

BODY OF REPORT

SEATO Medic Study No. 25 Studies on Opisthorchis viverrini in Thailand-
Morphology of Opisthorchis viverrini.

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Introduction: Little was known of hepatic trematodes in Thailand or the neighboring countries until 1953-1955 when Sadun (1955) reported that Opisthorchis viverrini was widespread in the northeast. Prior to his investigations, only a few scattered reports concerning Opisthorchis in Thailand were available. In 1908, Verdun and Bruyant noted that "Opisthorchis felineus" was found in Indo China, and in 1911 Leiper recovered "Opisthorchis viverrini from a human autopsy in Chiangmai (northwest) Thailand. Sixteen years later Prommas (1927)

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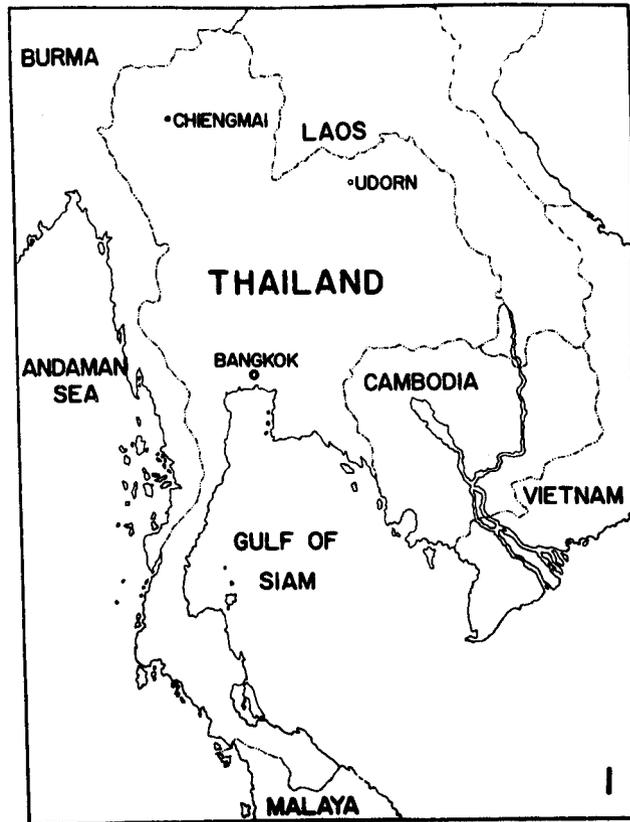
reported a single case of "O. felineus" from a human autopsy in northern Thailand, and in 1929 Bedier and Chesneau reported that "O. viverrini" was present in 25% of 1231 examinees in Takhek, and in 15% of 523 persons in Vientiane, both being Laotian cities immediately across the Mekhong River from Thailand. Inasmuch as Sadun (1955) reported only O. viverrini from Thailand it appears that the reports of "O. felineus" may have been in error. In any case, following the initial description of O. viverrini by Poirier (1886) uncertainty has surrounded the validity of the species. Erhardt (1935), on the basis of findings of other investigators, but without examining O. viverrini specimens himself, reported that this species was not synonymous with O. tenuicollis. This is in contrast to the opinions of Price (1932), Vogel (1932) Faust (1949) and Dawes (1956). It should be noted that O. tenuicollis was recovered from a sea-mammal, leading one to believe that marine intermediate hosts were involved. Ejamont (1937) explained this by stating that these mammals often enter estuaries to feed on cyprinoid fish infected with Opisthorchis metacercariae. He concluded that O. tenuicollis and O. felineus should be considered as subspecies. Watson (1960) reported that O. tenuicollis is synonymous with O. felineus, thus possibly making O. tenuicollis = O. felineus = O. viverrini. The issue is further broadened by the reports of Morgan (1927) and Price (1940), who believe that Opisthorchis and Clonorchis are not sufficiently different to warrant the use of separate genera. Erhardt, et. al. (1962) list over 20 other names which may be synonymous with O. felineus. In addition to the uncertainties mentioned above, even the specific name "viverrini" appears to be incorrect because the original specimens were recovered from the civet cat, Felis viverrini (not Felis viverrinus), thus requiring the parasite to be named "Opisthorchis viverrinae" (Stempell, 1938). Consequently it is not surprising that field workers have been troubled by this taxonomic confusion.

Various authors have reported that O. viverrini may be differentiated from O. felineus by a number of specific morphological characteristics. The flame cell patterns have been described for metacercariae of O. viverrini and O. felineus, respectively as 2 [(3+3) + (3+3+3)] (Vajrasthira, et. al., 1961) and 2 [(5+6) + (6+6+6)] (Komiya and Tajimi, 1941). The flame cell pattern for the cercariae of O. felineus is 2 [(5+5) + (5+5+5)] (Komiya and Tajimi, 1941). Although this was originally described by Vogel as 2 [(5)+(5+5+5+5)] he is now of the opinion that the formula of Komiya and Tajimi is correct (Vogel, 1963).

As a prerequisite for studying this infection in the endemic area, a field laboratory was established in Udorn Thani, a city of some 28,000 persons located in northeastern Thailand, 350 miles from Bangkok and 40 miles south of Laotian capital of Vientiane (Fig. 1).

Materials and Methods: Single stool specimens were collected from each human volunteer and concentrated by the formalin-ether technique (Ritchie, 1948). Similar examinations were made on domesticated cats and dogs.

Snails of the Family Bithyniidae were collected and identified by Dr. Rolf A. M.



Brandt (SEATO Lab.). Two species were found in the northeast: Bithynia (Digo-niostoma) goniomphalus and B. (Wattebledia) crosseana. They were placed in separate aquaria according to the type of cercariae they shed. While two of the types of cercariae shed by B. goniomphalus generally resembled the cercariae of O. felineus, none of those shed by B. crosseana were similar.

Fish for these investigations were obtained from lakes near Udon. They were identified by the Bangkok Department of Fisheries. Fish for experimental infection were purchased in Bangkok, where the infection is non-endemic.

Ten percent were examined and found to be free of natural infection. The remainder were placed in aquaria each containing snails shedding the different types of cercariae. After a period of from 4 to 8 weeks these fish were fed to cats and hamsters which had been first shown to be free of natural infection. After a period of from 2 to 4 months the animals were sacrificed and the hepatic trematodes fixed in Bouins solution, stained in acid carmine and examined.

