

EPIDEMIOLOGY, PATHOLOGY AND TREATMENT OF PARAGONIMIASIS IN VIETNAM

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Abstract. Studies on paragonimiasis have been carried out at 9 sites in 8 provinces of the northern mountainous regions of Vietnam, where the people have the habit of eating undercooked crab. From 1994 to 2000 with 6,576 persons in these endemic areas, the rate of eating raw-crab was 68.1%. Crab examination (species *Potamiscus tannanti*) showed an average infection rate with *Paragonimus* metacercaria of 53.6%. With 5,340 stool samples examined by Kato technique, the average infection rate of *Paragonimus* in humans was 1.9%. With 2,216 sputum samples examined by direct and centrifuge methods, the average infection rate of *Paragonimus* was 6.4%. Most of the patients were children (68.5%). The *Paragonimus* infection rate in dogs was 12.1%. Adult worms, collected from dogs in the field and from cats in the laboratory, were identified as *Paragonimus heterotremus* Chen et Hsia 1964 (by electron-microscope and PCR techniques). The main symptoms of paragonimiasis patients was cough (100%), hemoptysis (90.2%), interrupted development (97.7%), no fever (91.7%), chest pain (80.3%), pleural effusion (24.6%), neurological symptoms (4.5%), negative for TB (100%), eosinophilia (90.8%). Chest x-ray examination of the lungs showed nodular shadows (91.8%), ring shadows (90.2%), and more in the lower lobe (82.0%). Paragonimiasis patients were treated by: praziquantel 25 mg/kg/day x 3 days, the cure rate was 68.8%; praziquantel 50 mg/kg/day x 3 days, the cure rate was 75%; and praziquantel 75 mg/kg/day x 2 days, the cure rate was 99.6%.

INTRODUCTION

Paragonimiasis is a food-borne trematode disease. There are over 40 species in the *Paragonimus* genus. Over ten of these infect humans. There were 53 species in 21 genera of freshwater crustaceans and 40 species of freshwater snails, which are intermediate hosts of *Paragonimus* (Faust and Russell, 1964). Paragonimiasis is distributed in many countries of the world. Since 1995, paragonimiasis has been reported in 39 countries, especially China, Japan, Korea, Cameroon, Ecuador, and Peru (WHO, 1995; Darren *et al*, 1998; De *et al*, 1998).

The first case of paragonimiasis was reported in Vietnam, in 1906. From 1906 to 1992, over 30 cases of paragonimiasis in Vietnam were reported. The National Institute of Malariology, Parasitology, and Entomology, Ha Noi, commenced studies on the epidemiology, pathology, diagnosis, and treatment of paragonimiasis in some northern mountainous provinces of Vietnam in early 1994. The objectives of the studies were: 1) determination of the epidemiology of paragonimiasis in some northern mountainous areas of Vietnam; 2) Description of pathology of paragonimiasis for diagnosis; 3) Identification of *Paragonimus* species in Vietnam; and 4) Use of praziquantel to treat paragonimiasis in the field.

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MATERIALS AND METHODS

Study site

Sin Ho District of Lai Chau Province, Thuan Chau and Moc Chau districts of Son La Province, Da Bac District of Hoa Binh Province, Bao Yen District of Lao Cai Province, Ky Son District of Nghe An Province, Ha Giang City, Van Quan District of Lang Son Province and Luc Yen District of Yen Bai Province were chosen for study.

Epidemiological studies

Human examination. The relationship between the habit of eating undercooked crab and paragonimiasis infections in the population was studied. Stool samples were examined by Kato technique to find *Paragonimus* eggs; sputum examination by direct and centrifuge method to find *Paragonimus* eggs and by Ziehl-Neelsen stain to find TB (*Mycobacterium tuberculosis*).

Mammal examination. Stool samples of dogs were examined for *Paragonimus* eggs. Lungs of dogs and cats were examined for adult worms. Cats were experimentally infected with *Paragonimus* metacercariae collected from crabs (25-30 metacercariae/cat).

Crab examination. Crabs were collected and species identified, then examined by digestive method with pepsin-acid solution to find *Paragonimus* metacercariae (Thanh, 1995, 1997; Daren *et al*, 1997).

Snail examination. Snails were collected and species identified, then directly examined for *Paragonimus* cercariae.

