number and arrangement of papillae around the suckers⁵¹⁻⁵⁴.

Genotypic characteristics: Molecular characterization of genomic DNA of parasites can be performed using adult worms, larvae and ova. As the adult worm is not usually recovered in the clinical specimens from patients, molecular characterization of ova is the only option for accurate species identification of the causative agent. A phylogenetic analysis based on the ITS2 region of three species from Manipur and Arunachal Pradesh showed *P. westermani* from Manipur and Arunachal Pradesh in the same clade as *P. westermani* and *P. siamensis* from Thailand (Fig. 1). In addition, *P. heterotremus* from India were placed close to *P. heterotremus* from China and Thailand^{38,55}. *P. skrjabini* from Manipur, was closely related to *P. skrjabini* from China and *P. miyazakii* from Japan. Blair *et al*^{56,57} described that *P. skrjabini* from eastern China (Fujian Province) was phylogenetically very close to *P. miyazakii* from Japan and proposed that both the populations should be named as the same subspecies as *P. skrjabini miyazakii*. They also considered *P. szechuanensis* as the synonym of *P. skrjabini* and concluded that *P. skrjabini* represents a separate species complex. Recently, a new lung fluke species, *P. pseudoheterotremus* morphologically similar to but genetically distinct (different nucleotide sequence in *cox1* genes) from *P. heterotremus* was described from Thailand^{58,59}. This finding suggests possible occurrence of cryptic or sibling species to *P. heterotremus*.

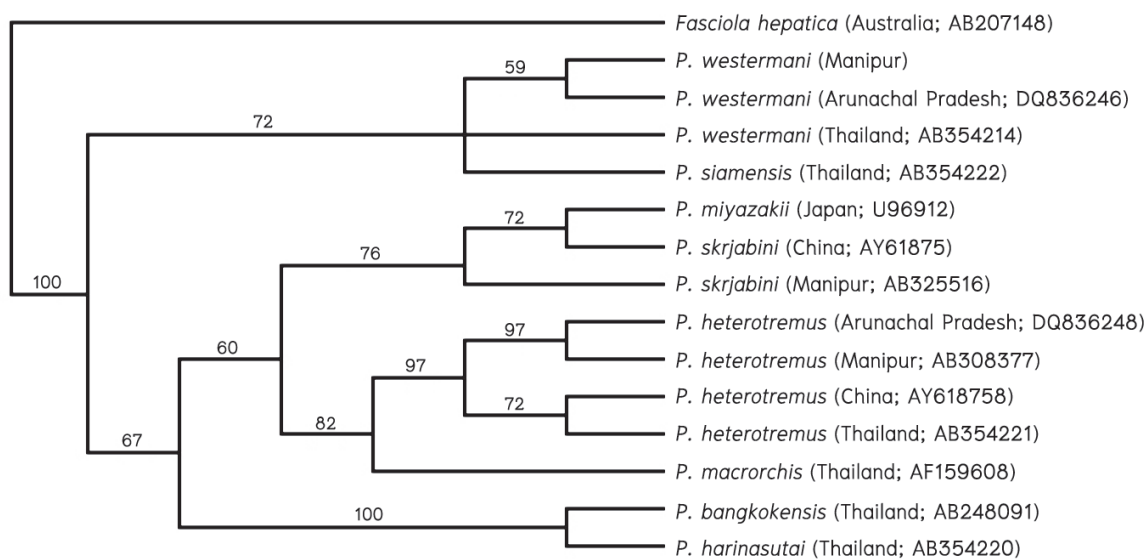


Fig. 1. Phylogenetic analysis of Indian *Paragonimus* spp. Numbers on the branches are bootstrap values (%) of 1,000 replicates. The sources of data, including geographical origins and GenBank accession numbers are indicated in parentheses.

Source: Ref. 37.

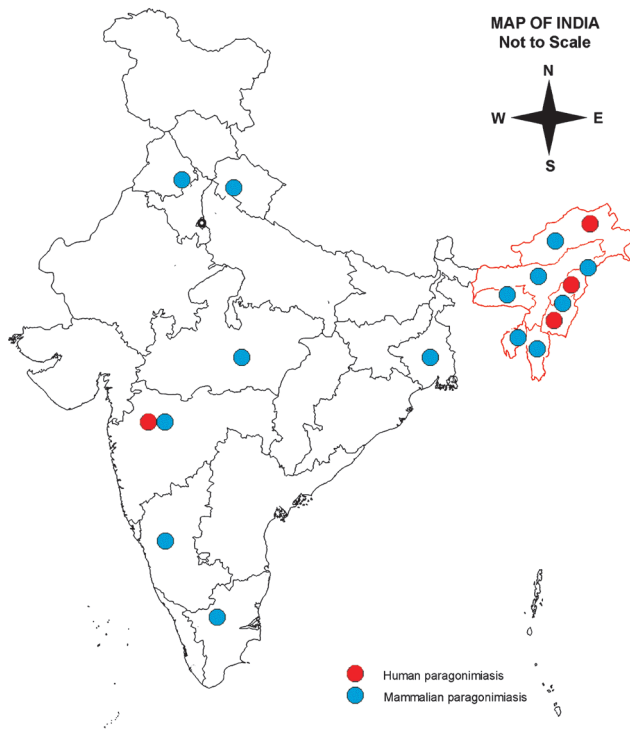


Fig. 2. Geographical distribution of paragonimiasis in India.

Experimental infection of laboratory animals

Experimental infection of laboratory animals is essential to determine the host-parasite relationship between various *Paragonimus* species and laboratory animals, to study the pathogenesis, to recover adult worms for detailed morphological and molecular characterization, to study immune responses during the course of parasite infection and to obtain antigen for developing immunodiagnostic tests. Therefore, selection of suitable laboratory animal species is a prerequisite for the successful recovery and good yield of the adult parasites. Generally, puppies and kittens are susceptible to all *Paragonimus* species in Asia and Southeast Asia and served to be the ideal animal model for experimental studies⁶⁰⁻⁶³. Puppies were found susceptible to all *Paragonimus* species occurring in Manipur^{23,24,64} whereas Wistar rats were found as a suitable rodent model for paragonimiasis in Arunachal Pradesh⁶⁵.

