

# FISH-BORNE TREMATODES IN VIETNAM

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**Abstract.** From 1976 to 2000, over 30,000 human stool samples from 38 communes in 15 provinces were examined. The average infection rate of *Clonorchis* and *Opisthorchis* in the provinces examined was 21%. In these provinces, the infection rates were distributed as follows: 0.6% in Ha Giang, 0.2% in Thai Binh, 16.3% in Bac Giang, 13.1% in Hai Phong, 5% in Hoa Binh, 16% in Ha Tay, 3-37.5% in Nam Dinh, 3% in Ha Nam, 20-30% in Ninh Binh, 11% in Thanh Hoa, 0.9% in Nghe An, 0.3% in Da Nang, 11.9% in Binh Dinh, 36.9% in Phu Yen, and 7.6% in Dak Lak. The infection rate of the liver flukes was 3 times higher in men than women, and higher in adults than children. The highest prevalence (50.2-51.6%) was recorded in the age group 40-50 years. In the north of Vietnam, *C. sinensis* infection was microscopically identified and confirmed by molecular approach (PCR technique). In the south of Vietnam, *O. viverrini* infection was microscopically detected only and has not been molecularly identified yet. The infection rates of *Clonorchis/Opisthorchis* in dogs and cats, animal reservoir hosts, were 28.6 and 64.2%, respectively, among which 814 flukes were particularly detected in the liver of one cat. Intermediate hosts, fish, have been examined for trematodes in the northern regions of Vietnam. Ten species of freshwater fish were examined, of which 7 species were positive for *Clonorchis* metacercariae. The main heavily-infected fish species was *Hypophthalmichthys molitrix*, at rates ranging from 44.5-92.9%, with an average of 75 metacercariae per fish. In the southern provinces, one (*Carassius carassius*) of 10 species of freshwater fish was infected with *Opisthorchis* metacercaria (10-29%). Snail examinations carried out in the northern (Red River Delta) region revealed that the infection rate of *Clonorchis* cercariae was 10.2% (in *Melanoides tuberculatus*) and 5.1% (in *Parafossarulus striatulus*). In the southern provinces, the cercarial infection rates of *Opisthorchis* were 2.6-8% (in *Melanoides tuberculatus*). The habit of eating raw fish is very common throughout Vietnam, with a long tradition. The rates of eating raw fish were 70-80% among populations in some provinces. In Vietnam, treatment of clonorchiasis/opisthorchiasis has been done by praziquantel 25 mg/kg/day x 3 days, with cure rates of 65-91%. Control of diseases in Vietnam by case management combined with IEC activities, reduced infection rates and intensity of infection.

## INTRODUCTION

Parasitic zoonoses are widespread in Southeast Asia, particularly Vietnam. Among fish-borne trematode infections caused by many species, only clonorchiasis and opisthorchiasis have been determined in Vietnam. The documented history of these parasites is quite long. As early as 1887, *Clonorchis sinensis* was reported from North Vietnam (Tonkin) by Grall (Bernard *et al*, 1924), with a prevalence of 28% in northern Vietnam (Mathis and Leger, 1910). *Opisthorchis felineus* was reported in Vietnam in 1907 by Verdun *et al* (1924), and *Opisthorchis viverrini* by De *et al* (1996) and Chuong *et al* (1997). Since 1976, epidemiological research on food-borne trematode infections, particularly fish-borne trematodes has been carried out in Vietnam by the National Institute of Malariology, Parasitology and Entomology (NIMPE) in Hanoi. These studies have contributed to strategies of food-borne trematode control in Vietnam.

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## MATERIALS AND METHODS

### Human prevalence surveys

Mass surveys in populations have been undertaken in areas where the local people have a habit of eating raw fish. Stool samples were analyzed by Kato and Kato-Katz techniques. For some human surveys, data on age, sex, and habit of eating raw fish were analyzed. Adult worms were collected from patients, stored in alcohol 70% in -20°C for molecular analysis (PCR).

