



Studies on Metacercariae
of
Opisthorchis spp. in Chiang Mai, Thailand

Anun Sujjanun, B.Sc. (Med. Tech) *
Prayuth Thitasut, M.D. **

Abstract

Five hundreds and twelve of fresh water fishes of **Puntius leiacanthus**, **Puntius orphoides** and **Esomus metallicus** species in Cyprinoid family collected in Chiang Mai areas were examined and the metacercarial forms of **Opisthorchis** spp. were observed in 259 fishes (51 per cent). The metacercarial cysts were 17 in average in each fish. The highest incidence of metacercariae was in **Puntius orphoides** (94 per cent) and there were 97 cysts in average in each of them. In **Esomus metallicus**, the incidence was 8 per cent and 11 cysts of metacercariae were observed in each. The average sized of the metacercarial cysts was 195×150 microns. The opisthorchis eggs were detected in feces after 35-41 days of infected hamsters and the percentage of infective incidence was 14-46.

Sadun (1955) (1) studied the incidence of **Opisthorchis viverrini** in Thailand and found that there were 19-55 and 4 per cent in the Northeast and the North respectively. He also noted that the incidence was 8 per cent around the Laotian border in the Northern part of Thailand. Vajrasuthira, S. and Harinasuta, C. (1957) (2)

reported that the incidence of liver fluke was 20.5 per cent in Chiang Mai, 19.9 per cent in Nan, 14.8 per cent in Lam-pang and 10.1 per cent in Prae. The people in those mentioned areas like to have the cooking fashion of raw fishes for meals. Studies of cercariae in snails, the first intermediate hosts and metacercariae

* Department of Pathology, Vajira Municipal Hospital, Bangkok, Thailand.

** Professor of Parasitology and Head, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

in fresh water fishes, the second intermediate hosts in the Northeastern part were reported by the Faculty of Tropical Medicine, Mahidol University. (3) Because of no treatment of choice for cases of opisthorchiasis at present, the preventive measures are so much important to handle this disease in Thailand.

Previously mentioned, the percentage of cases of liver fluke observed in Chiang Mai was 20.5 which was considered to be rather high. Brandt (1963) (5) studied snails in various areas in Thailand including Chiang Mai, he found that the cercariae of *Opisthorchis viverrini* were harboured and shed from the snails, *Digoniostoma funiculata* which was the first intermediate host. However, the study on metacercariae in fresh water fishes of Cyprinoid family was not done. Na-Bang Xang et al (1969) (4) reported the incidence of opisthorchiasis cases which were around 27.6 per cent in Amphur Sarapee.

The purpose of this study is to search for metacercariae of *Opisthorchis* spp. in some areas in Chiang Mai Province. The incidence of metacercariae of *Opisthorchis* spp. in fresh water fishes (Cyprinoid) and the morphologic studies of metacercarial cysts and adult liver flukes are also included.

Material and Methods :

The fresh water fishes (Cyprinoid) were collected from various Tambols (district) of Amphur Sarapee and San Kam pang. Most of them were *Puntius leia-*

canthus. The remainings were *Esomus metallicus* and *Puntius orphoides*.

Method of Examination of Fishes :

As we know, the metacercariae may be found in the chest, ventral and dorsal fins; tail, scales and flesh. So all mentioned specimens were separated and cut in small pieces which were transferred on the prepared drops of saline on the glass-slides (7.5×20.0×0.8 cm). Then the specimens were pressed by another glass-slides and examined under the dissecting microscope to look for the metacercarial cysts. It might be worthwhile to note that the flesh must be carefully prepared and free from skin and bones. The observed metacercarial cysts were removed by using the dissecting needles and kept in 0.85 per cent normal saline solution for further detailed morphologic studies of metacercariae and infested in the experimental animals

Procedures for Infestation in the Experimental Animals :

The experimental animals used in this study were white mice (150 gms. average weight) and hamsters (90-100gms. average weight). The stool examinations by formalin-ether concentration technique of all experimental animals were performed for seven consecutive days and they were absolutely considered free from opisthorchiasis. The animals were anaesthetized by ether inhalation method and hang them

by strings tied to their upper teeth. The polyethylene stomach tubes, one millimeter in diameter, were applied and exactly introduced into the stomach. Test for the proper in place of the stomach tube by placing the remaining end of the stomach tube underneath the water was performed. If the air bubbles occurred related to their respiration, the stomach tube was missed into the lungs. The application had to be again tried. Approximately 30-50 prepared metacercarial cysts were injected into the stomach tube. The syringe and stomach tube were rinsed several times with normal saline in order to confirm that all the metacercarial cysts were introduced.