Pathology

The pathologic lesions of paragonimiasis may be due to invasion and migration by the worms and toxic metabolites and host's immune responses to the invading parasites. Small haemorrhagic and inflammatory spots along the route of migration of

the immature worm(s), in the intestine, peritoneum, abdominal organs, diaphragm, thoracic cavity, and pleura, and sometimes, pericardium had been observed in the experimental animals^{23,24}. The similar observations were made by other workers and also expected to occur in humans^{61,62}. The inflammation may cause adhesion between intra-abdominal organs and peritoneal wall or diaphragm. Sometimes, granuloma may be formed around ova deposited by the migrating adult worms in the abdominal cavity. The basic pathology is the formation of a worm cyst primarily in the lungs. The worm cysts varying from 1 to 3 cm in size usually located superficially, sometimes deep in the lung parenchyma contained dark red or rusty brown viscous fluid, red blood cells, inflammatory cells, eosinophils, necrotic tissue, Charcot-Leyden crystals, and adult worms and ova. The adult worms are usually found at the periphery of the cyst, adhered to the cyst wall. Generally, a cyst contained paired worms, but single or more than two worms of the same or different species may be found^{54,64}. The cyst is communicated with bronchioles through which the cystic fluid, including the ova is discharged in the sputum to the exterior environment. If the sputum is swallowed as in case of young children, the ova can be detected in the faeces. Histopathologically, the worm cyst is composed of an outer fibrocollagenous cyst wall with focal haemosiderin-laden macrophages and an inner layer of congested blood vessels, inflammatory cells and normal or distorted ova.

Clinical manifestations

The incubation period may vary from 1 to 2 months or even longer. The shorter incubation period from 2 to 15 days and appearance of early symptoms were observed in 52 per cent of cases in an outbreak of *P. westermani* infection in Harbin, China⁶⁶. Clinically, paragonimiasis may be broadly classified into pulmonary, extra-pulmonary and pleuropulmonary forms. Others have categorized paragonimiasis, as acute paragonimiasis, chronic pleuropulmonary paragonimiasis, and ectopic paragonimiasis¹.

(i) *Pulmonary paragonimiasis*: Pulmonary paragonimiasis is the commonest clinical form of paragonimiasis occurring in 76-90 per cent of cases^{33,35}. Generally, most patients are ambulatory, apparently healthy and capable of performing normal physical activities. Initially, the patients may present with signs and symptoms of mild pleural effusion, pneumonitis, bronchiectasis or bronchopneumonia. Major presenting symptoms comprised pain or tightness in the chest,

difficult breathing, and coughing up rusty brown or blood-stained sputum or recurrent haemoptysis. Haemoptysis may be exacerbated on physical exertion, and massive requiring immediate medical attention and hospitalization. The chronic infection may be associated with fever, anaemia, weakness, and weight loss. Generally, pulmonary infection has high morbidity and low mortality unless complicated with infection in vital organs such as heart, and brain.

(ii) *Extra pulmonary paragonimiasis*: Extra pulmonary paragonimiasis is due to aberrant migrations of juvenile worms, which deviate from the normal route of migration to the lungs through the intestine, abdominal cavity, diaphragm and pleural cavity. It is likely to occur more commonly in children and in heavy infections. In a review of 45 cases of paragonimiasis in children in Manipur, 44.4 per cent of cases were extra pulmonary³⁵. In another report, extra pulmonary paragonimiasis in adults was seen in 2 per cent of 247 cases⁶⁷.

Pleural effusion: The migrating worms in the pleural cavity produce pleuritis and effusion; the severity may vary from a minimal to massive effusion depending on the number of worms, frequency of migration and duration. Frank effusion was found in up to 20 per cent cases of pulmonary paragonimiasis in which 60 per cent bilateral, 20 per cent unilateral and 20 per cent encysted were found^{32,35,19}. Uchiyama *et al*⁶⁹ reported 5 per cent bilateral and 35 per cent unilateral effusion, 45 per cent nodular or cavity lesion and 15 per cent pleuroparenchymal complex. Persistent effusion was reported in nine patients from Lao PDR⁶⁹. The main presenting symptoms of patients with pleural effusion were progressive dyspnoea, chest pain and cough with or without haemoptysis.

Cutaneous paragonimiasis: The skin is one of the common sites for ectopic paragonimiasis which may be seen in about 16 per cent of children³⁵. The typical lesion is a migratory subcutaneous nodule which usually appear on the anterior chest wall then migrating through the abdominal wall to the pelvic region or lower limbs without causing much discomfort to the patient except mild pain and itching. The abnormal migration is likely to occur in heavy infection in which the immature worm may invade any organ or tissue except bone. The factors which contribute to abnormal migration are not clearly understood, however, host specificity, and parasite species adaptability may play a major role in the pathogenesis. For example, the Chinese species *P. skrjabini* is well known to cause

extra pulmonary infections in brain, skin, peritoneal cavity and eye⁶⁶. The cutaneous infection caused by *P. skrjabini* has been estimated to be between 30 to 60 per cent of the infected patients⁷⁰. *P. skrjabini* is also expected to be one of the causative agents of cutaneous paragonimiasis in Manipur. Seven out of 45 cases in children in Manipur were found to be cutaneous paragonimiasis; presented with migratory subcutaneous nodules except in one³⁵. *Paragonimus* adult worm could not be isolated from the excised biopsy materials except in one case who presented with a subcutaneous swelling on the right anterior chest wall. The carmine stained worm was morphologically identified as an adult *P. heterotremus*⁷¹. Generally, the subcutaneous nodule is non tender, mobile, soft to firm in consistency, round to oval measuring 2-5 cm in diameter, and is usually not associated with obvious signs of inflammation or creeping eruption. Rarely, the nodule may remain localized without further migration. The immature or adult worm along with inflammatory exudates, granulomatous tissue, eosinophils, Charcot-Leyden crystals and sometimes *Paragonimus* ova may be detected in the excision biopsy of the nodule if performed before further migration of the worm.

Cardiovascular paragonimiasis: This is a rare but serious condition which may be associated with pleuropulmonary paragonimiasis. We have described three cases of pleuropulmonary paragonimiasis in children involving heart³⁵. Two of them presented with pericarditis and one child who presented with pericarditis, and myocarditis died of congestive heart failure in spite of praziquantel therapy.

Abdominal paragonimiasis: The symptoms during the prepatent period are usually mild that majority of patients do not recall if they had any gastrointestinal symptoms prior to pulmonary paragonimiasis. Some patients might present with vague abdominal pain, nausea, vomiting, mild diarrhoea and allergic dermatitis. Rarely, patient may present with diarrhoea and dysentery³³ and abdominal distension with hepatomegally³⁵.