Stained adult specimens of O. felineus from cats and humans were kindly supply by Professor Vogel. Specimens of O. viverrini were collected in north-eastern Thailand from cats, dogs and at human autopsies. Drawings were prepared with the aid of a camera lucida.

Results: Prevalence Studies. A total of 6316 persons have been examined for O. viverrini. Of these 5010 (79%) were found to be infected on the basis of a

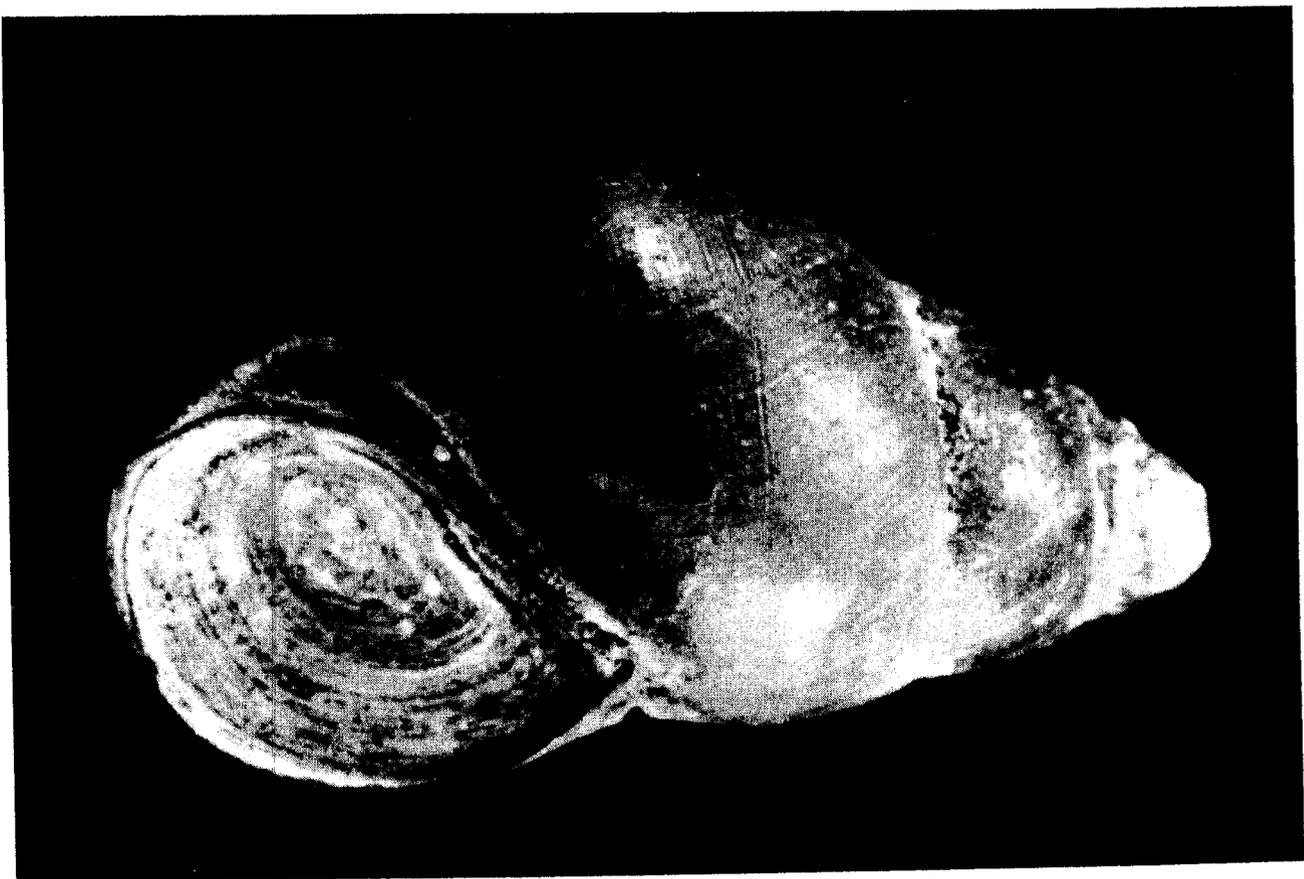
Table I
AGE-SEX DISTRIBUTION OF OPISTHORCHIS VIVERRINI INFECTIONS IN NORTH-
EAST THAILAND

| Age | Males | | | Females | | | Total | | |
|--------|-------|------|----|---------|------|----|-------|------|----|
| | No. | + | % | No. | + | % | No. | + | % |
| 0-5 | 451 | 162 | 36 | 424 | 147 | 35 | 875 | 309 | 35 |
| 6-10 | 451 | 328 | 73 | 377 | 354 | 94 | 828 | 682 | 82 |
| 11-15 | 411 | 332 | 81 | 373 | 295 | 79 | 784 | 627 | 80 |
| 16-20 | 353 | 321 | 91 | 434 | 395 | 91 | 787 | 716 | 91 |
| 21-25 | 269 | 241 | 90 | 256 | 220 | 86 | 525 | 461 | 88 |
| 26-30 | 276 | 242 | 88 | 262 | 230 | 88 | 538 | 472 | 88 |
| 31-35 | 194 | 172 | 89 | 156 | 146 | 94 | 350 | 318 | 91 |
| 36-40 | 218 | 188 | 86 | 176 | 160 | 91 | 394 | 348 | 88 |
| 41-45 | 156 | 130 | 83 | 122 | 106 | 87 | 278 | 236 | 85 |
| 46-50 | 170 | 146 | 86 | 112 | 107 | 96 | 282 | 253 | 90 |
| 51-55 | 107 | 95 | 89 | 78 | 67 | 86 | 185 | 162 | 88 |
| 56-60 | 108 | 89 | 82 | 92 | 81 | 88 | 200 | 170 | 85 |
| 61-65 | 68 | 59 | 87 | 43 | 39 | 91 | 111 | 98 | 88 |
| 66-70 | 48 | 43 | 90 | 36 | 32 | 89 | 84 | 75 | 89 |
| 70 | 54 | 47 | 87 | 41 | 36 | 88 | 95 | 83 | 87 |
| Total: | 3434 | 2559 | 75 | 2982 | 2415 | 81 | 6316 | 5010 | 79 |

single concentrated specimens. Of the 5441 examinees over the age of five, 4701 (86%) were positive, and the prevalence remained uniformly high for all remaining age groups (Table 1). Based on these findings it may now be conservatively estimated that over 3.5 million persons in Thailand harbor this parasite, and, because of geographical location and similar eating habits, several million more are likely infected in neighboring Laos, Viet Nam and China.

