

INTRODUCTION

Small liver fluke (SLF) and minute intestinal fluke (MIF) are of medical importance and public health significance in the community. It is estimated that more than one billion people are at risk and about 50-60 million people are infected with these trematodes worldwide. However, this number is underestimated because the clinical symptoms of fluke infection are not specific. Moreover, eggs of the two types of these flukes are highly similar leading to confusion so MIF has not been reported in humans for a long time.

In Vietnam 7 species of small flukes (SF) have been detected in humans. In many areas including Ninh Binh province, the reinfection rate of helminths in general and SF, in particular, is quite high. People living in two coastal districts of Ninh Binh province, Kim Son and Yen Khanh, customarily eat raw or improperly cooked fish and there have been some reports of SF infection among local people. In the past, diagnosis of SF in that area was made merely based on the observation of small fluke eggs in stool samples and it is difficult to identify the species by faecal examination under a light microscope so the exact prevalence of each species of SF in the community can not be determined. So the “*Study on epidemiological characteristics and species composition of small liver flukes and minute intestinal flukes in Kim Son and Yen Khanh districts, Ninh Binh province (2016-2019)*” was carried out with 2 objectives:

1. *Determine epidemiological characteristics of small liver fluke and minute intestinal fluke infection in Kim Son and Yen Khanh districts, Ninh Binh province (2016).*

2. *Determine species composition of small liver fluke and minute intestinal fluke by morphology and molecular biology techniques in study sites.*

*** NOVELTY AND SCIENTIFIC SIGNIFICANCE OF THE THESIS**

1. Add data on the situation of small fluke infection in Kim Son, Yen Khanh district, Ninh Binh province to the epidemiological map of small flukes in Vietnam which contributes to developing measures to effectively prevent the infection in areas where local people have the habit of eating raw fish.

2. Determine the species composition of small flukes that infect humans at the study site using molecular technique to analyse genetic characteristics of eggs in faecal samples.

3. Determine the situation of metacercariae infection in fish collected from the study site. With a very high rate of larval infection on freshwater fish, especially fish species that are commonly used for raw consumption such as common carps, grass carps and silver carps the health education to limit eating raw fish or apply food safety measures are of significance in the prevention of small fluke infection in the community.

THESIS STRUCTURE

The thesis consists of 125 pages divided into the following sections: Introduction (2 pages), Literature review (34 pages), Study subjects and methods (24 pages), Results (34 pages), Discussions (27 pages), Conclusions (2 pages), and Recommendations (1 page).

There are 43 tables and 24 figures, 176 references (42 in Vietnamese and 137 in English).

Chapter 1. LITERATURE REVIEW

1.1. Epidemiological characteristics of small flukes

More than 100 species of trematodes have been reported to infect humans and six main groups of are schistosomiasis, fascioliasis, paragonimiasis, SLF (opisthorchiasis, clonorchiasis) and MIF.

The species of SLF and MIF are transmitted by fish (fish-borne trematode - FBT) and their distribution is closely related to the habits of eating raw or undercooked fish of the people. FBT can complete their life cycle regardless of the presence of human because many animals or birds can be served as definite hosts for the flukes. These flukes are scattered throughout the world but the main endemic areas are Southeast Asia and the Far East. High prevalence of FBT has been reported in China, Korea, Thailand, Laos ... Currently, there are about 45 million people worldwide infected with FBT with the majority of them living in Asia (at least 35 million people infected and 200 million at risk of infection). All over the world, there are about 7 million people infected with MIF. Due to the similar epidemiological and infectious nature MIF can coexistence with SLF. About 26 species of the family Heterophyidae have been reported to be infected in humans and they are distributed throughout the world.

In Vietnam, *C. sinensis* and *O. viverrini* have been reported in many places. There have been no reports on the number of people infected with MIF but *H. pumilio*, *H. taichui*, *C. formosanus* and some other MIF species have been detected in areas of the Red River Delta.

There are many factors associated with small fluke infection.

Age: FBT infections are more common in adults with the rate and intensity of infection increases with age.

Gender: Most reports in the world and Vietnam have shown that the rate and intensity of FBT infection in men are higher than in women.