Cerebral paragonimiasis: Cerebral paragonimiasis has been described as the most common and serious form of extra-pulmonary paragonimiasis, which was reported mainly from China⁷⁰, Japan⁷², and Formosa^{73,74}. The infection has been described due to erratic migration in which the worms enter the cranial cavity through the jugular or carotid foramen and commonly invading the temporal and occipital lobes⁷⁵. The condition may be

confused with tubercular meningitis or tuberculoma or other space occupying lesions in the brain. The cerebral paragonimiasis is commonly seen in young adults who may present with symptoms of convulsive seizures, especially Jacksonian type, and may be associated with fever, headache, nausea, vomiting, visual disturbance, and motor weakness⁷⁶. In India, the first case of cerebral paragonimiasis mimicking tuberculoma in a child was reported from Nagaland⁷⁷. The imaging techniques such as X-ray, CT scans, and MRI are used for specific diagnosis of cerebral paragonimiasis⁷⁸.

(iii) *Pleuropulmonary*: The pleuropulmonary paragonimiasis usually occurs when both pleura and lung parenchyma are affected concomitantly. Most patients in the early stage of pleural infection show symptoms of pleurisy and pleural effusion of varying severity followed by parenchymal infection. The clinical manifestations are pain and tightness in the chest, dyspnoea, coughing up blood-stained sputum or haemoptysis and may be associated with fever. The condition was seen in 2.2 per cent of paragonimiasis patients in Manipur³³. A high seroprevalence (31.6%) of pleuropulmonary paragonimiasis was also reported from Arunachal Pradesh³⁹.

Laboratory diagnosis

A definitive diagnosis can be made by finding characteristic golden brown, ellipsoidal or oval operculated *Paragonimus* ova in the clinical specimens such as sputum, aspirated fluids and faeces by microscopy. Occasionally, adult worm may be detected in the excised tissue or cyst or at necropsy or pleural aspirate⁶⁹ or expectorated in the sputum⁷⁹. When the ova or worms are not detectable, serological tests play an important but adjunctive role for the diagnosis of extra pulmonary paragonimiasis.

Microscopy

Sputum: Morning sputum samples are ideal for a direct wet smear examination for the parasite ova. The rusty brown or blood-stained sputum usually contains numerous *Paragonimus* ova and Charcot-Leyden crystals. Earlier studies showed *Paragonimus* ova in 55.6 to 72 per cent of sputum specimens of pulmonary paragonimiasis cases^{33,80}. Devi *et al*³⁷ found ova positive sputum in 20.9 and 4.1 per cent of pleuropulmonary paragonimiasis in children and adults, respectively. The finding, however, is unusual because sensitivity of sputum examination is expected to be higher in adults than in children.

Pleural fluid: *Paragonimus* ova may be demonstrated in the centrifuged deposit of pleural fluid in about 10 per cent cases of pleural effusion⁸¹. Vidamaly *et al*⁶⁹ demonstrated *Paragonimus* ova in all the pleural aspirates of nine patients with persistent effusion and one of them discharged an adult worm through a draining chest tube. In the absence of demonstrable ova pleural fluid analysis showing glucose content less than 10 mg/dl, lactose dehydrogenase greater than 1000 IU/l, high-protein value, low pH and eosinophilia is indicative of pleuropulmonary paragonimiasis⁸².

Stool: Stool examination for *Paragonimus* ova is recommended in children who usually swallow sputum and in patients whose sputum samples are apparently negative for ova. Ideally, two to three stool samples collected at consecutive days should be examined by formolin-ether sedimentation or AMS III concentration technique. The recovery rate varied from 25.6 to 60 per cent, and is higher in children <10 yr of age. Komiya and Yokogawa⁸⁰ reported *Paragonimus* ova in 65.1 per cent in the faecal samples of 189 patients of pulmonary paragonimiasis by AMS III concentration technique.

Biopsy: Excision biopsy served as surgical treatment of subcutaneous nodule as well as laboratory diagnosis. Adult or immature worm may be found in a carefully dissected nodular or cystic lesion. Microscopic examination of the exudates will show inflammatory cells, eosinophils, *Paragonimus* ova and Charcot-Leyden crystals. Alternatively, histopathological section will reveal fibrocollagenous tissue, inflammatory cells, eosinophils, sections of the worms and distorted ova.

Immunodiagnosis

The serological tests are important in the diagnosis of extra pulmonary paragonimiasis and to differentiate it from tumours and cysts or nodular lesions caused by other parasites. Some of these tests are used by other researchers for evaluating the therapeutic responses to specific chemotherapy. The various immunological tests which have been evaluated are intradermal (ID) test, complement fixation test (CFT), immunodiffusion, indirect haemagglutination test (IHA), enzyme-linked immunosorbent assay (ELISA), dot-ELISA, and Western blot. ID test is simple, easy to perform, cheap, rapid and highly sensitive and was widely used over the past several years in Japan^{83,84}, China⁸⁵ and in India¹⁹ for mass screening and laboratory diagnosis. It is a valuable test in distinguishing pulmonary paragonimiasis from pulmonary tuberculosis, especially in the areas

where both are co-endemic. The test is performed by intradermal inoculation of 0.01 to 0.1 ml of the test antigen (saline extract or purified antigen of adult *P. westermani*) on the volar aspect of the forearm, and the wheal diameters are measured immediately and 15 min after the inoculation. A differential wheal diameter of ≥ 5 mm with erythema and pseudopodia indicates a positive test. Exceptionally, a large erythema of $\geq 45 \times 35$ mm in diameters without an appreciable wheal and pseudopodia may indicate a positive reaction. Whereas a negative skin test almost certainly rules out paragonimiasis, a positive test cannot differentiate between the past and the present infection as the test may remain positive as long as 10 to 20 years even after the successful chemotherapy or spontaneous recovery⁸⁶. There is also possible cross-reaction with other trematode infections such as schistosomiasis and clonorchiasis, if a crude antigen is used. However, the sensitivity and specificity of the test can be up to 100 per cent by using purified antigen⁸⁷.

CFT has been used in the diagnosis of active infection and to confirm ID positive cases. The test becomes negative within 3 to 9 months after successful treatment⁸⁷. It was recommended that ID test where used should be applied first in the epidemiological survey and followed by CFT or any other more specific test on individuals who showed positive or doubtful dermal reactions.

Biguet *et al*⁸⁷ first developed the immunodiffusion method for the diagnosis of paragonimiasis. Double immunodiffusion technique (Ouchterloney method), immunoelectrophoresis, and counter-current immunoelectrophoresis were reported to be highly sensitive and specific and can be used for speciation by demonstration of specific precipitin bands⁸⁸⁻⁹⁰. IHA is another simple, rapid and sensitive test. In Thailand, the test revealed a sensitivity of 88 per cent in the diagnosis of paragonimiasis heterotremas⁹¹.