Clinical studies

Clinical symptoms of patients infected with *Paragonimus*. The paragonimiasis patients were physically examined. The main clinical symptoms were cough, hemoptysis, chest pain, no fever, interrupted development, and pleural effusion.

Sub-clinical investigations of paragonimiasis patients. Besides stool and sputum samples examined to find *Paragonimus* eggs, blood samples were taken for examination of eosinophilia, and lung x-rays were done.

Treatment of patients infected with *Paragonimus*

Patients found to have *Paragonimus* eggs in sputum or stool were selected for study. All patients were treated with praziquantel 25 mg/kg/day x 3 days or 50 mg/kg/day x 3 days, and 75 mg/kg/day x 2 days. The efficacy of the regimen was determined by sputum and stool examination one month and one year after treatment.

Identification of species

Species were identified by morphology (electron-microscope) (Kino *et al*, 1995, De *et al*, 1997) and molecular technique (PCR with mitochondrial-encoded *cox1* gene) (Hoa *et al*, 2001). Samples collected from different hosts (human, reservoir host, and crab) in Lai Chau, Son La, Lao Cai and Hoa Binh provinces were amplified using polymerase chain reaction (PCR) and comparatively aligned with the known corresponding sequences of *Paragonimus*

heterotremus (geographical origin: China and Thailand) and representative species of the *Paragonimus* genus (GenBank and published data).

RESULTS

Human infection rate

The total number of people reviewed was 6,505 of which 68% (range 40.1-90.0%) answered that they ate raw crab. Stools of 5,340 individuals were examined with 1.9% positive for *Paragonimus*. Sputums (2,216 samples) were also examined, 6.4% gave positive results. The rate of raw-crab eating and results of stool and sputum examinations in each study site are presented in Table 1.

At the 9 study sites, the prevalence of paragonimiasis was 0.3-15.7% (Fig 1). Of the 90 sputum samples examined, 100% were negative for TB.

Infection rates in snails, dogs and crabs

At all 9 study sites, *Potamicus* crab infection by *Paragonimus* metacercariae was 8.7-98.1%. The percentage of *Melanoides* snail-infected *Paragonimus* cercariae was 1.4-5% in 5 of the 9 study sites; the infection rate for *Canis familiaris* was 2.9-26.1% in 5 of the 9 study sites (Table 2). For 145 *Parathephusa* crabs in the study sites, no *Paragonimus* metacercaria was found.

Species identification of parasite and intermediate host

Adult worms were identified as *Paragonimus heterotremus* Chen et Hsia 1964.

Potamicus tannanti crab and *Melanoides* sp were intermediate hosts of *Paragonimus* in Vietnam.

Table 1
The rate of eating raw-crab and prevalence of paragonimiasis in humans.

Study site	Eating raw-crab		Stool examination		Sputum examination	
	No. of people reviewed	Answered "yes" (%)	No. examined	% (+)	No. examined	% (+)
Sin Ho, LC	3,350	66.1	1,754	2.7	1,329	4.6
Thuan Chau, SL	510	90.0	500	0.2	74	9.5
Moc Chau, SL	360	72.2	326	3.4	134	15.7
Da Bac, HB	460	73.0	451	3.3	71	11.3
Bao Yen, LC	401	67.8	400	4.5	103	3.9
Luc Yen, YB	197	64.0	892	0.9	128	10.9
Ky Son, NA	364	97.3	313	0	177	0.6
Tx Ha Giang	464	40.1	340	2.1	100	1.0
Van Quang, LS	400	42.0	364	0.3	100	0
Total	6,506	68.1	5,340	1.9	2,216	6.4

Table 2
The rate of paragonimiasis in intermediate hosts and reservoir hosts.

Study site	<i>Potamicus</i> crab		<i>Melanoides</i> snail		Domestic dog	
	Examined	% positive	Examined	% positive	Examined	% positive
Sin Ho, LC	52	98.1	0	0	37	24.3
Thuan Chau, SL	36	88.9	0	0	34	5.9
Moc Chau, SL	80	52.5	182	2.7	2	1/2
Da Bac, HB	67	55.2	140	3.6	23	26.1
Bao Yen, LC	45	95.6	100	5.0	34	2.9
Luc Yen, YB	38	60.5	200	2.5	0	0
Ky Son, NA	31	9.7	131	0	34	0
Tx Ha Giang	24	12.5	366	1.4	34	0
Van Quang, LS	46	8.7	100	0	24	0
Total	419	53.5	1,219	1.7	222	12.1

LC = Lai Chu, SL = Son La, HB = Hao Binh, YB = Yen Bai, NA = Nghe An, LS = Lang Son.