Knowledge: Results of a study in China show that knowledge of prevention related to FBT infection. Many authors recommend the need for raising the awareness of people. Research in Nga Son, Thanh Hoa found a relationship between knowledge, attitude and practice of using hygienic latrines and FBT infection. A study carried out by Nguyen Van Chuong and colleagues found that specific treatment combined with health education to increase people's understanding about SLF was effective in controlling SLF infection. In the intervention commune, the prevalence of *O. viverrini* infection decreased by 74.1% and the intensity decreased by 76.75% compared to before the intervention.

Attitude: Although FBT can be prevented simply by eating only cooked fish, it has been difficult for millions of people to change their centuries-old habits of eating raw fish.

Practice: All studies on FBT in the world and Vietnam have similar results that eating raw fish is a strong risk factor of FBT infection. Some factors related to this behaviour such as location, type of fish... affect the risk of infection. People eating fish at a restaurant is considered to be at a higher risk of getting an infection because the majority of the fish is processed from locally grown fish.

Sanitary conditions: People living in houses with fish ponds are at risk of infection. The prevalence of FBT infection among people living near freshwater sources is 2.15 times higher compared to those

far from water sources. Pig stables and toilets near to ponds, sewer system leading waisted water into ponds make these water storages polluted. The use of night soil for fish farming is related to infection. Controlling the infection in FBT reservoirs like dogs, cats.. and other fish-eating animals play a role in controlling the human infection.

1.2. Methods for the detection and identification of small fluke

Various methods can be used to diagnose FBT infection such as ova and parasite test, immunological or molecular tests.

Useful characteristics for species identification based on the morphological characteristics of eggs are shape, size, shell, opercula, shoulder of the eggs and embryo in the eggs. The main features that help classify adult flukes are the shape, size, features of the abdominal cavity, oral and ventral suckers, internal organs especially the genital organs. Features commonly used in the identification of metacercariae are oral and ventral suckers, tegumental spines, oral spines, stylets, pharynx, oesophagus and ceca, flame cells, seminal vesicle and receptacle, testicles, uterus, vitellaria, gonotyl, hermaphroditic duct,...

Due to the overlapping distribution and the morphological similarity of FBT eggs, the exact identification based on merely morphological characteristics is difficult. The molecular technique has great potential in species determination. Many techniques have been applied to identify flukes such as PCR technique with species-specific primers; multi-primer PCR; PCR-RFLP and sequencing. The genetic markers commonly used in identification are nuclear segments such as ITS1, ITS2 and mitochondrial genes (COX1).

1.3. Symptoms, diagnosis and treatment of FBT infection

Clinical symptoms of SLF infection depend on the number of helminths in the body. Patients with mild infections often have no symptoms. The clinical manifestations of severe infections are often apparent with different symptoms. Diagnosis of human SLF infection is based on epidemiological factors (history of eating raw fish and living in an epidemic area), clinical symptoms and laboratory tests. Praziquantel is a drug of choice.

Mild infection with Heterophyidae usually has no symptoms and progresses mild and fast. People with severe infections may experience symptoms such as diarrhoea, mucus hypersecretion, abdominal pain, loss of appetite, indigestion, nausea and vomiting. Symptoms usually subside after 1 month but can last up to 1 year. Praziquantel is the effective drug for all species of Heterophyidae.

1.4. Prevention of small liver fluke and minute intestinal fluke

Prevention should focus on measures to reduce and eliminate factors relating to transmission: active detection and treatment of infected people; protection of fish ponds and other aquaculture systems against the risk of contamination from stools or egg disposal sources; proper management and handling of stools from human dogs, cats and other infected animals will reduce the risk of egg contamination in water sources, thereby helping to prevent infection with FBT; Controlling snails in ponds and lakes; Strengthen communication and education activities to eliminate the habit of eating raw fish and promote processing and using fish properly.

Chapter 2. STUDY SUBJECTS AND METHODS

2.1. Subjects of the study

- For the study of epidemiological characteristics:
 - + Persons live in the study sites, are capable of answering the interview and agree to provide stool samples. The inclusion criteria are people aged 15 years or older irrespective of gender, occupation or ethnic group.
 - + Fish commonly used for raw consumption in the study sites.
- For determining the species composition of flukes:
 - + Eggs and adult flukes collected from infected people.
 - + Metacercariae obtained from fish.