ELISA test for paragonimiasis was first developed by Quicho *et al* in Thailand⁹². Since then ELISA based on different techniques and with different antigen preparations have been developed and evaluated for diagnosis of paragonimiasis⁹³⁻⁹⁶. The overall specificity of IgG ELISA using the saline extract of adult worms as an antigen was found to be 97 per cent. A 100 per cent sensitivity and specificity could be obtained in an indirect ELISA using F1 antigen fraction to detect antibody against *P. heterotremus* infection⁹⁷. The various antigenic components such as 27 kDa possibly excretory/secretory (E/S) products of *P. westermani*⁹⁸ and 31.5 kDa substance of *P. heterotremus* were used

as antigens for specific diagnosis of paragonimiasis and for serodiagnosis of human paragonimiasis heterotremas and therapeutic evaluation of praziquantel therapy⁹⁹, both by ELISA and Western blot, respectively. An enzyme-linked immunoelectrotransfer blot was developed for differential diagnosis between *P. heterotremus* and *P. westermani* infections¹⁰⁰. Other ELISA techniques are sandwich ELISA using monoclonal antibodies-based antigen detection assay¹⁰¹ and multiple dot-ELISA⁹⁵ now used in Japan. Generally, ELISA tests are used to detect parasite specific IgG antibodies but the detection of specific IgE antibodies was proven to reduce cross-reactions with other trematode infections and detection of parasite specific IgM antibodies was recommended in the diagnosis of infection at the early stage^{102,103}. In India, Regional Medical Research Centre (RMRC, ICMR), Dibrugarh had developed IgG ELISA using E/S antigen for diagnosis of paragonimiasis and was reported to be highly sensitive and specific¹⁰⁴. The ELISA tests are now most widely used for serological diagnosis of paragonimiasis due to their high sensitivity and specificity. The tests are also applicable to mass screening. However, ELISA tests are more expensive, time-consuming and require costly equipment and experienced persons and all the reagents, antigens, in particular, are not commercially available.

Rapid test: Recently, dot-immunogold filtration assay (DIGFA) kit was developed in China for anti-*P. westermani* antibody detection. This is found to be simple and rapid and does not require any special devices and/or experienced technicians and the results are obtained within 10 min. This kit was prepared using *P. westermani* antigen and reported in China to have the sensitivity and specificity up to 99 and 92 per cent, respectively¹⁰⁵.

Haematological investigation

Leucocytosis with relative lymphocytosis, eosinophilia and increased ESR were common findings in patients with paragonimiasis³³. Most patients have haemoglobin value within normal limits in spite of frequent spitting of blood and recurrent haemoptysis. Eosinophilia (absolute eosinophil count $>1000/\mu\text{l}$) and increased ESR up to 104 mm at the end of 1st h (Westergren) were consistently found in children with paragonimiasis in Manipur³⁵.

X-rays and other imaging technique

The common radiographic findings were patchy air-space consolidation or opacity with associated pleural reaction or thickening, cystic or cavitory lesions, pleural

effusion (usually bilateral) and nodular opacities, which were difficult to differentiate from the similar lesions of tubercular origin. The chest radiographs showed patchy consolidations in 62-71 per cent, pleural thickening in 28 per cent, cystic or cavitory lesions in 11-14 per cent, effusion in 9-10 per cent, and nodular lesions in 8-13 per cent³³⁻³⁵. Persistent pleural effusion in nine patients was reported from Lao PDR of whom in 44.4 per cent patients showed bilateral effusion⁶⁹. Compared to the chest X-ray, computerized tomography (CT) was found as a better technique for visualization of the lesions in the lungs¹⁰⁶. Burrows and tunnels joining the cystic lesions have been described in broncho-tomogram or pulmonary CT^{107,108}. Chest radiographs may be normal in symptomatic patients^{31,32}. Higher rate of normal chest roentgenograms in pulmonary paragonimiasis was also reported from endemic areas in Nigeria¹⁰⁹. CT and MRI of brain of cerebral paragonimiasis patients will show conglomerates of multiple ring-shaped shadows called the 'grape cluster' or 'soap bubble appearance'^{110,111} or isodense lesion mimicking tuberculoma⁷⁷ in one hemisphere of the cerebral cortex.

Treatment

Three major antihelminthic drugs are currently available for treatment of paragonimiasis. Praziquantel is the drug of choice for both pulmonary and extra pulmonary paragonimiasis¹¹². The recommended dose is 25 mg/kg body weight administered orally three times a day after meals for three days without any appreciable side effects. With this regimen relapse occurred in about 2 per cent cases. A 100 per cent cure rate was obtained when the therapy was extended up to 5 days³⁴. Bithionol, 2, 2'-thiobis [4, 6-dichlorophenol] was the drug used before the praziquantel was available for the treatment of paragonimiasis. It is given in doses of 40 mg/kg body weight in two equally divided doses on alternate days for a course of 10 to 20 doses³³. The most common side effect was urticaria, which was observed in about 50 per cent of patients after 2 or 3 doses of bithionol. Rarely, urticaria may be very severe requiring hospitalization and parenteral antihistaminics. The other side effects such as nausea, vomiting, diarrhoea and itching were generally few, mild and transitory. Zhong *et al*¹¹³ had used bithionol to treat paragonimiasis in doses of 50 mg/kg body weight per day on alternate days for 20 doses and found a cure rate of 97.1 per cent. Recently, triclabendazole {5-chloro-6 (2,3-dichlorophenoxy)-2methylthio benzimidazole}, a drug used in veterinary medicine was evaluated for the treatment of paragonimiasis in humans. Control trials

have shown that triclabendazole when administered in a single dosage of 10 mg/kg body weight had comparable efficacy, safety and tolerability with praziquantel^{114,115}.