Results :

The fishes examined were in *Cyprinoid* family and they were *Puntius leiakanthus*, *Esomus metallicus* and *Puntius orphoides*. All 512 fishes were caught from various areas of Amphur Sarapee and San Kampang. The results were as follows :

1. Thirty-seven out of 512 fishes collected from Tambol Chompoo, Amphur Sarapee were found to harbour metacercariae, so the incidence was 24.2 per cent. There were 12 metacercarial cysts in average in each fish.

2. The metacercarial cysts were observed in 61 out of 141 fishes obtained from Tambol Sansai, Amphur Sarapee, so the percentage of incidence was 43.2 and

the average metacercarial cysts per fish was 20.5.

3. There were 195 fishes caught from canals along the Lampang-Chiang Mai Hight way at the kilometer 12. The metacercarial cysts were seen in 147 fishes, so the incidence was 75.3 per cent. The average metacercarial cysts per fish was 17.4.

4. The fishes collected from Tambol San Kowng, Amphur San Kampang were 24 in number. Fourteen of them were positive for metacercariae, so it was 58.3 per cent incidence and the average numbers of metacercarial cysts in each fish were 4.7.

The total numbers and species of fishes obtained from several areas in Amphur Sarapee and San Kampang, numbers of the metacercarial cysts observed and the average numbers of the metacercarial cysts in each were tabulated in Table I and II.

The average size of living 100 metacercarial cysts was 195×150 microns as shown in Table III.

The duration of the experimental feeding of metacercarial cysts in three white mice and nine hamsters was 30 days. The fecal examinations by simple and concentration methods were performed every day and the opisthorchis ova were negative in white mice but the opisthorchis ova were positive in hamsters after

35-41 days after inoculation. The stool examinations in hamsters were continued for seven days, and the two hamsters were sacrificed. Fourteen and nineteen adult liver flukes were identified in both. The numbers of the metacercarial cysts fed in these two hamsters were 30 and 50 respectively, so the incidence of infestation was 46.6 and 38.0 per cent. Another two hamsters fed with 50 metacercarial cysts were dead with unknown cause after 35 days of experimental feeding. The autopsies were performed carefully. There were 21 and 7 adult liver flukes identified respectively, therefore, the percentage of infective incidence was 42 and 14. The remaining five hamsters were kept feeding for further study. The details about the numbers of metacercariae developed to be adult liver flukes in hamsters, areas and species of fishes that harboured metacercariae, duration of positive stool examination for opisthorchis ova after the experimental feeding were presented in Table IV.

Discussion :

The metacercariae observed in fresh water fishes (Cyprinoid) collected from various areas of Amphur Sarapee and San Kampang showed the same morphologic characteristics as reported by Vajrasthira, (1961). The morphologic characteristics are the body of metacercariae are folded within the cyst and frequently appears to be C-shaped. The mature larvae move

vigorously at room temperature. When the metacercariae are at rest, the characteristic excretory corpuscles and the brownish-yellow pigment scattered throughout the body are clearly visible. The excretory bladder appears as an oval area composed of masses of dark granules. The oral and ventral suckers are usually also clearly seen (Picture I). The total numbers of metacercarial cysts were measured and they were ranged from 160-245 microns in length and 116-225 microns in width. Therefore, the average size was 195×150 microns (Table III). In comparison, the above average metacercarial size was smaller than the size of metacercariae, 204×145 , 201×167 and 202×168 microns reported by Harinasuta, C. (1960) (7), Vajrasthira, S. (1961) (6) and Wykoff, D.E. (1965) (8), respectively. The authors believed that the metacercariae were observed in different areas and fishes that Vajrasthira, S. (1961) (6) and Wykoff, D.E. (1965) (8) examined were *Cyclocheilichthya* spp., *Puntius* spp., *Esomus* spp., and *Hampale* spp. The incidence of metacercarial cysts in fishes obtained from Tambol Sansai was higher than Tambol Chompoo, Amphur Sarapee (Table I). It might be mentioned that the author's findings are relatively comparable with findings reported by Na-Bang Xang et al (1969) (4). The incidence of cases of opisthorchiasis may be concluded

that the population around these areas have their meals with raw or insufficiently cooked of fresh water fishes haboured so many metacercarial cysts and caught from these areas. Besides, the evidence of metacercariae in fishes in Tambol SanKowng, Amphur SanKampang was quite high (Table I). Recently, there are no available reports on cases of opisthorchiasis in this area, but the authors would guess that the evidence of this disease in the population in this area might be high and interested.