Table 3
Efficacy of praziquantel in paragonimiasis patients.

Dosage of praziquantel	No. treated	No. cured	Cure rate (%)
25 mg/kg/day x 3 days	16	11	68.8
50 mg/kg/day x 3 days	12	9	75.0
75 mg/kg/day x 2days	178	177	99.4

Pathological information

Paragonimiasis patients. The 178 paragonimiasis patients included 68.5% children (under 15 years old). Positive for *Paragonimus* eggs was 100%, chronic cough 100%, hemoptysis 90.2%, interrupted development 97.7%, no fever 91.7%, chest pain 80.3%, no weight loss 99%, pleural effusion 24.6%, eosinophilia 90.8%, nodular shadow 91.8%, ring shadow 90.2%, lung lesion in the lower lobe 82.0%.

Infected cats. *Paragonimus* eggs appeared in the stools of infected cats 50-52 days post- infection. Most adult worms parasitized the lower lobes (90.5%). Most lung abscesses contained 2 adult worms (13/19 abscesses).

Efficacy of praziquantel

The efficacy of praziquantel at different dosages is presented in Table 3. Some side-effects of praziquantel treatment were noted: dizziness, headache, nausea, and vomiting (4.1-12.5%), which disappeared some hours later.

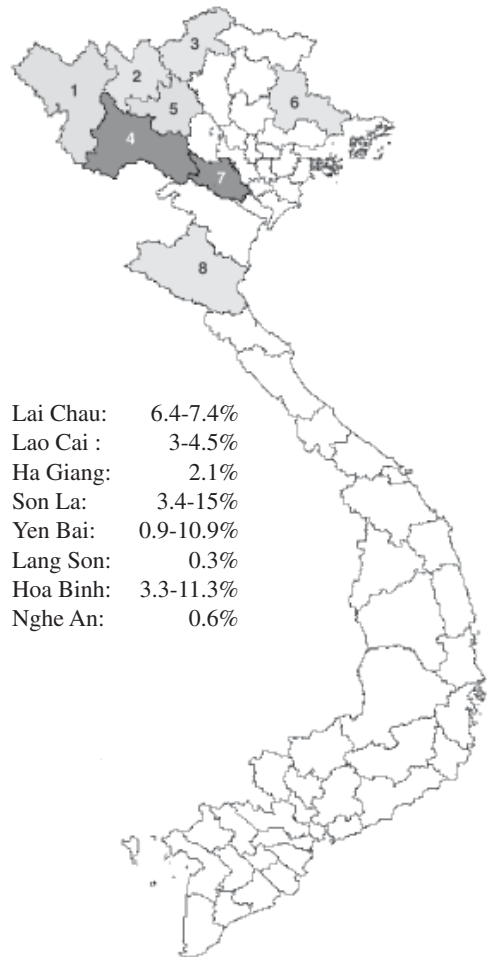


Fig 1- Distribution of paragonimiasis in Vietnam.