2.2. Time of the study: From 2016 to 2019.

2.3. Sites of the study

- Field: Kim Dong, Kim Tan communes in Kim Son district; Khanh Thanh, Khanh Thuy communes in Yen Khanh district, Ninh Binh province.
- Laboratory: Department of Parasitology, National Institute of Malaria, Parasitology and Entomology; Department of Parasitology, Department of Microbiology and Pathogens, Institute of Biomedicine and Pharmacy, Vietnam Military Medical University.

2.4. Study design

Cross-sectional study and experimental laboratory.

2.5. Sample size

- For ova exam and KAP interview: according to the formula for a ratio the sample size of 185 for one district was calculated and about 200 persons each district were involved in the study.

- To study the metacercaria infection rate in fish: the calculated sample size was 32 for one species of fish. 345 fish belong to 6 species commonly used by local people for raw consumption were tested including 5 freshwater fish and one brackish water fish.

- To determine the species composition of flukes
 + All faecal samples that were positive for eggs.
 + Adult flukes collected from 10 persons with the highest intensity of infection after taking antihelminth drugs.

- All metacercaria in the tested fish.

2.6. Content of the study

- Determine the rate and intensity of human infection with FBT.
- Determine factors related to FBT infection.
- Determine the rate and intensity of metacercaria infection in fish.
- Determine the species composition of flukes in human and fish.

2.7. Variables

+ Democratic characteristics of the subjects: age, gender, level of education, occupation ...

+ Prevalence of infection: the number of positive persons/ total number of examined persons x 100 (expressed by the percentage (%)).

+ Intensity of infections: the number of eggs per gram of faeces (EPG) and was classified into 3 levels that were mild (<1,000), medium (1,000 - 10,000 EPG) and heavy infection (> 10,000 EPG).

+ Knowledge, attitude and practice of local people to prevent FBT.

+ Factors related to human FBT infection at the study sites.

+ The rate of fish infected with metacercaria: (number of fish infected with metacercaria) / (total tested fish) x100.

The ratio of each fish species infected with metacercaria: (number of fish of the same species infected with metacercaria) / (total number of tested fish of the same species) x 100.

+ Infection intensity: (number of metacercaria) / (total gram of fish).

+ Some morphological characteristics of eggs, adults and metacercaria of SLF and MIF.

+ Some characteristics of ITS2 region and COX1 gene obtained from eggs, adults and metacercaria of SLF and MIF.

2.8. The techniques applied in the study

- Formalin ether test for ova exam
- Interviewing participants with questionnaires (KAP).
- Digestion technique for metacercaria in fish.
- Collect and stain the adult fluke with carmin.
- Extract DNA from eggs, adult and metacercariae.
- Amplification and sequencing ITS2 region and COX1 gene.

2.9. Data analysis: biomedical statistics by SPSS 16.0 software.

Chapter 3. RESULTS

3.1. Prevalence and related factors to small fluke infection in Kim Son and Yen Khanh districts, Ninh Binh province 2016

400 people were involved in the study, 244 men (61.0%) and 156 women (39.0%). The average age was 46.8 ± 11.57 year old.

The majority of subjects were farmers (79%) with limited educational attainment and only 1% were graduated from universities or colleges. Most participants used hygienic toilets (87.25%). The majority of them lived near the river (67.25%) or with a fish pond (57%), had dogs (71.5%) or cats 253 (63.25%).

Table 3.3. Knowledge of participants on the mode of fluke infection (n = 400)

Mode	Number	Rate (%)
Eating raw fish	275	68.8
Eating raw vegetables	233	58.3
Skin transmission	75	18.8
Not know	11	2.8

Comments: 275 participants (68.8%) gave the right answer on the mode of fluke infection; others answered that human could be infected with FBT through eating raw vegetables (58.3%) or skin transmission (18.8%). 2.8% of participants did not know.

Table 3.5. The distribution of age and sex of participants who knew that eating raw fish would make people infected with small flukes

Participant groups		n	Know	%	p
Age groups n = 400	15 - 29	30	18	60	0.336
	30 - 39	70	45	64.29	
	40 - 49	120	84	70	
	50-59	132	92	69.70	
	≥60	48	36	75.00	
Gender n = 400	Male	244	166	68.03	0.782
	Female	156	109	69.87	

Comments: The rate of participants being aware of the mode of fluke infection through eating raw fish gradually increased with age group, however, the difference was not statistically significant. There was no difference in knowledge between the two genders.