Although many types of non-domesticated animals were trapped in the endemic area, none were found to harbor O. viverrini. Domesticated cats and dogs were examined by stool concentration. Approximately 60% of the cats and 40% of the dogs were found to be naturally infected.

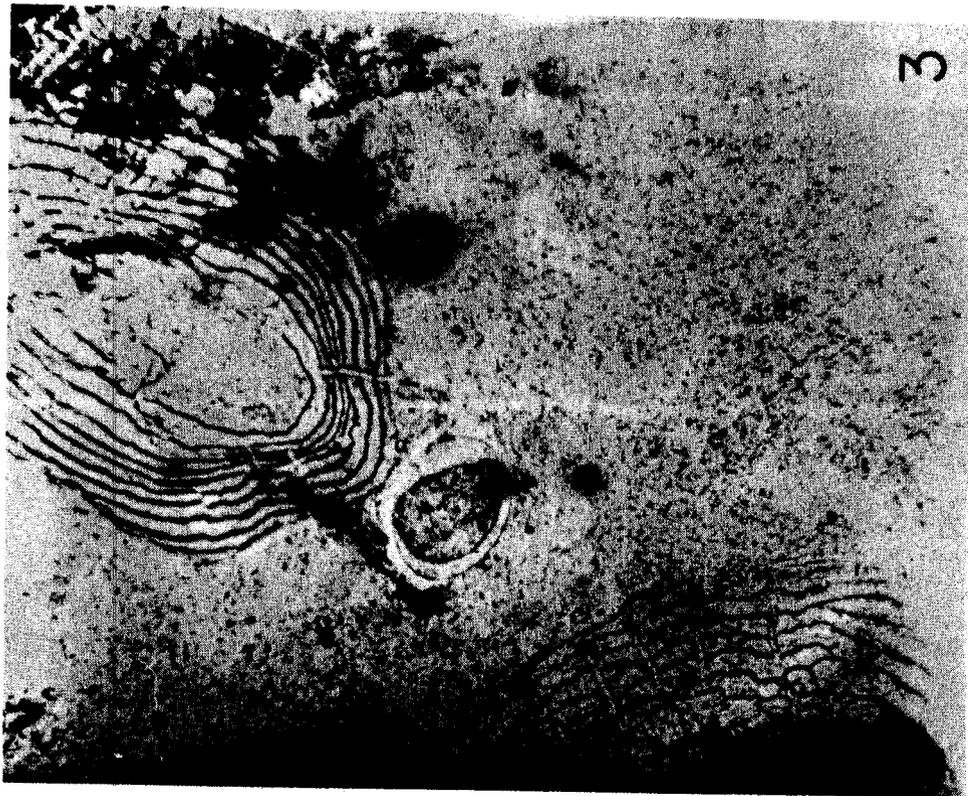
The Snail Host. The snails harboring the cercaria of O. viverrini were identified as being Bithynia goniomphalus Morelet, 1866 (Fig. 2). This species belongs to the subgenus Digoniostoma Annandale the type species of which is B. cerameopoma Benson from East India. Bithynia goniomphalus is also found in collections under the names laevis Lea, subcarinata Kobelt and funiculata Walker. Bithynia laevis, is, in our opinion, only an ecologic form of B. goniomphalus which is found in running water containing considerable vegetation. The specimens remain smaller in such an environment, are not covered with a brownish layer and corrode less frequently. B. subcarinata and funiculata are easily distinguished by their more open umbilici and by having sharp basal keels.



Concerning B. goniomphalus, the shell is elevated and turreted, regularly tapering to the apex. There are 5-6 slightly convex whorls, the protoconch, however, usually being corroded (but truncate) leaving only 3-4 whorls. The color is olive greenish but the shell is covered by a brownish layer when adult. The umbilicus is very narrow with a feeble basal keel around a shallow canal at the base of the aperture. The aperture is oval with an angle at the upper margin. The peristome is continuous and attached to the penultimate whorl, not expanded and without a lip. The operculum is calcareous with concentric lines around the subcentric nucleus.

After determining that B. goniomphalus was the intermediate host in northeast Thailand, observations were extended into areas where this species was not present, but where cases of O. viverrini were found. It was then determined that in the northeast B. (Digoniostoma) funiculata acts as intermediate host while B. (Digoniostoma) laevis is the responsible snail.

Intramolluscan Stages. The mother sporocyst was difficult to isolate in snails harboring older infections. The mature sporocysts are extremely thin-walled. They average 1.1 x .65 mm, and are generally coiled, containing a large number of developing rediae. The developing rediae show distinct contractions which assist them in breaking from the thin-walled sporocyst. The redia measures from 0.18 to 1.1 mm in length by 0.08 to 0.28 mm in width (mean 0.54 x 0.12mm). Each possesses a distinct pharynx but is without an annulus. The integument is



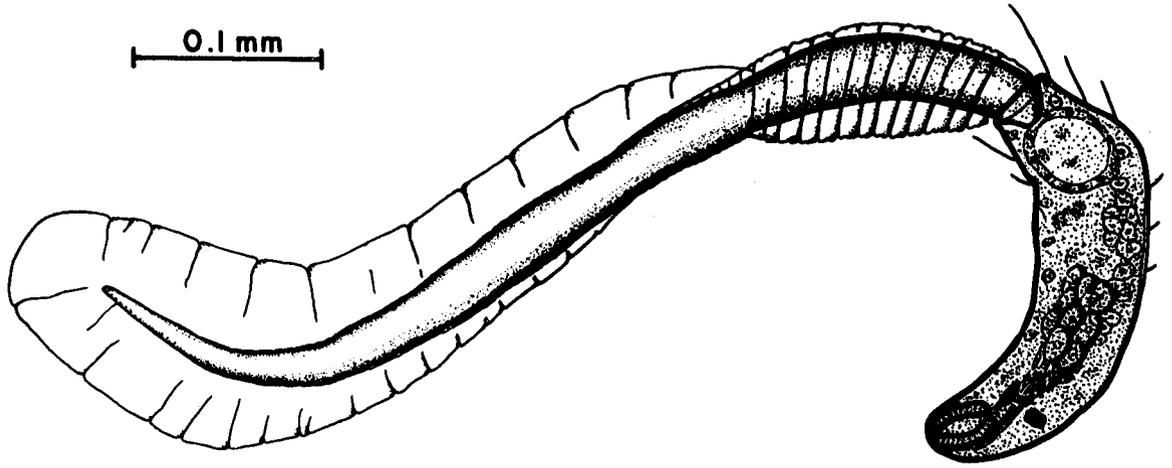
thick. Within the mature redia are as many as 15 developing cercariae. These, immediately upon leaving the rediae, have an average length of 207 μ .