*	20	*	40	*	60	*	80	*	94
PhetChina	:	TTTAATTTTGCCTGGATTGGTGTGTGAGACATATCGCATGACTTTGACTAATAAAGATTCTTTGTTCGGTTATTATGGCTTGGTTTTGCC	:	94					
PhetThai	:	:	94					
PHB1seq	:	:	94					
PHB383	:	:	94					
PhVN1	:	:	94					
PhVN3	:	:	94					
PhVN4	:	:	94					
Pbangkok_H	:	.C.G.C.A.C.G.G.....T.C.T.....G.C.T.....G.G.C.T	:	94					
P_harinasu	:	.G.A.G.....A.A.T.....T.....G.C.T.....C.G.G.C.T	:	94					
P_siamensi	:	.G.....T.....GA.....T.T.....G.....T.....C.....G.A.G.G	:	94					
Piloktsuen	:	.A.A.....GA.....T.T.....C.A.....G.....T.....A.G.T	:	94					
Pmacorchi	:	.A.A.G.....T.T.....G.A.....C.C.....T.A.....G	:	94					
PMexEcuado	:	.G.....C.....G.....T.....T.....T.....T.....G.A.G	:	94					
PohiraiKin	:	.A.A.....GA.....T.T.....C.A.....G.....T.....A.G.T	:	94					
PohiraiTan	:	.A.A.....GA.....T.T.....C.A.....G.....T.....A.G.T	:	94					
	100	*	120	*	140	*	160	*	180
PhetChina	:	ATGGGGCTATTGTGTGTTGGGGAGGGTGTGTTGAGCGCACCATATGTTATGGTTGGTTTAGAIGTCAAGACTGCTGTTTTTTAGTTCTG	:	188					
PhetThai	:	:	188					
PHB1seq	:	:	188					
PHB383	:	:	188					
PhVN1	:	:	188					
PhVN3	:	:	188					
PhVN4	:	:	188					
Pbangkok_H	:	.A.G.....T.....T.T.....G.G.T.T.....G.....T.....C.G.G.....A.....	:	188					
P_harinasu	:	.G.....T.....T.T.....G.....C.T.....T.....C.G.G.....A.....	:	188					
P_siamensi	:	.A.....A.T.....G.G.....T.....G.....T.....G.....T.....A.A.....C.A.....	:	188					
Piloktsuen	:	.G.....T.....A.T.....C.G.C.....G.....T.....A.A.....C.A.....	:	188					
Pmacorchi	:	.T.....T.....G.....G.....T.....C.T.....T.....C.....	:	188					
PMexEcuado	:	.C.A.....A.A.....A.T.....G.....T.....C.....G.....	:	188					
PohiraiKin	:	.G.....A.....T.....C.G.C.....G.....T.....A.A.....C.A.....	:	188					
PohiraiTan	:	.G.....A.....T.....C.G.C.....G.....T.....A.A.....C.A.....	:	188					
	200	*	220	*	240	*	260	*	280
PhetChina	:	TTACTGGGTTGATTGGGATCCACAGGGATTAAGGTTTTTCTTGGTTGTTATGTTGGGGGGGACTCGTTTACGGTTTTGAGATCCGGTGGT	:	282					
PhetThai	:	:	282					
PHB1seq	:	:	282					
PHB383	:	:	282					
PhVN1	:	:	282					
PhVN3	:	:	282					
PhVN4	:	:	282					
Pbangkok_H	:	.G.....A.T.....C.G.....T.....G.....T.....T.....AA.....	:	282					
P_harinasu	:	.A.....T.....C.G.....C.....T.....C.....G.....T.....AA.....	:	282					
P_siamensi	:	.C.....T.....A.T.....G.....T.....A.A.....C.....A.T.....G.....G.....TC.....G.....T.....CT.....	:	282					
Piloktsuen	:	.G.....A.....T.....T.....A.....A.....C.....C.....G.....T.....AA.....	:	282					
Pmacorchi	:	.G.....T.....T.....G.....T.....C.....A.....C.....G.....C.....AA.....	:	282					
PMexEcuado	:	.T.....T.....T.....G.....T.....A.....T.....T.....G.....A.....C.....T.....	:	282					
PohiraiKin	:	.G.....A.....T.....A.....A.....T.....T.....C.....G.....T.....AA.....	:	282					
PohiraiTan	:	.G.....A.....T.....T.....A.....A.....T.....C.....G.....T.....AA.....	:	282					
	300	*	320	*	340	*	360	*	376
PhetChina	:	TTGGTGAATTTAGGCTTATTTTCTTTTACTATTGGTGGTGAACCTGGGATATTGTTGCTTCTTCTATTTTGGATAGTCTGTTACATGAT	:	376					
PhetThai	:	:	376					
PHB1seq	:	:	376					
PHB383	:	:	376					
PhVN1	:	:	376					
PhVN3	:	:	376					
PhVN4	:	:	376					
Pbangkok_H	:	.A.....G.....C.....G.....C.....G.....G.....A.....C.....C.....T.....G.....C.....	:	376					
P_harinasu	:	.A.....G.....G.....G.....G.....G.....A.....G.....T.....G.....C.....	:	376					
P_siamensi	:	.G.....G.....C.T.....T.C.....CT.G.....A.....G.....G.....C.....T.....G.....C.....A.A.....T.....G.....	:	376					
Piloktsuen	:	.A.A.....C.T.....G.....C.....T.G.....A.A.G.....A.....C.....C.....T.A.....G.....C.....	:	376					
Pmacorchi	:	.A.....G.....A.....A.....G.....G.....G.....A.....T.....G.....	:	376					
PMexEcuado	:	.G.A.G.....G.....C.....A.....A.....A.....C.....T.....C.....	:	376					
PohiraiKin	:	.A.A.....C.T.....G.....C.....T.G.....A.A.G.....A.....C.....C.....T.A.....G.....C.....	:	376					
PohiraiTan	:	.A.A.....C.T.....G.....C.....T.G.....A.A.G.....A.....C.....C.....T.A.....G.....C.....	:	376					

Fig 2- Nucleotides of the mitochondrial-encoded *cox1* gene of *Paragonimus* sp.