Table 3.6: The proportion of participants knowing that eating cooked fish could prevent fluke infection (n=400)

Participant groups		n	Know	%	P
Age groups	15 - 29	30	19	63.33	0.087
	30 - 39	70	47	67.14	
	40 - 49	120	81	67.50	
	50 -59	132	93	70.45	
	≥ 60	48	37	77.08	
Gender	Male	244	162	66.39	0.271
	Female	156	115	73.72	

Comments: 69.3% of people knowing that eating cooked fish could prevent small fluke infection, this proportion tended to increase with age and in women, but the difference was not significant.

Table 3.8. The proportion of participants knowing the harmful effects of fluke infection (n=400)

Harmful effects	Know	Rate (%)
Abdominal pain	212	53.0
Pain in the liver region	166	41.5
Anemia	66	18.0
Biliary tract infection	60	15.0
Biliary tract cancer	52	13.0
Gallstones	54	13.5
Itching	41	10.3

Comments: The most known harmful effects of fluke infection were abdominal pain (53%), pain in the liver region (41.5%).

Table 3.10: People's attitudes to small fluke infection

Attitudes		n	%
Attitude if infected with small flukes	Consult a doctor	387	96.8
	Self-medication	9	2.2
	Let self-healing	4	1.1
If knowing that he/she can be infected with dangerous pathogens when eating raw fish	Continue eating raw fish	13	3.3
	Stop eating raw fish	297	74.3
	Reduce the frequency of eating raw fish	90	22.4

Comments: The majority (96.8%) of the participants would consult a doctor if they were infected with small flukes. 74.3% of respondents would stop eating raw fish if they know this habit could make them infected with dangerous pathogens.

Table 3.11: Proportion of people eating raw fish at the study site

District		Eating raw fish			p1-2
		Yes	No	Total	
Kim Son (1)	N	154	45	199	> 0.05
	Rate (%)	77.4	22.6	100	
Yen Khanh (2)	N	139	62	201	
	Rate (%)	69.2	30.8	100	
Total	N	293	107	400	
	Rate (%)	73.3	26.7	100	

Comments: There are 73.3% of persons having the habit of eating raw fish at the study site. These rates were not significantly different between the two districts.

Table 3.12: Proportion of people eating raw fish by age group and gender (n = 400)

Group		N	Eating raw fish	%	p
Age group	15 – 29	30	18	60.00	> 0.05
	30 – 39	70	53	75.71	
	40 – 49	120	90	75.00	
	50 – 59	132	94	71.21	
	≥ 60	48	38	79.17	
Gender	Male	244	209	85.7	< 0.001
	Female	156	84	53.8	

Comment: The rate of people eating raw fish was not different by age groups but men eating raw fish more often than women.

Table 3.13: Reasons and places to eat raw fish

Reasons and places		n	Rate (%)
Reasons	Eat when feeling like it	110	27.50
	Along with alcohol drinking	107	26.75
	When invited	67	16.75
	Receiving guests	50	12.50
Places	At home	233	58.25
	At restaurants	124	31.00
	Near to the fish pond	23	5.75
	In friend's houses	3	0.75
	Other places	56	14.00

Comment: local people usually ate raw fish whenever they liked (27.5%) or drank alcohol (26.8%). They often ate at home (58.25%) or a restaurant (31%).

Table 3.14: Frequency of eating raw fish by gender

Frequency	Male (n=209)		Female (n=84)		Total	
	n1	%	n2	%	n	%
1 time /month	137	65.55	55	65.48	192	65.53
2 - 3 times /month	53	25.36	28	33.33	81	27.65
≥ 4 times /month	19	9.09	1	1.19	18	6.14
Total	209	100.00	84	100.00	20	0.68
p	0.032				293	100

Comments: The majority of people ate raw fish once a month (65.6%). Men tended to eat more frequently than women ($p < 0.05$).