### Intermediate host surveys

Fish examinations for metacercariae were performed either by direct compression of muscle or fins between glass for microscopic viewing, or by pepsin-HCl digestion of 10 gram muscle samples (WHO, 1995). Snails examined for cercariae were crushed, and a drop of the released liver-pancreatic fluid was added and examined with a microscope.

### Reservoir host surveys

Potential mammal reservoir hosts for zoonotic trematodes were obtained from known endemic areas; these included cats and dogs. Cats and dogs were necropsied and their organs (liver/bile ducts) searched for adult worms. Adult worms were fixed, stained,

and prepared for taxonomic studies according to standard methods (Schell, 1970).

### Experimental infections

Metacercariae recovered from either fish were administered to laboratory animals (cats or dogs), to obtain adult stages for species identification.

### Taxonomic determinations

The species determinations of eggs (feces), cercariae, metacercariae or adult trematodes were made by reference to the taxonomic literature (for example, Schell, 1970). Morphological identification was done and confirmed by PCR technique.

### Food habit surveys

People were interviewed about their habits of eating raw or undercooked fish, using questionnaires.

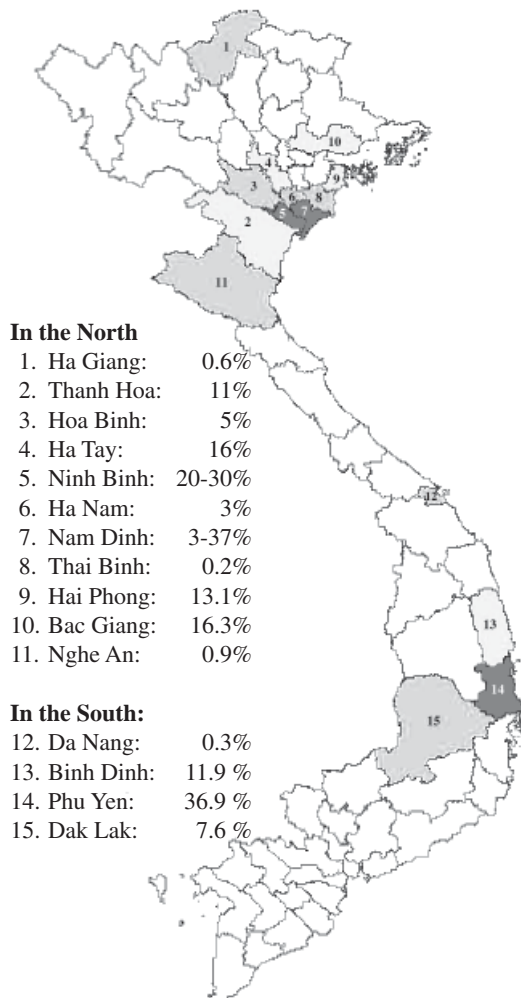


Fig 1- Distribution of clonorchiasis/opisthorchiasis in Vietnam, by province (up to 2002).

### Statistical analysis

When relevant, the data collected were analyzed statistically using SAS version 8, and chi-square and SeqEd 1.0.3; Assembly LIGN 1.0.9c for sequencing.

## RESULTS

### Human infection

From 1976 to 2002, over 30,000 human stool samples from 38 communes in 15 provinces were examined. The average infection rate of *Clonorchis/Opisthorchis* in the provinces examined was 21%, among which Thai Binh Province showed the lowest (0.2%) and Nam Dinh the highest (37.5%). These provinces were (Fig 1): Ha Giang: 0.6%; Thai Binh: 0.2%; Thanh Hoa: 11%; Hai Phong: 13.1%; Bac Giang: 16.3%; Hoa Binh: 5%; Nam Dinh: 3-37.5%; Ninh Binh: 20-30%; Ha Nam: 3%; Ha Tay: 16%; Nghe An: 0.9%; Da Nang: 0.3%; Binh Dinh: 11.9%; Phu Yen: 36.9%; Dak Lak: 7.6%.

The Infection rate of the above liver flukes was 3 times higher in men than in women, and higher in adults than in children. The highest prevalence (50.2-51.6%) was recorded in the age group 40-50 years (Fig 2).