The Cercaria. Two types of cercariae shed by the snail Bithynia goniomphalus were both oculate and pleurocercous. One, when at rest, assumed a characteristic pipe-form shape. The other remained straight with the flat surface of the body at a 45° angle to the bottom of the container. The body of the latter cercaria was more spade-shaped and a little wider than that of the former kind.

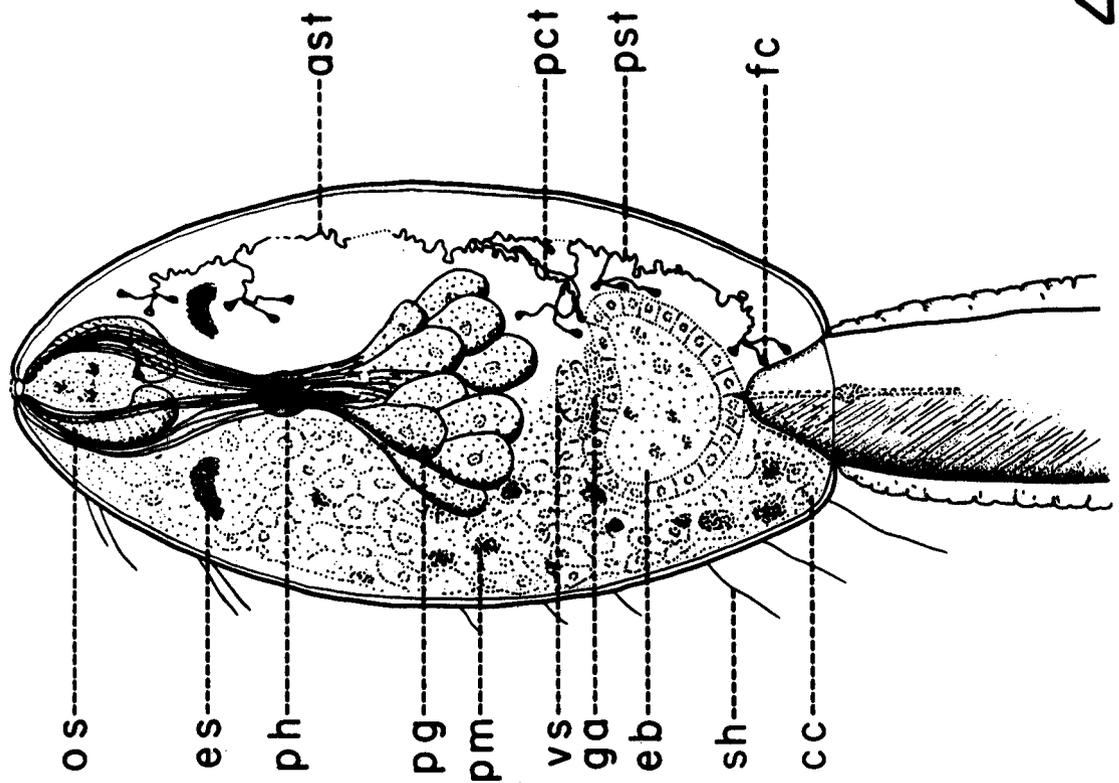
Both types were placed in beakers with Cyprineus carpio fish. While both kinds of cercariae penetrated beneath the scales and fins, the pipe-form type was more active. Four hours after penetration, the more active type had lost their tails, and resembled the mature metacercariae of O. viverrini, except that the eye spots were still visible (Fig. 3).

The lesser active or spade-body type cercaria produced metacercariae which did not resemble those of O. viverrini. After a period of from 2 to 6 months, the fish harboring the metacercariae were fed to hamsters, cats and rabbits, after making certain that these animals were free of natural infection. After a minimum period of 60 days, the animals were sacrificed. Those which had been fed the metacercariae from the pipe-shaped, active-swimming cercariae were found to harbor hepatic trematodes, while those fed metacercariae from the slower penetrating cercariae failed to produce liver flukes in the experimental definitive

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hosts. The trematodes were removed from the livers, stained and identified as being Opisthorchis viverrini. The O. viverrini cercariae, immediately after being shed from the snails and fixed in hot formalin, were found to vary in total length from 490 to 565 u (mean 532) (Fig. 4). The length of the body averaged 154 u. (range 140 to 183). The width of the body varied from 61 to 96 u (mean 75 u), while that of the tail was 26 u. The cercaria assumed the characteristic pipe-form, resting on the bottom or briefly hanging in the water with its head down (Fig. 5). This behaviour is similar to that of Clonorchis sinensis reported by Komiya and Tajimi, 1940, and O. felineus reported by Vogel, 1934. The cercariae of O. viverrini are both geo- and photo-tropic. The surface of the body is covered with minute spines. A conspicuous pair of eye spots is located laterally between the posterior margin of the oral sucker and the pharynx. The exact location varies somewhat among the specimens studied, some being close to the sucker, and others almost lateral to the pharynx. Brownish pigment is scattered in a bilaterally symmetrical pattern throughout the body. At least ten sensory hairs are present on each side of the body. Two are anterior to the eye, two are at the eye level, one is approximately half way between the pharynx and the ventral sucker and the others are to the posterior.