Table 3. 16: Prevalence of small fluke infection

Infection		Yes	No	Total	p1-2
District					
Kim Son (1)	n	40	159	199	0.861
	(%)	20.1	79.9	100	
Yen Khanh (2)	n	38	163	201	
	(%)	18.9	81.1	100	
Total	n	78	322	400	
	(%)	19.5	80.5	100	

Comments: The rate of infection at the study site was 19.5% and was not significantly different between the two districts.

Table 3.21: Intensity of small trematode infection

Groups		n (%)	Mean ±SE	p
Total		78 (100)	517.06 ± 124.9455	
Intensity	Mild	68 (87.17)		
	Moderate	10 (12.83)		
	Severe	0 (0)		

District	Kim Son	40	723.00 ± 231.5450	0.194
	Yen Khanh	38	396.84 ± 75.6782	

Comments: The average intensity of fluke infection was 517.06 EPG. The majority were mild infection and no one was severely infected.

Table 3. 26: Relationship between eating raw fish and fluke infection

Eating raw fish \ Infection		Yes	No	Total	OR (CI 95%)	P
		Yes	N	73		
	Rate (%)	24.9	75.1	100		
No	N	5	102	107		
	Rate (%)	4.7	95.3	100		

Comments: People eating raw fish had 6.769 times higher risk of infection than those without this habit (OR = 6.769; p < 0.001).

Table 3. 27: The frequency of eating raw fish and prevalence of fluke infection

Frequency	n	Infection	Rate (%)	p
Never (1)	107	5	4.67	p1-2;3;4 < 0.05 p2-3 > 0.05; p2-4 < 0.05; p3-4 < 0.05
1 time a month (2)	192	43	22.40	
2 – 3 times a month (3)	81	21	25.93	
≥ 4 times a month (4)	20	9	45.00	

Comment: Frequency of eating raw fish was related to infection. The rate of FBT infections in people who frequently ate raw fish (≥ 4 times a month) was significantly higher than those who ate less frequent (1 - 3 times a month (p < 0.05)).

Table 3.28: Prevalence of small fluke infection by gender

District / Gender		n	Infected	%	OR (CI95%)	P
Kim Son n=199	Male	112	31	27.68	3.32 (1.48 - 7.42)	0.004
	Female	87	9	10.34		
Yen Khanh n=163	Male	132	34	25.76	4.44 (1.51 - 13.03)	0.001
	Female	69	4	5.80		
Total n = 400	Male	244	65	26.6	3.99 (2.12-7.54)	< 0.001
	Female	156	13	8.3		

Comment: the prevalence of FBT infection in men was significantly higher than that of women (OR 3.99; $p < 0.001$).

Table 3. 29: Gender, raw fish intake and infection

Raw fish intake/ Gender		Infect ed	Not infected	Total	OR (CI95%)	p
Eat raw fish	Male	62	147	209	2.799 (1.390 – 5.636)	0.003
	Female	11	73	84		
Do not eat raw fish	Male	3	32	35	3.281 (0.522 – 20.607)	0.196
	Female	2	70	72		
Total n = 400	Male	65	179	244	3.99 (2.12-7.54)	<0.001
	Female	13	143	156		

Comment: in people who dit not eat raw fish salad, the difference of small fluke infection between men and women was not significant, but in people who ate raw fish, the prevalence of infecton in male was 2.799 times higher than that in female ($p < 0.01$).

Table 3. 26: The prevalence of metacercariae in fish

Fish species		Infected	Rate (%)	p
Silver carp (1)	87	58	66.7	(1-3) > 0.05
Mud carp (2)	53	8	15.1	(3-4;1) > 0.05

Grass carp (3)	51	40	78.4	(2-4;5) < 0.05
Common carp (4)	52	45	86.5	(2-3;5) < 0.05
Tilapia (5)	52	1	1.9	(1-2;5) < 0.05
Dotted gizzard shad	50	0	0	(2-5) > 0.05
Total	345	152	44.1	

Comments: 44.1% of tested fish were infected with metacercariae. All 5 species of freshwater fish were infected with metacercariae but not for brackish water fish. The highest infection rate was among common carp (86.5%); grass carp (78.4%) and silver carp (66.7%).