Conclusion

In the recent years, paragonimiasis has emerged as an important food-borne parasitic disease in India, mainly in the Northeastern States of India. Failure to recognize pulmonary paragonimiasis has resulted in over diagnosis of pulmonary tuberculosis and unwarranted antitubercular therapy, which will have a negative impact on the outcome of the Revised National Tuberculosis Control Programme, especially in the tuberculosis endemic areas. Mahajan emphasized the need to generate awareness among the clinicians and public regarding paragonimiasis and to consider this disease in the differential diagnosis of PTB in places where both co-exist¹¹⁶. *P. heterotremus* has been identified as the causative agent of human paragonimiasis in the northeast India. *Potamiscus manipurensis*, *M. lugubris* and *A. superciliosum* were identified as second intermediate crab hosts of *Paragonimus* in these regions. Further research work is required to determine the first intermediate snail hosts of *Paragonimus* species in India and the role of *P. skrjabini* and *P. westermanni* in human paragonimiasis. The control strategies for paragonimiasis should include the epidemiological surveys to determine the magnitude of the problem, training of public health care providers about the diagnosis and management of paragonimiasis, screening of all patients attending TB clinics, DOTS microscopy centers, hospitals and rural health centers for both tuberculosis and paragonimiasis. People should be educated not to consume fresh and improperly cooked crabs and crayfish, and to clean hands, utensils, cutlery boards, strainers, knives, *etc.* after processing fresh crabs and crayfish. Public health authority should ensure the continuous supply of praziquantel in the hospitals and dispensaries. The problem of paragonimiasis is likely to continue in India unless awareness among public and medical practitioners is spread and appropriate control measures taken.

References

1. Procop WW. North American paragonimiasis (caused by *Paragonimus kellicotti*) in the context of global paragonimiasis. *Clin Microbiol Rev* 2009; 22 : 415-46.
2. Miyazaki I. Lung flukes in the world: Morphology and life history. In: *Symposium on Epidemiology of Parasitic Disease*. International Medical Foundation of Japan; 1974. p. 101-34.

3. Doanh PN, Le NT, Tat D. *Paragonimus* and paragonimiasis in Vietnam. In: Arizono N, Chai JY, Nawa Y, Takahashi Y, editors. *Asian Parasitology 1. Food-borne helminthiasis in Asia*. Chiba, Japan: Federation of Asian Parasitologists; 2005. p. 149-53.
4. Chung HL, Holy HHCP, Ts'ao WJ. Recent progress in studies in *Paragonimus* and paragonimiasis control in China. *Chinese Med J* 1981; 94 : 483-94.
5. Miyazaki I, Hirose H. Immature lung flukes first found in the muscles of wild boar in Japan. *J Parasitol* 1976; 62 : 836-7.
6. Cobbold TS. On some new forms of Entozoa. *Trans Linn Soc London* 1859; 22 : 363-6.
7. Kerbert C. Knowledge to Trematodes. *Zool Anz* 1878; 1 : 271-3.
8. Vevers GM. Observations on the genus *Paragonimus* Braun, 1899 with a redescription of *P. compactus* (Cobbold, 1859). *J Helminth* 1923; 1 : 9-20.
9. Gulati A. On the occurrence of a lung fluke *Paragonimus edwardsi*, n.sp. in a palm civet (*Paradoxurus grayi*) in Kumaon hills. *Mem Dept Agri India Vet Ser* 1926; 3 : 187-90.
10. Blair D, Xu Z-B, Agatsuma T. Paragonimiasis and the genus *Paragonimus*. *Adv Parasitol* 1999; 42 : 174-8.
11. Rao MAN. Lung flukes in two dogs in Madras Presidency. *Indian J Vet Sci Anim Hus* 1935; 5 : 30-2.
12. Srivastava HD. The occurrence of *Paragonimus westermani* in the lungs of cats in India. *Indian J Vet Sci Anim Hus* 1938; 8 : 255-7.
13. Singh NP, Somvanshi R. *Paragonimus westermani* in tigers (*Panthera tigris*) in India. *J wildlife Dis* 1978; 14 : 322-4.
14. Dutta SC, Gupta PP. Paragonimiasis in a bear cat (*Articus binturong*). *Ann Trop Med Parasitol* 1978; 72 : 391-3.
15. Ravikumar S, Vijyalakshmi V, Hanumantha Rao K. Trematodes of the mongoose *Herpestes edwardsii* Geoffroy from Visakhapatnam District. *Proc Indian Acad Sci B* 1979; 88 : 421-4.
16. Gaur SNS, Tewari HC, Sethi MS, Prakash OM. Helminth parasites from tiger (*Panthera tigris*) in India. *Indian J Parasitol* 1980; 4 : 71-2.
17. Parihar NS, Shrivastava SN. Bronchial hyperplasia in a tiger (*Panthera tigris*). *Indian J Anim Sci* 1988; 58 : 230-3.
18. Sano M, Agrawal MC, Kotwal PC, Gopal R. *Paragonimus* infection in tigers at Kanha National Park. *J Parasitol Appl Anim Biol* 1994; 3 : 115-6.
19. Singh TS, Mutum S, Razaque MA, Singh YI, Singh EY. Paragonimiasis in Manipur. *Indian J Med Res* 1993; 97 : 247-52.
20. Takeda M, Sugiyama H, Singh TS. Some freshwater crabs from Northeast India bordered on Myanmar. *J Teikyo Heisei Univ* 2012; 23 : 199-213.
21. Singh TS, Singh YI. Three types of *Paragonimus* metacercariae isolated from *Potamiscus manipurensis* in Manipur. *Indian J Med Microbiol* 1997; 15 : 159-62.
22. Singh TS, Singh YI, Singh KN. A case of paragonimiasis in a civet cat with a new *Paragonimus* subspecies in Manipur, India. *Indian J Pathol Microbiol* 1998; 41 : 351-3.
23. Singh TS. Occurrence of the lung fluke *Paragonimus hueit'ungensis* in Manipur, India. *Chinese Med J (Taipei)* 2002; 65 : 426-9.
24. Singh TS. Occurrence of the lung fluke *Paragonimus heterotremus* in Manipur, India. *Chinese Med Sci J* 2003; 18 : 20-5.
25. Singh TS, Singh ID, Sugiyama H. Possible discovery of Chinese lung fluke, *Paragonimus skrjabini* in Manipur, India. *Southeast Asian J Trop Med Public Health* 2006; 37 (Suppl 3): 53-6.
26. Narain K, Devi Rekha K, Mahanta J. *Paragonimus* and paragonimiasis - A new focus in Arunachal Pradesh, India. *Curr Sci* 2003; 184 : 985-7.
27. Singh TS, Sugiyama H, Umehara A, Hiese S, Khalo K. *Paragonimus heterotremus* infection in Nagaland: A new focus of paragonimiasis in India. *Indian J Med Microbiol* 2009; 27 : 123-7.
28. Tandon V, Prasad PK, Chatterjee A, Bhutia PT. Surface fine topography and PCR-based determination of metacercariae of *Paragonimus* sp. from edible crabs in Arunachal Pradesh, Northeast India. *Parasitol Res* 2007; 102 : 21-8.
29. Devi KR, Rekha, Narain K, Agatsuma T, Blair D, Nagataki M, Wickramashinghe S, *et al.* Morphological and molecular characterization of *Paragonimus westermani* in northeastern India. *Acta Tropica* 2010; 116 : 31-8.
30. Surveyer. Case of Distoma Disease in India. *Indian J Med Res (special Indian Sci congress)* 1919; 1 : 214-6.
31. Singh YI, Singh NB, Devi SS, Singh YM, Razaque M. Pulmonary paragonimiasis in Manipur. *Indian J Chest Dis Allied Sci* 1982; 24 : 304-6.
32. Patil SD, Jindani IB, Bansal MP, Gaiwad KD. Paragonimiasis of the lung. *Ann Trop Med Parasitol* 1984; 78 : 445-8.
33. Singh TS, Mutum SS, Razaque MA. Pulmonary paragonimiasis: Clinical features, diagnosis and treatment of 39 cases in Manipur. *Trans R Soc Trop Med Hyg* 1986; 80 : 967-71.
34. Razaque MA, Mutum SS, Singh TS. Recurrent haemoptysis: Think of paragonimiasis. *Trop Doc* 1991; 21 : 153-5.
35. Singh TS, Singh PI, Singh LBM. Paragonimiasis: Review of 45 cases. *Indian J Med Microbiol* 1992; 10 : 243-7.
36. Singh TN, Singh HR, Devi KS, Singh NB, Singh YI. Pulmonary paragonimiasis. *Indian J Chest Dis Allied Sci* 2004; 46 : 225-7.
37. Singh TN, Kananbala S, Devi KS. Pleuropulmonary paragonimiasis mimicking pulmonary tuberculosis-a report of three cases. *Indian J Med Microbiol* 2005; 23 : 131-4.
38. Singh TS, Sugiyama H, Rangsiruji A, Devi KR. Morphological and molecular characterizations of *Paragonimus heterotremus*, the causative agent of human paragonimiasis in India. *Southeast Asian J Trop Med Public Health* 2007; 38 (Suppl 1): 82-6.
39. Devi KR, Rekha, Narain K, Bhattacharya S, Negmu K, Agatsuma T, Blair D, *et al.* Pleuropulmonary paragonimiasis due to *Paragonimus heterotremus*: molecular diagnosis, prevalence of infection and clinicoradiological features in an endemic area of northeastern India. *Trans R Soc Trop Med Hyg* 2007; 101 : 786-92.