Our findings that the incidence of metacercarial cysts were highest in *Puntius orphoides* and lowest in *Esomus metallicus* (Table II) were quite comparable with Wykoff's studies (1965) (8) that the incidence of metacercarial cysts he found in the *Puntius orphoides* and *Esomus metallicus* obtained in the Northeast. That is, the *Puntius orphoides* are the better second intermediate hosts than other fresh water fishes. In our study, we found that the metacercarial cysts were identified in 259 out of 512 fishes and 17 metacercarial cysts per fish (Table II). This incidence would be considerably high in comparison with Harinasuta's report (1961) (8) which the incidence was 36 per cent and the average cysts were 9 per fish investigated in six provinces (Udorn, Sakol Nakorn, Nakorn Panom, Kalasin, Mahasarakarm and Khon Kean) in the Northeast. Because of flat

and damped areas and also good irrigation of Chiang Mai City may be the good reasons for breeding of the fishes that are the second intermediate hosts for opisthorchiasis. It was interesting to note that there were no opisthorchis ova detected in feces of three mice after 30 days in this experiment. The authors would express that the white mice are not practically unable for this particular study. The studies of Harinasuta, C. (1963-1964) (3) were noted that the opisthorchis ova were found in hamsters' stools after 22-27 days of infection and the infective incidence was 10-80 per cent, but in our study, the opisthorchis ova were observed in the same species of the experimental animals after 35-41 days and the infective incidence was 14-46 per cent (Table IV). According to this experiment, the hamsters were practically excellent. However, the longer duration of infection and lower infective incidence of our study are needed for further investigation.

The general morphologic studies of adult form of *Opisthorchis* spp. indentified from our experimental animals were carefully made and found that they were similar to the *Opisthorchis viverrini* which was lancet-shaped with rather small anterior end and blunt or rounded posterior end. There were no spines on the body surface. The oral sucker and acetabulum were the same size. The testes that com-

posed of two lobulated masses were located dorsally along the body length and distal to the ovaries. One of the testicular masses was slightly deviated from the other one. The ovaries were located proximal to the testes and the anterior one showed many lobulations in character. The vitelline glands were arranged in groups and located on both sides between the acetabulum and ovaries (Picture II). (1, 11, 12) By the above morphologic findings, the adult form of *Opisthorchis viverrini* was not sharply differentiated from the *Opisthorchis felinus*.

Conclusion :

In several areas in Chiang Mai Province, the metacercariae were detected in three species of fishes in Cyprinoid family. They were 388 of *Puntius leiocanthus*, 18 of *Puntius orphoides* and 106 of *Esomus metallicus*. The metacercarial cysts were observed in 233 of *Puntius leiocanthus*, 17

of *Puntius orphoides* and 9 of *Esomus metallicus*; therefore, the incidence was 50.5 per cent (259 out of 512 fishes examined). The average numbers of the metacercarial cysts in each fish were 3.5 minimum and 124.7 maximum. Because of the cooking habits of raw or medium fresh water fishes caught around the Northern areas for normal meals of the population, the epidemiologic problems of opisthorchiasis are urgent needed to be solved.

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Table I: Shows the evidence of the metacercariae of some Tambols, Amphur SanKampang and S

Place	Species of fishes examined	Local name	Number fishes examined
Tambol Chompoo, Amphur Sarapee	<i>Puntius leiocanthus</i> (Bleeker)	Pla Tapien	1
	<i>Esomus metallicus</i> (Ahl.)	Pla Siew	
	<i>Puntius orphoides</i> (Cuo. + Val.)	Pla Gam Shum	
Total			1
Tambol Sansai, Amphur Sarapee.	<i>Puntius leiocanthus</i> (Bleeker)	Pla Tapien	
	<i>Esomus metallicus</i> (Ahl.)	Pla Siew	
	<i>Puntius orphoides</i> (Cuo. + Val.)	Pla Gam Shum	
Total			1
Canals along the Lampang Chiang Mai High Way at twelveth kilometer, Amphur Sarapee.	<i>Puntius leiocanthus</i> (Bleeker)	Pla Tapien	1
	<i>Puntius orphoides</i> (Cuo. + Val.)	Pla Gam Shum	
Total			1
Tambol SanKowng, Amphur SanKam- pang.	<i>Puntius leiocanthus</i> (Bleeker)	Pla Tapien	
	<i>Puntius orphoides</i> (Cuo. + Val.)	Pla Gam Shum	
	<i>Esomus metallicus</i> (Ahl.)	Pla Siew	
Total			
Grand Total			5

of *Opisthorchis* spp. in cyprinoid fishes in
Sarapee, Chiang Mai Province.

Number of fishes examined	Numbers of fishes harboured metacercariae	Percentage of fishes harboured metacercariae	Number of metacercariae observed	Average numbers of metacercarial cysts per fish
109	28	25.6	142	5.0
37	4	10.8	14	3.5
6	5	83.3	289	57.8
152	37	24.2	445	12.0
71	54	76.0	957	17.7
68	5	7.9	81	16.2
2	2	100.0	216	10.8
141	61	43.2	1,254	20.5
186	138	73.8	1,447	10.4
9	9	100.0	1,123	124.7
195	147	75.3	2,570	17.4
22	13	59.0	48	3.6
1	1	100.0	19	19.0
1	-	-	-	-
24	14	58.3	67	4.7
512	259	50.5	4,336	16.7

Table II: Shows the number
including n

Species	Local N
Puntius leiacanthus (Bleeker)	Pla Tapie
Puntius orphoides (Cuo. + Val.)	Pla Gam S
Esonus metallicus (Ahl.)	Pla Siew
Total	

and species of cyprinoid fishes that harboured metacercariae,

numbers of metacercarial cysts in those fishes.