Molecular-based analysis revealed that the *Paragonimus* sp of Vietnam is *Paragonimus heterotremus* Chen et Hsia, 1964, showing high nucleotide similarity to the China and Thailand strains (over 99%) (Fig 2). Phylogenetic analysis uniquely grouped the Vietnamese *Paragonimus* sp with the *Paragonimus heterotremus* of China and Thailand (Fig 3).

DISCUSSION

In the 9 study sites, the majority of the population had the habit of eating raw crab (68.1%). Crabs in 9/9 studied sites were infected with *Paragonimus* larva (78.7-98.1%). This habit is a risk for paragonimiasis, and threatens many people

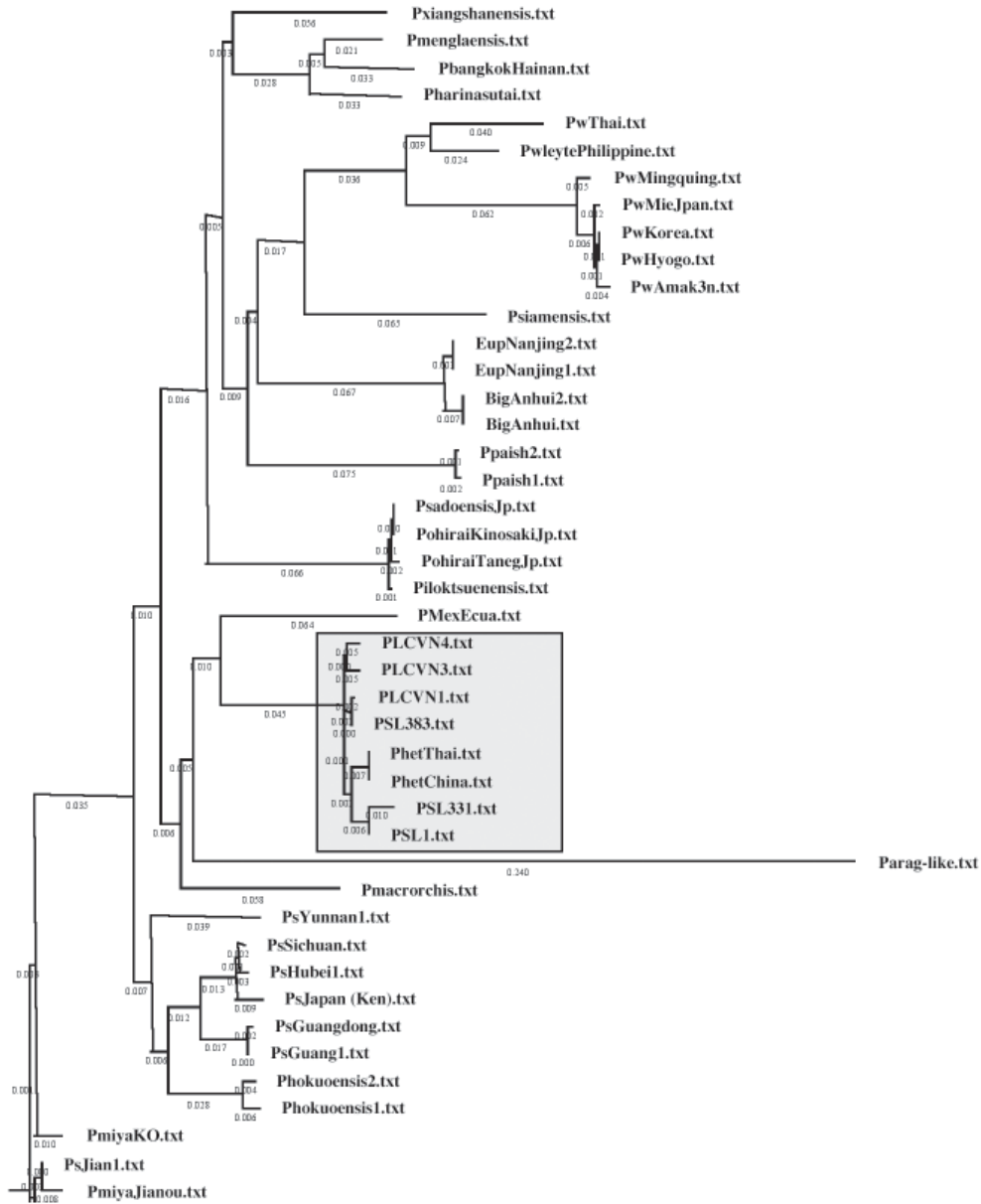


Fig 3- Phylogenetic analysis of unique groups of Vietnamese *Paragonimus* sp with the *Paragonimus heterotremus* of China, Thailand, and others.

living in endemic areas. Moreover, the infection rate of reservoir hosts (dogs) was also high (2.9-26.1%) in 5/9 of the studied sites. This is a major local health problem that is very difficult to prevent. The prevalence of paragonimiasis in humans at 9/9 studied sites was 0.3-15.7%.

The main symptoms of patients were chronic cough (100%), hemoptysis (90.2%), and tuberculosis-

like chest pain (80.3%). Those symptoms are discontinuously developed over time (97.7%). All patients (100%) in the study were negative for TB, 91.7% had no fever, none lost weight, and eosinophilia was present in 90.8% of the patients; the result of Uchiyama *et al* (1999), was 81.7% for eosinophilia.

Nodular ring shadows in the lungs were shown by chest x-ray examination as in tuberculosis but more

often in the lower lobes, as in the laboratory results with infected cats, similar to the result of Toscano *et al* (1995).

Among paragonimiasis patients treated with anti-tuberculosis drugs, the youngest paragonimiasis patient was 1.5 years old and the oldest was 73 (De *et al*, 1999).