40. Liu Q, Wei F, Liu W, Yang S, Zhang X. Pragonimiasis: An important food-borne zoonosis in China. *Trends Parasitol* 2008; 24 : 318-23.
41. Shimazu T. Experimental completion of the life cycle of the lung fluke, *Paragonimus westermani* in the laboratory. *Jpn J Parasitol* 1981; 30 : 173-7.
42. Gyoten J. Experimental infection of the fresh water crab, *Geothelphusa dehaani*, with the cercariae of *Paragonimus miyazakii*. *Jpn J Parasitol* 1986; 35 : 183-9.
43. Xie BW, Yang YS, Li QZ, Tanf LY. *Rana boulengeri* as the paratenic host of *Paragonimus skrjabini*. *Chinese J Parasitol Parasitic Dis* 1985; 3 : 157-9.
44. Shibahara T. The route of infection of *Paragonimus westermani* (diploid type) cercariae in the fresh water crab, *Geothelphusa dehaani*. *J Helminthol* 1991; 65 : 38-42.
45. Shibahara T, Nishhida H, Sasaki N. A case of multiple lung fluke (*Paragonimus westermani*) infection of a raccoon dog in Japan. *Jpn J Parasitol* 1985; 34 : 513-6.
46. Shibahara T, Nishhida H. An epidemiological survey of the lung fluke, *Paragonimus* spp. in wild mammals in northern part of Hyogo Prefecture, Japan. *Jpn J Vet Sci* 1985; 47 : 911-9.
47. Miyazaki I, Habe S. *Paragonimus westermani filipinus* Miyazaki, 1978, stat. n. occurring at Jaro, Leyte, the Philipinus. *Med Bull Fukuoka Univ* 1979; 6 : 447-62.
48. Habe S, Lai KPF, Agatsuma T, Ow-Yang C K, Kawashima K. Growth of *Paragonimus westermani* (Kerbert, 1878) in mammals and mode of transmission of the fluke among mammals. *Jpn J Trop Med Hyg* 1996; 24 : 225-32.
49. Valez I, Velasquez LE, Velez ID. Morphological description and life cycle of *Paragonimus* spp. (Trematoda: Troglotrematidae): causal agent of human paragonimiasis in Colombia. *J Parasitol* 2003; 89 : 749-55.
50. Guan XH. Paragonimiasis. In: LI YL, Guan XH, editors. *Human parasitology*, People's Public Health Press; 2005. p. 109-13.
51. Chen HT. The occurrence of a new type of *Paragonimus* and some clinical problems related to lungfluke in China. *Annual Report of the Chung Shang Medical College*; 1958. p. 192-3.
52. Chen HT. The Chinese species of paragonimid trematodes, their phylogenetic relationship and faunal distribution. *Acta Parasitologica Sinica* 1964; 1 : 53-68.
53. Chung HL, Hsu CP, Ho LY, Kao P C, Shao L, Chiu, FH, *et al.* Studies on a new lung fluke: *Paragonimus hueit'ungensis* sp. Nov. *Chinese Med J* 1977; 3 : 379-94.
54. Miyazaki I. *Illustrated book of Helminthic zoonoses*, 1st ed. Tokyo: International Medical Foundation of Japan; 1991.
55. Prasad PK, Tandon V, Biswal DK, Goswami LM, Chatterjee A. Phylogenetic reconstruction using secondary structures and sequence motifs of ITS2 rDNA of *Paragonimus westermani* (Kerbert, 1878) Braun, 1899 (Digenea: Paragonimidae) and related species. *BMC Genomics* 2009; 10 (Suppl 3): S25.
56. Blair D, Agatsuma T, Watanobe T, Okamoto M, Ito A. Geographical genetic structure within human lung fluke, *Paragonimus westermani*, detected from DNA sequences. *Parasitology* 1997; 115 : 411-7.
57. Blair D, Chang Z, Chen M, Cui A, Wu B, Agatsuma T, *et al.* *Paragonimus skrjabini* Chen, 1959 (Digenea: Paragonimidae) and related species in eastern Asia: A combined molecular and morphological approach to identification and taxonomy. *Syst Parasitol* 2005; 60 : 1-21.
58. Waikagul J. A new species of *Paragonimus* (Trematoda: Troglotrematidae) from a cat infected with metacercariae from mountain crabs *Larnaudia larnaudii*. *J Parasitol* 2007; 93 : 1496-500.
59. Thaenkham U, Waikagul J. Molecular phylogenetic relationship of *Paragonimus pseudoheterotremus*. *Southeast Asian J Trop Med Public Health* 2008; 39 : 217.
60. Kawashima K, Sugiyama H, Ketudat P. *Paragonimus* in Asia: Biology, genetic variation and speciation. *Paragonimus Research Report* Number 2: Kawashima K, editor. Fukuoka, Japan: Kyushu University School of Health Sciences; 1989.
61. Shibahara T. Studies on lung fluke, *Paragonimus westermani* diploid type: In: Northern part of Hyogo Prefecture, Japan. III. Experimental oral infection with metacercariae of rats, with reference to juvenile worms removed from the muscle. *Jpn J Parasitol* 1984; 33 : 119-32.
62. Keiser J, Utzinger J. Emerging food-borne trematodiasis. *Emerg Infect Dis* 2005; 11 : 1507-14.
63. Toscano C, Yu SH, Nunn P, Mott KE. Paragonimiasis and tuberculosis, diagnostic confusion: A review of the literature. *Trop Dis Bull* 1995; 92 : R 1-R 26.
64. Singh TS, Sugiyama H, Devi Ranjana KH, Singh LD, Binchai S, Rangsiruji A. Experimental infection of laboratory animals with *Paragonimus heterotremus* metacercariae occurring in Manipur, India. *SEA J Trop Med Public Health* 2010; 1 : 47-51.
65. Narain K, Devi KR, Mahanta J. A rodent model for pulmonary paragonimiasis. *Parasitol Res* 2003; 91 : 517-9.
66. Chung HL, Ts'ao WJ. *Paragonimus westermani* (Szechuan variety) and a new species of lung fluke- *Paragonimus szechuanensis*, Part II. Studies on clinical aspects of paragonimiasis szechuanensis - a new clinical entity. *Chin Med J* 1962; 81 : 419-34.
67. Kulka F, Barabas M. Clinical aspects and X-ray diagnosis of paragonimiasis. *Acta Med* 1955; 7 : 371-90.
68. Uchiyama F, Moromoto Y, Nawa Y. Re-emergence of paragonimiasis in Kyushu, Japan. *Southeast Asian J Trop Med Public Health* 1999; 30 : 686-91.
69. Vidamaly S, Choumlivong K, Keolouangkhon V, Vannavong N, Kanpitaya J, Strobel M. Paragonimiasis: A common cause of persistent pleural effusion in Lao PDR. *Trans R Soc Trop Med Hyg* 2009; 103 : 1019-23.
70. Lei HH, Yen CK. Pathologic changes of paragonimiasis: A report of five cases. *Chin Med J* 1957; 75 : 986-1003.
71. Singh TS, Vashum H. Cutaneous paragonimiasis: A case report. *Indian J Pathol Microbiol* 1994; 37 : S33-4.
72. Higashi K, Aoki H, Takebayashi K, Morioka H, Sakata Y. Cerebral paragonimiasis. *J Neurosurgery* 1971; 34 : 515-28.
73. Chung YC, Wu TJ, Lee TC, Yang TC. Four cases of cerebral paragonimiasis. *J Formosan Med Assoc* 1953; 52 : 801-14.