Table 3. 37: Intensity of metacercariae infections in freshwater fish (metacercariae / gram of tested fish)

Fish species	N	Mean	SD	p
Silver carp (1)	87	0.1529	0.4056	(1-2) > 0.05
Mud carp (2)	53	0.0585	0.3443	(1-3;4) < 0.001
Grass carp (3)	51	6.3769	11.8058	(1-5) < 0.01
Common carp (4)	52	0.4677	0.5706	(2-3; 4) < 0.001
Tilapia (5)	52	0.0004	0.0028	(2-5) > 0.05
Total	295	1.2406	5.4208	(3-4;5) < 0.01 (4;5) < 0.01

Comments: The density was 1.24 metacercariae/gram of fish; The highest density was in grass carp and the lowest was in mud carp.

3.2. Species composition of small fluke

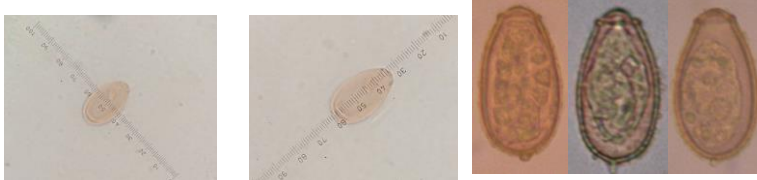


Figure 3.3. Eggs of small flukes in faeces

Under light microscopy obtained eggs had:
 Oval shaped with one smaller end and embryos inside the eggs;
 Thin eggshells with rough or smooth surface;
 An operculum at the smaller end and a raised shoulder around the operculum; a small comma-shaped knob on the larger end;
 The size of the eggs were comparable to that of SLF and MIF.

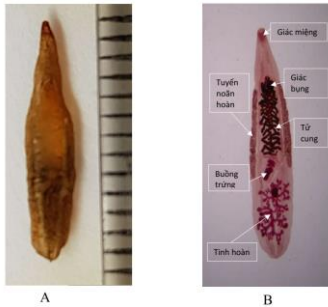


Figure 3. 2: Images of adult flukes

(A: fresh and unstained flukes, B carmine-stained flukes)

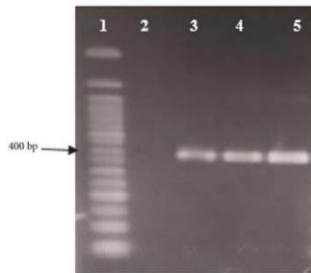


Figure 3.8. Electrophoresis of PCR products in stool samples

Band 1: 50 bp DNA marker, band 2: negative control, band 3-5: samples.

Comment: The size of the PCR products were about 400 bp.

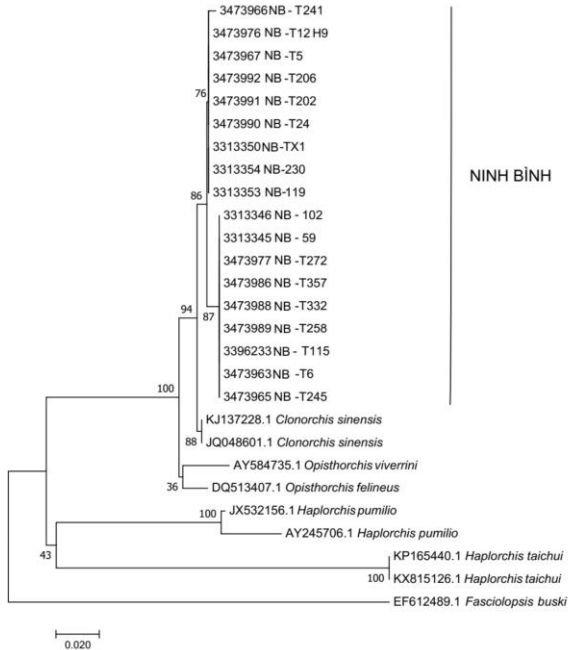


Figure 3. 4: A phylogenetic tree of small flukes based on ITS2 obtained from eggs in human stools

Comment: All the samples yielding DNA products were of *C. sinensis*.