The oral sucker is oval, measuring 36-37 by 34-51 u (mean 36 to 43). It is protrusible with the opening somewhat ventral to the anterior end of the body. The sucker possesses several rows of small tooth-like structures. A round weak pharynx is generally visible. The center portion of the cercariae is filled by at least five pairs of secretory (penetration) glands. Each cell possesses a rather distinct round nucleus. The cephalic secretory gland ducts from each side proceed anteriorly to the posterior portion of the oral sucker where they separate, some following the outer lateral margin of the oral sucker, others proceeding along the inner aspects of this organ. It is quite difficult to see the ventral sucker, which is located on the median line of the body, slightly anterior to the excretory bladder. The bladder is roughly spherical. It is epithelial in origin, with thick walls composed of a thin layer of epithelium covering the muscular layer. When the cercaria elongates, the lateral walls become concave. The main excretory canals empty into the anterior corners of the bladder, and extend laterally to a point somewhat anterior to the mid section of the body. Here, each receives an anterior and posterior collecting tube. The anterior tube receives two tertiary collecting tubules, one terminating with 3 flame cells anterior to each eye spot, the other ending with 3 flame cells located posterior to the eye spot. The posterior collecting tube subdivides into 3 tertiary collecting tubules, each terminating with 3 flame cells. These are located as follows: one group in the lateral 2/3 of the body at the level of the acetabulum; one group near the area where the primary collecting tube enters the excretory bladder; and the last group in the subcaudal region posterior to the excretory bladder. The flame cell pattern is 2 [(3+3) + (3+3+3)] *. The secondary and tertiary collecting tubules can be recognized only in young cercariae, for in older specimens the scattered

The authors are indebted to Dr. Yoshitaka Komiya, NIH Tokyo, Japan, for his * assistance in determining the flame cell pattern.

pigment tends to obscure them. The cystogeneus glands are found dorsolaterally on both sides of the body. A nerve commissure is located just posterior to the pharynx. The genital primordium consists of a group of cells dorsal to the acetabulum. The excretory bladder apparently drains through a tube located in the posterior portion of the bladder which extends into the tail. The excretory pores in the tail were not visualized. The tail measures 350-437 u (mean 392 u) and has a transversely lined cuticular covering. The lateral margins of the tail are drawn out into a thin almost transparent fin-like membrane. It is present on the dorsal side for about 1/3 the tail length from the proximal end, and on the ventral side it begins slightly more posteriorly.

Recently, Ito, et. al. (1962) in reporting on various cercariae from Thai snails, described and illustrated a cercaria which they postulated may be that of O. viverrini, although they noted that they had no opportunity to carry out animal infections. The cercaria presented does not fit the characteristics of the type described above, and is most likely the straight flat-surfaced larva from which the actual cercaria was differentiated during the present investigation. The reader of that paper should not be confused by the photograph of the snail host (identified as Digoniostoma funiculata) for it is the same which the present authors believe to be correctly named Bithynia goniomphalus. The photograph has been accidentally reversed making the snails appear sinistral instead of dextral.

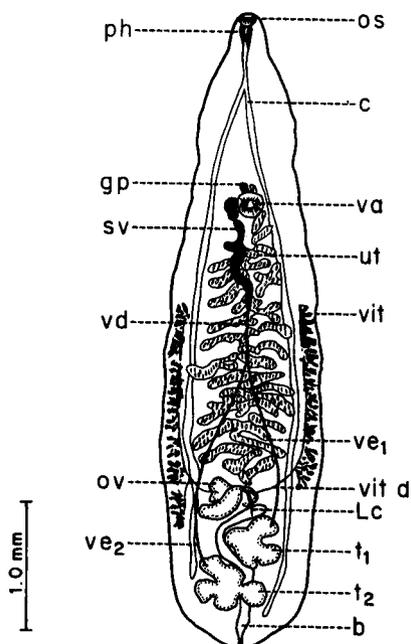
The Fish Intermediate Host. A total of 1764 fish taken from the endemic area and Bangkok have been examined (Table II). The species containing the largest average number of O. viverrini metacercariae (79 per fish) was Puntius orphoides. The highest frequency of infection (74%) was in Hampula dispar. Fish from Bangkok were free of these metacercariae. The season of the year appears to have definite influence on the intensity of infection in fish in the northeast. During the dry season (starting in November and ending around May), the ground becomes increasingly parched, wells go dry and water becomes a valuable commodity. Whereas during the rainy season bodies of water act as convenient latrines, in the dry season the feces are deposited on the ground and the eggs of O. viverrini fail to come in contact with the snail hosts. In the few remaining ponds, as the water level falls, fishermen use large nets to remove the confined fish.

As the end of the dry season approaches, extremely few fish are left and most snails have either died or have buried themselves beneath the surface. The trematode ova die without reaching water. In May, when the rains start again, the drainage ditches, ponds and canals begin to fill. The snail population increases quickly and fish soon refill the ponds. At this time fresh feces are washed or deposited into the water, and the snails eat the eggs. For a period of at least a month, while the cercariae begin to develop in snails, the only source of human infection is the very few fish which may have been previously infected with metacercariae and which may have survived the drought. After this time, cercariae penetrate new fish and again a period of at least 21 days is required for the full maturation of the metacercariae. As soon as the metacercariae become infective, human re-infection commences. In addition to those eggs already being

Table II

EXAMINATION OF FRESH WATER FISH FROM NORTHEAST THAILAND FOR THE METACERCARIAE OF OPISTHORCHIS VIVERRINI.

| Scientific Name | Common Name | Number examined | With O.v. No. | % | Ave. No. Per Fish |
|--------------------------------|----------------------|-----------------|---------------|----|-------------------|
| <i>Puntius orphoides</i> | Pla Pok | 426 | 276 | 65 | 79 |
| <i>Hampula dispar</i> | Pla Suud | 205 | 152 | 74 | 22 |
| <i>Cyclocheilichthys siaja</i> | Pla Measadaeng | 214 | 110 | 51 | 26 |
| <i>Esomus metallicus</i> | Pla Sew | 164 | 17 | 10 | 1 |
| <i>P. gonionotus</i> | Pla Drapein Kau | 34 | 1 | 3 | 3 |
| <i>P. proctozysron</i> | Pla Gramong | 112 | 9 | 8 | 1 |
| <i>P. viehoever</i> | Pla Drapein Sai | 204 | 44 | 22 | 6 |
| <i>Osteochilus sp.</i> | Pla Na Mong | 46 | 2 | 4 | - |
| <i>Labiobarbus lineatus</i> | Pla Sau Sai | 28 | 1 | 3 | - |
| <i>P. spilopterus</i> | Pla Howa Kaeng | 93 | - | - | - |
| <i>O. hasseltii</i> | Pla Soi Nok Kau | 100 | - | - | - |
| <i>Cirrhinas sp.</i> | Pla Soi Kriept Daeng | 77 | - | - | - |
| <i>Cirrhinas sp.</i> | Pla Soi Kriept Kau | 18 | - | - | - |
| <i>Datnoides microlepis</i> | Pla Sua | 38 | - | - | - |
| <i>Notopterus notopterus</i> | Pla Chalad | 5 | - | - | - |



passed from old infections, the newly developed worms also begin passing ova. By the end of the rainy season, large numbers of eggs are reaching the aquatic snails, thousands of cercariae are being shed, and numerous fish are being infected. As the waters recede, fish are more easily caught and the number of metacercariae being eaten by the human host is at a seasonal high.