74. Oh SJ. Cerebral paragonimiasis. *Trans Am Neurol Assoc* 1967; 92 : 275-7.
75. Choo JD, Su BS, Lee HS, Song CJ, Shin DW, Lee YH. Chronic cerebral paragonimiasis combined with aneurismal subarachnoid haemorrhage. *Am J Trop Med Hyg* 2003; 69 : 466-9.
76. Miyazaki I, Nishimura K. Cerebral paragonimiasis. In: Hornabrook RW, editor. *Topics on tropical neurology. Contemporary Neurology Series*. vol. 12. Philadelphia: FA Davis Company; 1975. p. 109-32.
77. Singh TS, Khamo V, Sugiyama H. Cerebral paragonimiasis mimicking tuberculoma: First case report in India. *Trop Parasitol* 2011; 1 : 39-41.
78. Chang KH, Cha SH, Han MH, Kim HD, Cho SY, Kong Y, *et al.* An imaging diagnosis of cerebral paragonimiasis: CT and MR findings and correlation with ELISA antibody test. *J Korean Radiol Soc* 1993; 29 : 345-54.
79. Vanijanota S, Radomyos P, Bunnag D, Harinasuta T. Pulmonary paragonimiasis with expectoration of worms: A case report. *Southeast Asian J Trop Med Public Health* 1981; 12 : 104-6.
80. Komiya Y, Yokogawa M. The recovery of *Paragonimus* eggs from stools of paragonimiasis patients by the AMS III centrifugation technique. *Jpn J Med Sci Biol* 1953; 6 : 207-12.
81. Singh SI, Singh LP, Sahu RP, Singh TS, Singh TD. *Paragonimus* ova in pleural fluid (a rare finding). *J Med Soc* 1994; 17 : 54-6.
82. Romeo DP, Pollock JJ. Pulmonary paragonimiasis: Diagnostic value of pleural fluid analysis. *South Med J* 1986; 79 : 241-3.
83. Yokogawa, M. *Paragonimus* and paragonimiasis. *Adv Parasitol* 1965; 3 : 99-158.
84. Sawada I, Takei K, Kato S, Hatsuyama S. Studies on the immunodiagnosis of paragonimiasis II. Intradermal test with fractionated antigen. *J Infect Dis* 1968; 118 : 235-9.
85. Yokogawa M, Tsuji M. Immunological diagnosis as the screening method of paragonimiasis in the endemic area of paragonimiasis. In: *Proceedings of the first regional symposium on scientific knowledge of tropical parasites*, University of Singapore. Singapore: UNESCO; 1962. p. 194-206.
86. Yokogawa M. Epidemiology and control of paragonimiasis. In: Sasam M, editor. *Parasitic diseases. Symposium on epidemiology of parasitic disease*. Tokyo International Medical Foundation of Japan; 1974. p. 137-49.
87. Biguet J, Rose F, Capron A, Tranvaky P. Contribution of immunoelectrophoretic analysis to the knowledge of worm antigens: Practical effects on their standardization, their purification & diagnosis of helminthiasis by immunoelectrophoresis. *Rev Immunol Ther Antimicrob* 1965; 29 : 5-30.
88. Katamine D, Imai J, Iwamoto I. Immunological study on paragonimiasis. Agar-gel precipitation reactions in paragonimiasis. *Jpn J Trop Med Hyg* 1968; 10 : 28-38.
89. Tsuji M, Capron A, Yokogawa M. Immunodiffusion test in the diagnosis of paragonimiasis. Second international congress of parasitology. Colloquium on Immunodiagnosis, Washington D.C.; 1970. p. 6-12.
90. Tsuji M. On the immunoelectrophoresis for helminthological researches. *Jpn J Parasitol* 1974; 23 : 335-45.
91. Pariyanonda S, Maleewong W, Pipitgool V, Wongkham C, Morakote M, Intapan P, *et al.* Serodiagnosis of human paragonimiasis caused by *Paragonimus heterotremus*. *Southeast Asian J Trop Med Public Health* 1990; 21 : 103-7.
92. Quicho LP, Thamaparlerd N, Tharabanij S, Vajrasthira S. Human immune response of cats to *Paragonimus* infection. *Southeast Asian J Trop Med Public Health* 1981; 12 : 364-7.
93. Imai J. Evaluation of ELISA for the diagnosis of paragonimiasis westermani. *Trans R Soc Trop Med Hyg* 1987; 81 : 3-6.
94. Waikagul J. Serodiagnosis of paragonimiasis by enzyme-linked immunosorbent assay and immunoelectrophoresis. *Southeast Asian J Trop Med Public Health* 1989; 20 : 243-51.
95. Ito M, Sato S. Multidot enzyme-linked immunosorbent assay for serodiagnosis of trematodiasis. *Southeast Asian J Trop Med Public Health* 1990; 21 : 471-4.
96. Ikeda T, Oikawa Y, Nishiyama T. Enzyme-linked immunosorbent assay using cysteine proteinase antigens for immunodiagnosis of human paragonimiasis. *Am J Trop Med Hyg* 1996; 55 : 434-7.
97. Indrawati I, Chaicumpa W, Setasuban P, Ruangkunaporn Y. Studies on immunodiagnosis of human paragonimiasis and specific antigen of *Paragonimus heterotremus*. *Int J Parasitol* 1991; 21 : 395-401.
98. Sugiyama H, Sugimoto M, Akaska K, Horiuchi T. Characterization and localization of *Paragonimus westermani* antigen stimulating antibody formation in both the infected cat and rat. *J Parasitol* 1987; 73 : 363-7.
99. Wongkham C, Maleewong W, Intapan P, Morakote N, Chaikumpa W. Partially purified antigens of *Paragonimus heterotremus* for serodiagnosis of human paragonimiasis. *Southeast Asian J Trop Med Public Health* 1994; 25 : 176-80.
100. Dekumyoy P, Waikagul J, Eom KS. Human lung fluke *Paragonimus heterotremus*: Differential diagnosis between *Paragonimus heterotremus* and *Paragonimus westermani* infections by EITB. *Trop Med Int Health* 1998; 3 : 52-6.
101. Zhang ZH, Zhang YJ, Shi ZM, Sheng KF, Liu LT, Hu ZG, *et al.* Diagnosis of active *Paragonimus westermani* infections with monoclonal antibody-based antigen detection assay. *Am J Trop Med Hyg* 1993; 49 : 329-34.
102. Ikeda T, Oikawa Y, Ohashi M, Nawa Y. Parasite specific IgE and IgG levels in the serum and pleural effusion of paragonimiasis westermani patients. *Am J Trop Med Hyg* 1992; 47 : 104-7.
103. Nakamura-Uchiyama F, Onah DN, Nawa Y. Clinical features of paragonimiasis cases recently found in Japan: parasite-specific immunoglobulin M and G antibody classes. *Clin Infect Dis* 2001; 32 : 171-5.
104. Narain K, Devi KR, Mahanta J. Development of enzyme-linked immunosorbent assay for serodiagnosis of human paragonimiasis. *Indian J Med Res* 2005; 121 : 739-46.
105. Gan Xiaoxian, Shi Xiaohua, Wang Yue, Wang Peng-peng, Shen Hui-ying, Zhu Ming-dong, *et al.* Development of rapid diagnostic kit (Dot Immunogold Filtration Assay) for detection of antibodies against *Paragonimus westermani*. *Chinese J Zoon* 2005; 21 : 988-90.