Table 3.35. Some ITS2 sequences registered in the gene bank

Code of samples	Species	Genbank code
59-NB	<i>Clonorchis sinensis</i>	MN128615
102-NB	<i>Clonorchis sinensis</i>	MN128616
119-NB	<i>Clonorchis sinensis</i>	MN128617
TX1-NB	<i>Clonorchis sinensis</i>	MN128618

Comments: all the faecal samples yielding DNA products were of *C. sinensis*.

Table 3. 36: Similarity of sample 115 with some sequences

	Code	Source	Level of similarity (%)	Species
1	EU652407	Vietnam	99.52	<i>C. sinensis</i>
2	MN116478	Russia	99.01	<i>C. sinensis</i>
3	MN116477	Russia	99.01	<i>C. sinensis</i>
4	MN116476	Russia	99.01	<i>C. sinensis</i>
5	MN116475	Russia	99.01	<i>C. sinensis</i>
6	KY564177	Korea	99.01	<i>C. sinensis</i>
7	KJ204622	Vietnam	99.01	<i>C. sinensis</i>
8	KJ204600	Vietnam	99.01	<i>C. sinensis</i>
9	KJ204582	Russia	99.01	<i>C. sinensis</i>
10	KJ204590	Russia	99.01	<i>C. sinensis</i>

Comment: Cox1 gene sequence of sample 115 coincided > 99.0% with some sequences of *C. sinensis*.

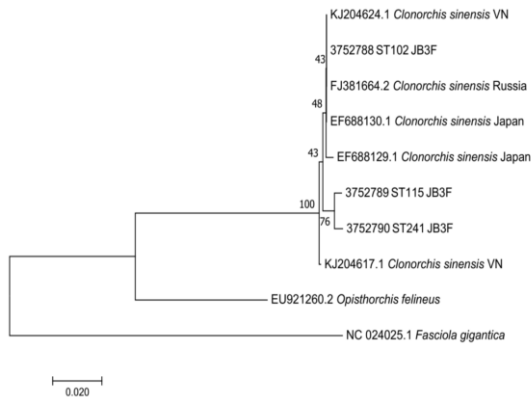


Figure 3.12. A phylogenetic tree of small flukes based on CoxI obtained from eggs in human stools

Comments: All eggs from human stool were *C. sinensis*.

3.2.2. Species composition of metacercariae in fish

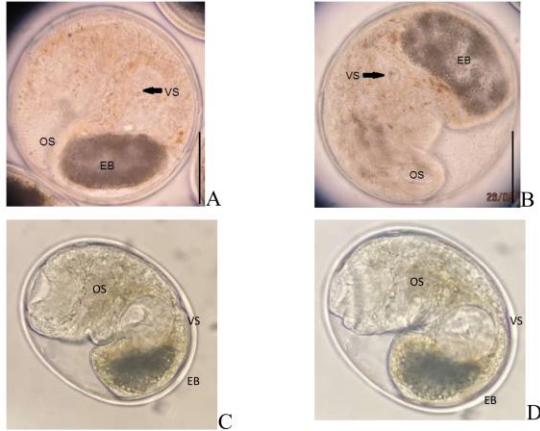


Figure 3.13. Metacercariae in fish

A *H. pumilio*; B *H. taichui*; C-D *C. sinensis*

Comments: *H. pumilio* (A), and *H. taichui* (B) metacercariae had small I-shaped spines arranged inside the ventral sucker. The metacercariae of *C. sinensis* (C-D) had no spines in the ventral sucker.

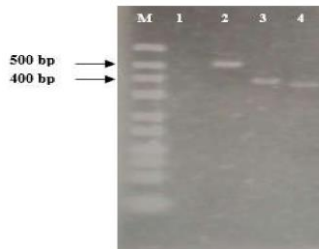


Figure 3.14. PCR products from metacercariae

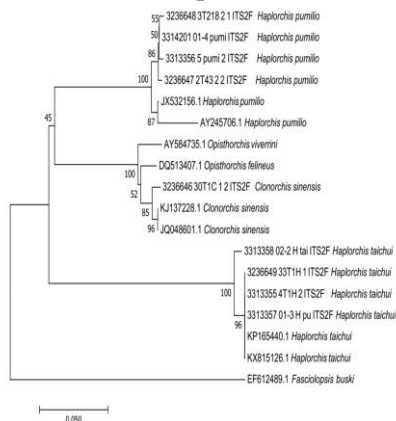
M. Marker 50 bp, 1. The negative control, 2. *H. taichui*, 3. *H. pumilio*, 4. *C. sinensis*

Comment: After DNA extraction, samples amplified with ITS2 primers had expected sizes of 530 bp (*H. taichui*), 380 bp (*H. pumilio*), and 390 bp (*C. sinensis*).