Diagnosis of paragonimiasis is confirmed by identification of *Paragonimus* eggs in sputum, stool or pleural effusion samples. However, the number of paragonimiasis patients may be larger than this study indicated because the rate of positive samples was 40% (Faust, 1949; Toscano, 1995).

Adult worms were identified as *Paragonimus heterotremus* by electron-microscope and confirmed by PCR technique (Hoa, 2001). This species was described as distributed in China (Chen Hsia, 1964), Thailand (Miyazaki and Harinasuta, 1966) and Lao PDR (Miyazaki and Fontan, 1970; Vanijanonta, 1984). The efficacy of praziquantel for patients infected with *Paragonimus* was high, and its use was safe in the field.

Conclusion

The prevalence of paragonimus in the 9 study sites of 8 northern mountainous provinces of Vietnam was 0.3-15.7% (Lai Chau, Son La, Hoa Binh, Lao Cai, Ha Giang, Lang Son, Yen Bai and Nghe An), where 42-97.3% of people had the habit of eating raw crab. Adult worms were identified as *Paragonimus heterotremus* (Chen et Hsia, 1964). The infection rate of *Paragonimus* larvae in *Potamiscus tannanti* crabs was 53.6% (8.7-98.1%). The infection rate of *Paragonimus* cercaria in *Melanooides* snail was 1.7% (0-5.0%).

The main symptoms of paragonimiasis were hemoptysis (90.2%), with interrupted development (97.7%), no fever (91.7%), chest pain (80.3%), pleural effusion (24.6%), negative for TB (100%), eosinophilia (90.8%), nodular shadows (91.8%), ring shadows (90.2%), and lesion in the lower lobes (82.0%) of the lungs by chest x-ray examination.

Paragonimiasis patients were treated with praziquantel 25mg/kg/day x 3 days, and the cure rate was 68.8%; by praziquantel 50mg/kg/day x 3 days, 75%, and by praziquantel 75mg/kg/day x 2 days, 99.4%, and safe.

Molecular-based analysis confirmed that the *Paragonimus* sp of Vietnam is *Paragonimus hete-*

rotremus Chen et Hsia, 1964. Phylogenetic analysis uniquely groups the Vietnamese *Paragonimus* sp with the *Paragonimus heterotremus* of China and Thailand.

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