	Numbers of fishes, examined	Numbers of fishes, harboured metacercariae	Incidence of infected fishes (per cent)	Total numbers of metacercarial cysts observed	Average of metacercarial cysts per fish
	388	233	60	2,594	11
hum	18	17	94.4	1,647	96.8
	106	9	8.4	95	10.5
	512	259	50.5	4,336	16.7

Table III: Shows the size of the living metacercaria

No.	Length	Breadth	No.	Length	Breadth
	160 - 245	116 - 225		160 - 245	116 - 225
1	204	174	51	205	140
2	100	144	52	175	140
3	198	162	53	170	175
4	168	168	54	185	140
5	198	180	55	910	185
6	198	156	56	185	165
7	188	156	57	175	150
8	198	180	58	190	165
9	198	162	59	175	125
10	204	150	60	180	135
11	192	150	61	210	165
12	210	168	62	175	165
13	186	168	63	185	150
14	204	156	64	235	200
15	216	168	65	205	160
16	204	180	66	225	180
17	204	150	67	230	185
18	210	180	68	215	200
19	192	138	69	900	175
20	198	168	70	185	140
21	204	180	71	190	160
22	216	168	72	200	175
23	204	156	73	190	160
24	216	180	74	185	165
25	204	156	75	185	150
26	204	168	76	170	150
27	204	168	77	200	187
28	222	198	78	200	150
29	238	180	79	200	175

30	204	168	80	207	185
31	204	186	81	230	185
32	216	156	82	210	175
33	186	144	83	195	180
34	210	198	84	190	140
35	198	138	85	175	130
36	180	132	86	165	140
37	210	180	87	190	150
38	210	204	88	230	140
39	180	162	89	185	180
40	185	160	90	230	225
41	200	175	91	175	145
42	200	185	92	190	130
43	235	215	93	190	165
44	245	210	94	175	140
45	185	170	95	175	125
46	200	150	96	175	185
47	185	140	97	190	160
48	180	130	98	165	115
49	185	150	99	160	180
50	170	125	100	185	140
Mean				195.75	150.17

Table IV: Shows the duration and incidence

Date	Animals	Numbers of metacercarial cysts infested	Species of cyprinoid fishes harboured
Dec. 2, 69	White mice	22	<i>Puntius leiakanthus</i> , Tambol Chom
Dec. 9, 69	White mice	100	<i>Puntius leiakanthus</i> , Tambol Sansai
Dec. 9, 69	White mice	50	<i>Esomus metallicus</i> , Tambol Sansai
Dec. 19, 69	Hamster	30	<i>Puntius leiakanthus</i> , Tambol Sansai
	Hamster	30	<i>Puntius leiakanthus</i> , Tambol Sansai
Dec. 23, 69	Hamster	50	<i>Puntius leiakanthus</i> , Canals along C Lampang High Way, Amphur
Dec. 25, 69	Hamster	50	" " " "
	Hamster	50	" " " "
Jan. 6, 70	Hamster	50	<i>Puntius orphoides</i> , Canals along C Lampang High Way, Amphur
	Hamster	50	" " " "
	Hamster	50	" " " "
Jan. 7, 70	Hamster	50	<i>Puntius leiakanthus</i> , Canals along C Lampang High Way, Amphur

Incidence of Opisthorchiasis in the experimental Animals

Hosts and places	Opisthorchis ova observed in feces in days	Date Sacrificed	Numbers of adult liver flukes	Incidence of adult liver flukes per cent	Remarks
Amphur Sarapee	--	--	--	--	Negative for Ova
Amphur Sarapee	--	--	--	--	"
Amphur Sarapee	--	--	--	--	"
Amphur Sarapee	41	Feb. 4, 70	14	46.6	
Amphur Sarapee	41	--	--	--	
Chiang Mai -- Sarapee	37	Feb. 12, 70	19	38.0	
"	35	Feb. 10, 70	21	42.0	Expired
"	35	--	--	--	
Chiang Mai -- Sarapee	35	Feb. 2, 70	7	14.0	Expired
"	35	--	--	--	
"	35	--	--	--	
Chiang Mai -- Sarapee	35	--	--	--	



PICTURE I
METACERCARIA OF OPISTHOCHIS SPP.



PICTURE II
ADULT LIVER FLUKE

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