Table 3.37: The sequences from metacercariae registered in Genebank

Genbank code	Species	Code of samples
MK453254	<i>H. pumilio</i>	2T43
MK453255	<i>H. pumilio</i>	218
MK780187	<i>C. sinensis</i>	30T1C
MK790157	<i>H. taichui</i>	33T1

Comment: metacercariae of three species of flukes had been detected.

**Figure 3.15. The phylogenetic tree of sequenced strains based on ITS2**

Comments: Metacercariae of three species of flukes had been detected: *C. sinensis*, *H. pumilio*, *H. taichui*.

CONCLUSION

1. Epidemiological characteristics of small fluke infection in Kim Son and Yen Khanh districts, Ninh Binh province

1.1. Small fluke infection in human

Through analysis data from 400 people aged 15 years or older and living in the study site, the study has the following conclusions:

- The prevalence and intensity of infection with small flukes is 19.5%

and not different between the two districts. The prevalence of infection is higher in men (26.6%) than in women (8.3%).

- + The average intensity of infection is 517.06 eggs/g faeces and higher in men than in women; the majority (87.2%) of subjects are lightly infected, and no one is severely infected.

- Knowledge, attitudes and practices of people at the study site

- + The rate of people who are aware of flukes is relatively high (72.5%), 68.8% of participants know that these flukes are transmitted through eating raw fish and 69.3% know that eating cooked fish can prevent disease. 74.3% of subjects will stop eating raw fish if knowing that they can be infected with dangerous pathogens through this habit.

- + 73.3% of subjects eat raw fish and this proportion in men is higher than that of women. The fish commonly used for raw consumption are dotted gizzard shad (62.25%), silver carp (52.75%), common carp (34.75%), and grass carp (32%). People eat raw fish for many reasons, as well as fish from many different sources.

- Factors related to fluke infection in humans

- + People eating raw fish are 6.769 times more likely to get infected with fluke than who do not eat raw fish.

- + There is no relationship between age groups, occupation, education level, living conditions (living near water sources; having hygienic latrines, dogs or cats), some behaviours (eating raw vegetables; drinking unboiled water, walking barefoot; defecating into the pond) and infection.

1.2. Metacercariae infection in fishes

- Results of studying 345 fish of 6 species show that 44.1% of the tested fish are infected with metacercariae. Common carp (86.5%),

grass carp (78.4%), and silver carps (66.7%) are the three fish species with the highest infection rate. All 5 freshwater fish species are infected with metacercaria but not for brackish water fish.

- The intensity of infection is 1.24 metacercaria/gram of freshwater fish; The highest density is in grass carp (6.4 metacercariae/gram) and the lowest is in mud carps (0.0004 metacercaria/gram).

2. Species composition of small liver fluke and minute intestinal fluke

2.1. Species composition of small fluke in humans

- Based on characteristics of ITS2 region and cox1 from 42.85% faecal samples yielding PCR products all eggs are of *Clonorchis sinensis*.

- All adult flukes obtained from people are *Clonorchis sinensis*.

2.2. Species composition of small fluke in fish

18,323 metacercariae are belonging to 3 species of flukes collected. *Haplorchis pumilio* metacercariae account for 99.84%, *Haplorchis taichui* 0.14% and *Clonorchis sinensis* 0,02%.

Haplorchis pumilio metacercariae occur in all 5 species of freshwater fish. Grass carps harbour metacercariae of all three flukes.

The highest intensity is of *Haplorchis pumilio* and the lowest is of *Clonorchis sinensis* (0.0002 metacercariae/gram fish).

RECOMMENDATION

- It is necessary to strengthen communication and education to improve people's knowledge, reduce behaviours related to the transmission of small fluke in the community.

- Increase the application of techniques capable of accurately identifying small flukes such as molecular techniques to have more precise data on the epidemiology of small flukes in Vietnam.