106. Vanijanota S, Bunnag D, Harinasuta T. Radiological findings in pulmonary paragonimiasis heterotremus. *Southeast Asian J Trop Med Public Health* 1984; 15: 122-8.
107. Suwanik R, Harinasuta C. Pulmonary paragonimiasis: An evaluation of roentgen findings in 38 sputum positive patients in an endemic area in Thailand. *Am J Roentgenol* 1959; 81 : 236-44.
108. Im JG, Kong Y, Shin YM, Yang SO, Song JG, Han MC, *et al.* Pulmonary paragonimiasis: Clinical and experimental studies. *Radiographics* 1993; 13 : 575-86.
109. Nokolo C. Outbreak of paragonimiasis in Eastern Nigeria. *Lancet* 1972; 1 : 32-3.
110. Li HZ, Xie FW, Sun SC. CT findings in fresh cerebral paragonimiasis. *No Shinkel Geka* 1992; 20 : 91-7.
111. Cha SH, Chang KH, Cho SY, Han MH, Kong Y, Su DC *et al.* Cerebral paragonimiasis in early stage: CT and MR features. *AJR Am J Roentgenol* 1994; 162 : 141-5.
112. Rim HJ, Chang YS. Chemotherapeutic effect of niclofan and praziquantel in the treatment of pulmonary paragonimiasis. *Korea Univ Med J* 1980; 17 : 113-8.
113. Zhong H L, He LY, Xu ZB, Cao WJ. Recent progress in studies of *Paragonimus* and paragonimiasis control in China. *Chin Med J* 1981; 94 : 483-94.
114. Belizario VY, Mallari AO, De Leon WU, Lucero AC. Assessment of efficacy, safety, and tolerability of praziquantel and triclabendazole in the treatment of paragonimiasis. *Southeast Asian J Trop Med Public Health* 2007; 38 (Suppl 1): 97-105.
115. Calvopina MH, Guderian Ronald H, Paredes Wilson Y, Cooper Philip J. Comparison of two single day regimens of triclabendazole for the treatment of human pulmonary paragonimiasis. *Trans Roy Soc Trop Med Hygiene* 2003; 9 : 451-4.
116. Mahajan RC. Paragonimiasis: An emerging public health problem in India. *Indian J Med Res* 2005; 121 : 716-8.

Reprint requests: Dr T. Shantikumar Singh, Professor & Head, Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, Gangtok 737 102, Sikkim, India
e-mail: shantikumar_singh@rediffmail.com