Thus, most human infections are acquired toward the last third of the rainy season and the first third of the dry, for it is during this time (September to February) that the fish harbor the largest number of metacercariae and toward the end of this season that they are most easily caught. Snails shed increasing numbers of cercariae until the ponds once more become dry, in March.

The Metacercaria. The metacercaria has been described by Vajasthira et al. (1961). It is contained in a double-walled cyst, lying longitudinally between the muscle bundles. The walls of the cyst are thin, the outer being 3-8 u and the inner so thin it can be recognized only after the parasite has escaped. The size of the average cyst is 202 x 168 u. The characteristic excretory corpuscles in the bladder, the brownish-yellow pigment, and the oral ventral suckers are usually clearly seen. When the metacercaria is excysted, the body averages 558 x 145 u.

The Adult. The adult O. viverrini has been described in general by other investigators and a full description is not warranted here. The main characteristics are, however, depicted in Fig. 6. Of one hundred specimens recovered from a single human autopsy, the size ranged from 5.5 to 9.55 mm. (mean 7.4) in length, by 0.77 to 1.65 mm (mean 1.47) in width.

Differentiation from O. felineus. The following characteristics of O. viverrini are reported to differentiate it from O. felineus: 1. Greater proximity of ovary and testes; 2. Aggregation of vitellaria into a few clusters of cells containing granular material; 3. Different size and shape of its eggs; 4. Deeper lobulation of the testes (the preceding 4 reported in Faust, 1949); 5. The location of the posterior testes near the tip of the cecum; 6. The more elongated esophagus (quoted from Price in Sadun, 1955); 7. The more lobed ovary (Leiper, 1911); and 8. The shorter and less winding seminal vesicle (Vogel, 1963). Vogel further reported that characteristics other than the seminal vesicle are of limited value in differentiation of the two species.

Each characteristic has been examined in over 200 stained adult specimens of O. viverrini and compared with specimens of O. felineus. It was found that far from being constant criteria, the characteristics varied considerably, most likely representing the extent of contraction at the time of fixation. Eggs of O. felineus measure from 21 to 36 by 11 to 17 u (mean 30 x 14). The size of 1450 O. viverrini eggs were 22 to 32 by 11 to 22 u (mean 28 x 16). The differences in size and shape of eggs are not sufficient to differentiate the two species. While it may be said that some of the characteristics (such as the nature of the seminal vesicle) may tend to be present more frequently in one than in the other species,

the differences are neither consistent nor specific enough to permit certain differentiation. It is possible that future investigations may reveal other characteristics by which the adults of these species may be differentiated, but the present investigators have been unable to do so.

The flame cell pattern of the O. viverrini cercaria remains constant from the time of its emersion from the snail through its transformation into the metacercarial stage. This pattern, 2 [(3+3) + (3+3+3)], is quite distinct from that reported for O. felineus, 2 [(5+5) + (5+5+5)] for the cercaria and 2 [(5+6) + (6+6+6)] for the metacercaria (Komiya and Tajimi, 1941). The validity of the observations on both stages of both species is strengthened by the fact that all were either reported, or the observations were assisted, by Prof. Komiya. Based on these differences, O. viverrini and O. felineus cannot be synonymous in spite of the lack of differentiating characteristics in the adult stage.

It is believed that "O. viverrini" should correctly be written "O. viverrinae" but since the former has been widely published and the spelling error does not tend to confuse the reader to any considerable degree, it is advised that the "viverrini" spelling should be retained.

Summary. Studies on Opisthorchis viverrini were carried out in northeast Thailand. It can be conservatively estimated that 3.5 million persons in this country harbor this parasite, with several millions more likely infected in neighboring Laos, Viet Nam and China. There appears to be no difference in prevalence by sex and after age 15. Some 90% of the examinees were positive. The cercaria is described in detail, the flame cell pattern being 2 [(3+3) + (3+3+3)]. The authors believe that positive differentiation of O. viverrini from O. felineus adults is extremely difficult, if not impossible. Absolute differentiation can be made only on the basis of the cercarial or metacercarial flame cell pattern.

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LEGENDS FOR FIGURES

Fig. 4

Cercaria of Opisthorchis viverrini

ast - anterior secondary collecting tubule
cc - cytoogenous cell
eb - excretory bladder
es - eye spot
fc - flame cell
ga - genital anlage
os - oesophagus
pct - primary collecting tubule
pg - penetration gland
ph - pharynx
pm - pigment masses
pst - posterior secondary tubule
sh - sensory hair
vs - ventral sucker

Fig. 6

Opisthorchis viverrini

b - excretory bladder
c - cecum
gp - genital pore
lc - Laurer's canal
os - oral sucker
ov - ovary
ph - pharynx
sv - seminal vesicle
t₁t₂ - testes
ut - uterus
va - ventral acetabulum
vd - vas deferens
ve₁ve₂ - vasa efferentia
vit - vitellaria
vit d- vitelline duct

FURTHER MORPHOLOGICAL STUDIES ON THE DIFFERENTIATION OF O. VIVERRINI AND O. FELINEUS

Detailed descriptive drawings have been prepared for each of the characteristics reported to differentiate O. viverrini from O. felineus. Drawings have been made with the aid of a camera lucida.