### Food habits

A recent survey in Nam Dinh and Phu Yen provinces revealed that the rates of eating raw fish were 80.4 and 46.0%, respectively; and the infection rates of *Clonorchis/Opisthorchis* were 37.5 and 36.9%, respectively. In Hoa Binh Province, the rate of eating raw fish was 4.7% and it seemed to correlate with the *Clonorchis* infection rate of 5%.

### Animal reservoir hosts

The infection rate of *Clonorchis/Opisthorchis* in dog and cat was 28.6% and 64.2%, respectively, among which 814 flukes were particularly detected in the liver of one of the above cats.

### Intermediate hosts

Fish examination for trematodes has been conducted in the northern regions of Vietnam. Ten species of freshwater fish were examined, of which 7 species were positive with *Clonorchis* metacercaria. The main fish species heavily infected is *Hypophthalmichthys molitrix* with the rate ranging from 44.5 to 92.9% and 75 metacercariae per fish in average. Others species of fish, the infection rate of *Clonorchis* metacercaria was 32% in anabas (*Anabon testudineus*), 25% in carp (*Cyprinus carpis*), 15.6% in crucian carp (*Carassius carassius*), 13.9% in major carp (*Cirrhina molitorella*), 13.3% in amur (*Mylopharyngodon piceus*), 1/1 in tilapia (*Tilapia mossambica*), and negative in snakehead (*Ophicepalus maculatus*), cat fish (*Clarias fucus*), and loach. In the

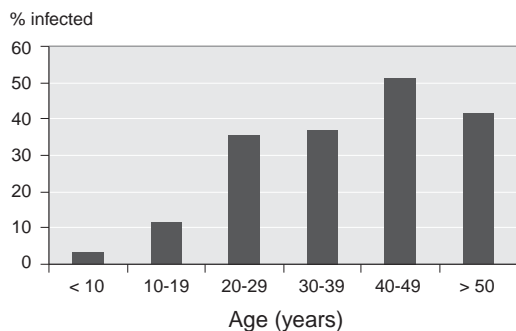


Fig 2- Age distribution.

Southern provinces, one (*Carassius carassius*) of 10 species of freshwater fish was infected with *Opisthorchis* metacercaria (10-29%).

Snail examination carried out in the northern (Red River Delta) region revealed that, the infection rate of *Clonorchis* cercaria was 10.2% (in *Melanoides tuberculatus*) and 5.1% (in *Parafossarulus striatulus*). In the southern provinces, the cercarial infection rate of *Opisthorchis* was 2.6-8% (in *Melanoides tuberculatus*).

#### Identification of species

By morphological methods and molecular methods (PCR technique with mitochondrial genome), adult flukes from humans and cats, and metacercaria from fish were identified as *Clonorchis sinensis* in the north and *Opisthorchis viverrini* in the south of the country.

#### DISCUSSION

In the north of Vietnam, *C. sinensis* infection was microscopically identified and confirmed by molecular approach. In the south of Vietnam, *O. viverrini* infection was also microscopically detected and has been molecularly identified. The data accumulated over the past decade show that the liver flukes, *C. sinensis* and *O. viverrini*, are important food-borne trematode zoonoses in Vietnam. The prevalences of *C. sinensis* were comparable with those reported for endemic areas in neighboring China (Li *et al*, 2001), and Hong Kong (Ko, 1991), and exceeded those reported for Korea (Rim, 1997) and Taiwan (Chen, 1991), and *O. viverrini* compared with Thailand (Hinz *et al*, 1994).

With the data obtained, epidemiologically, clonorchiasis/opisthorchiasis in Vietnam should be considered a risky public health problem. There is a great need that all steps be taken to instigate control programs and to screen potential reservoirs and vectors of these important diseases.

To date, liver flukes have been detected in more than 15 provinces. However, the distribution of these diseases is not limited to those provinces, due to the very common habit of eating rawfish in Vietnam. Overall survey for screening and serious study, are urgently needed in all other provinces with molecular confirmation of their taxonomic status.

In conclusion, clonorchiasis and opisthorchiasis are widespread in Vietnam (in more than 15 provinces). *Clonorchis sinensis* is distributed in northern, and *Opisthorchis viverrini* in southern, Vietnam.

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