As noted in the preceding paper, the following characteristics of O. viverrini are reported to differentiate it from O. felineus: 1. Greater proximity of ovary and testes; 2. Aggregation of vitellaria into a few clusters of granular material; 3. Different size and shape of its eggs; 4. Deeper lobulation of the testes (the preceding 4 reported in Faust, 1949); 5. The location of the posterior testes near the tip of the cecum; 6. The more elongated esophagus (quoted from Price in Sadun, 1955); 7. The more lobed ovary (Leiper, 1911); and 8. The shorter and less winding seminal vesicle (Vogel, 1963). Vogel further reported that characteristics other than the seminal vesicles are of limited value in differentiating the two species.

Each characteristic has been studied and comparative studies made. The range of characteristics of O. felineus depicted in Plate I are drawn from only 12 specimens. The likelihood of greater variation being found in a large number of specimens is clear.

The length of the esophagus in both species varies considerably, making this criterion of differentiation valueless (Fig. 1). The seminal vesicle does appear to be a little more winding in O. viverrini but the differences are such that this does not seem to constitute a characteristic by which the species can be accurately differentiated (Fig. 2). The vitellaria in both species varies from separate clusters to an almost uniform distribution (Fig. 3). Lobulation of the ovary varies considerably (Fig. 4) and no differentiation can be made on the basis of the proximity of ovary testes (Fig. 5). The testes vary from almost round to deeply lobed (Fig. 6), and the proximity of the testes to the tip of the cecum varies considerably (Fig. 7). Finally, the extent to which the cecum is posteriorly directed varies considerably (Fig. 8). The size of O. felineus eggs is 21-32 x 10-174 (Erhardt, et. al., 1962). Sadun (1955) reported that of 300 O. viverrini eggs examined, the sizes were 19-29 x 12-17 u (mean 26.7 x 15 u). The present authors have measured 1500 eggs under 900x magnification. The sizes of these were 22-32 x 12-22 u (mean 28 x 16 u). Thus, differentiation of species based solely on egg measurements is open to question.

While some of the above mentioned characteristics may tend to be present more frequently in one than in the other species, the differences are neither consistent nor great enough to permit absolute differentiation. As previously mentioned, the authors believe that a positive identification may be made only on the basis of the flame cell patterns in the cercarial or metacercarial stages.

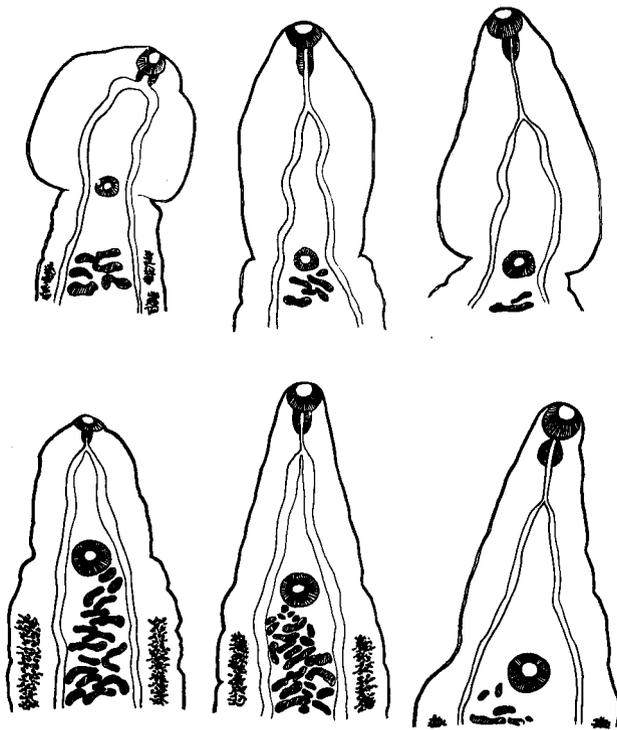


Figure 1 - ESOPHAGUS
Above - *O. VIVERRINI*
Below - *O. FELINEUS*

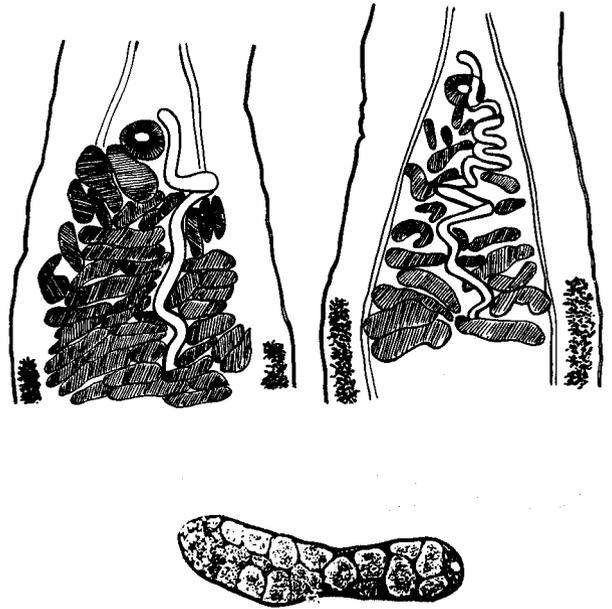


Figure 2 - SEMINAL VESICLE AND REDIA
Above Left - *O. VIVERRINI*
Above Right - *O. FELINEUS*
Below - REDIA of *O. VIVERRINI*

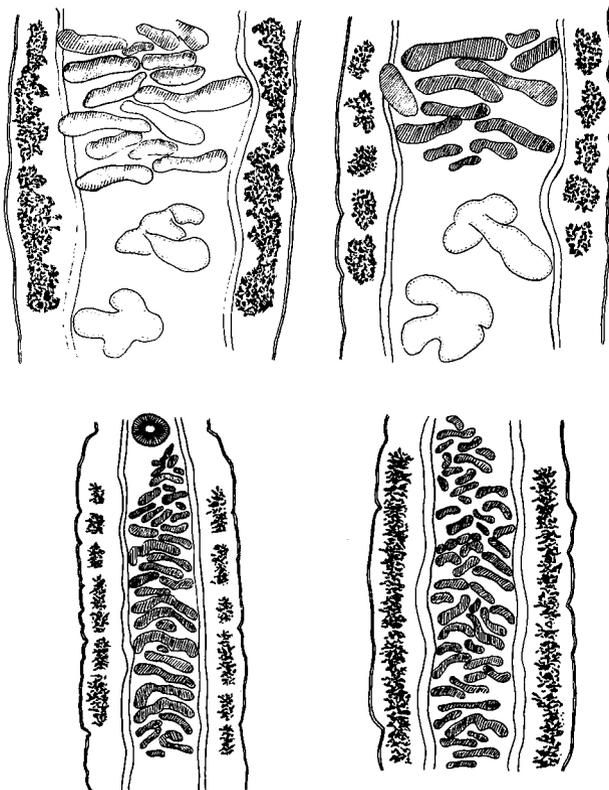


Figure 3 - VITELLARIA
Above - *O. VIVERRINI*
Below - *O. FELINEUS*

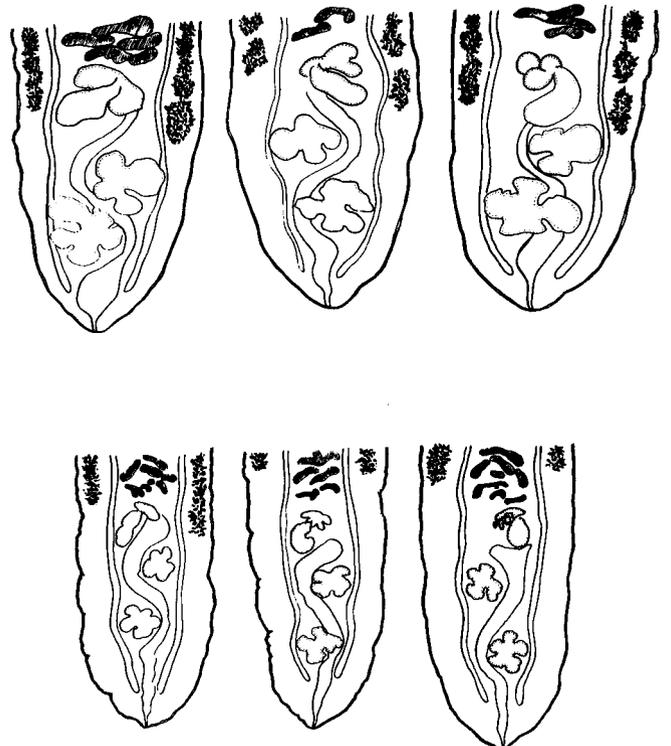


Figure 4 - LOBULATION OF OVARY
Above - *O. VIVERRINI*
Below - *O. FELINEUS*

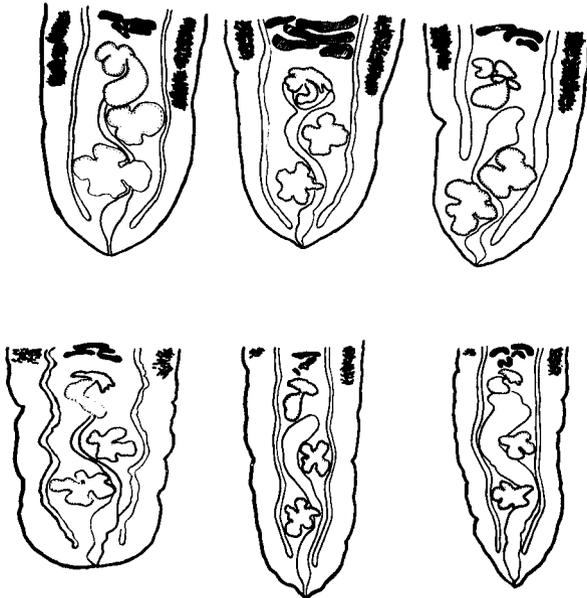


Figure 5 - PROXIMITY OF OVARY & TESTES
 Above - O. FELINEUS
 Below - O. VIVERRINI

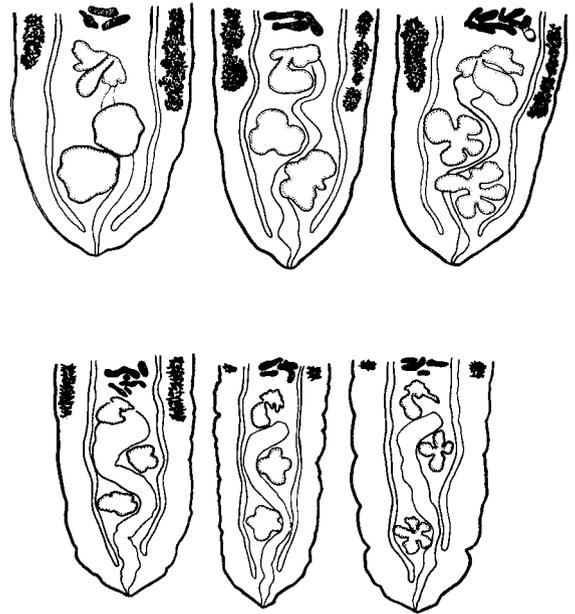


Figure 6 - LOBULATION OF TESTES
 Above - O. VIVERRINI
 Below - O. FELINEUS

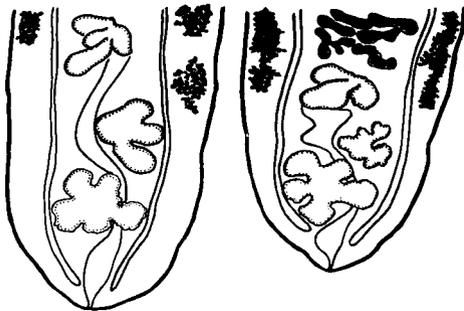


Figure 7 - TESTES AND TIP OF CECUM
 Above - O. FELINEUS
 Below - O. VIVERRINI

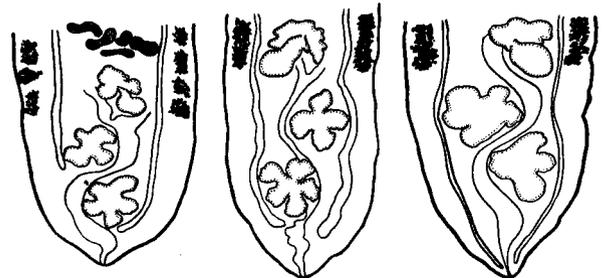


Figure 8 - POSTERIOR CECA
 Above - O. FELINEUS
 Below - O. VIVERRINI