COMPARATIVE STUDIES ON THE MORPHOLOGY OF THE EGGS OF *OPISTHORCHIS VIVERRINI* AND LECITHODENDRIID TREMATODES

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Abstract. Iodine staining and scanning electron microscopy were used to determine the morphological features distinguishing *Opisthorchis viverrini* from lecithodendriid eggs in human feces. The embryonated eggs of lecithodendriid trematodes differ from *O. viverrini* by the presence of an iodophilic body, a large mass at the posterior end of miracidium that stains brown in 0.2% iodine solution and the curved miracidium of *Phaneropsolus bonnei.* All forms of lecithodendriid eggs can be differentiated from those of *O. viverrini* by a set of morphological features of the shell surface, the knob, the operculum, the shoulder, the shape and size. On the basis of these differences, it is possible to perform differential egg counts.

INTRODUCTION

The consumption of raw or undercooked food is a dietary tradition in Northeast Thailand. Due to this habit the region is an endemic area of many food-borne parasites, such as *Opisthorchis viverrini,* minute intestinal flukes (MIF), *Echinostoma* spp. and *Taenia* sp. (Preuksaraj *et al,* 1982; Radomyos *et al,* 1984). The human liver fluke, *O. viverrini,* is the most common, infecting an estimated 7 million people (Preuksaraj, 1984). This parasite is an important regional health problem because moderate to heavy infections are associated with hepatobiliary disease and cholangiocarcinoma (Sonakul *et al,* 1978; Kim, 1984; Elkins *et al,* 1990). Diagnosis is usually based on the observation of eggs in the feces (Preuksaraj *et al,* 1982). However, *O. viverrini* is commonly found in mixed infection with MIF, which release morphologically similar eggs (Manning *et al,* 1971; Radomyos *et al,* 1984). Because of these similarities, the epidemiology of MIF is unknown and determining the effectiveness of campaigns to control *Opisthorchis* is difficult.

The MIF are classified into 2 families : Heterophyidae and Lecithodendriidae. Four species of heterophyid *(Haplorchis taichui, H. yokogawai, H. pumilio* and *Stellantchasmus falcatus)* and 3 species of lecithodendriid trematodes *(Phaneropsolus bonnei, P. spinicirrus* and *Prosthodendrium molenkampi)* have been reported from man in Thailand (Manning *et al,* 1971; Radomyos *et al,* 1983; Radomyos *et al,*1990; Kaewkes *et al,*1991). Some comparative studies on the surface ultrastructure of opisthorchiid and heterophyid eggs have been reported (Ishii, 1972; Fujino *et al,* 1989; Ditrich *et al,* 1990), but this method is complicated and not suitable for field or routine laboratory work. Comparison of opisthorchiid with lecithodendriid eggs have not been made although the lecithodendriids are recovered in mixed infections with *O. viverrini* in a higher prevalence than the heterophyids (Manning *et al,* 1971; Radomyos *et al,* 1984; Kaewkes, unpublished). In this paper we compare the eggs of *O. viverrini* and lecithodendriid flukes *(P. bonnei* and *P. molenkampi)* using light microscopy for iodine-stained eggs and confirm the shell surface structure by scanning electron microscopy.

MATERIALS AND METHODS

Collection and recovery of trematode eggs and worms: This work was part of a larger study on

the epidemiology of human trematode infections in Yodgang Village, Kalasin Province, Northeast Thailand. In October 1988, stool samples were collected from 341 villagers and examined for parasitic infection by the simple smear technique and preserved in 10% formalin. Those found positive for *O. viverrini* or *O. viverrini*-like eggs (150 people) were treated with praziquantel (Biltricide, Bayer Germany) in a single dose of 40 mg/kg body weight followed by magnesium sulphate purgative (Bunnag and Harinasuta, 1981; Radomyos *et al*, 1984). Whole stools were collected for 48 hours following treatment and preserved in 10% formalin. The post-treatment stools were gently washed and strained through 14, 60 and 120 mesh screens (1.25, 0.25 and 0.125 mm apertures). The sediment was examined under a stereoscopic microscope and the recovered parasites were counted and identified. Stool samples collected before treatment were processed from 20 patients harboring only *O. viverrini* (10 cases) or lecithodendriid adults (10 cases) using the formalin-ethyl acetate concentration technique. The sediment was mixed with either distilled water or 0.2% iodine solution and the morphological features of 400 eggs per species were recorded.

Choice of morphological features for comparison: The adult worms of *O. viverrini, P. bonnei* and *P. molenkampi* collected from post-treatment stools were dissected and the uterine eggs removed. The eggs were then either mixed with distilled water or stained with 0.2% iodine solution. The size and morphological features of the eggs were observed under a light microscope. Differences which were consistently observed for each species were then recorded (Table 1). These same criteria were used to differentiate the eggs recovered from the pre-treatment fecal samples. Each morphological characteristic was then quantitatively scored and summed for each species.

Processing of specimens for studying ultrastructure: The formalin-preserved adult worms of *O. viverrini, P. bonnei* and *P. molenkampi* were washed in 0.1 M phosphate buffer pH 7.4 and post-fixed in 1% osmium tetroxide for 2 hours at 4°C. The post-fixed worms were rewashed with the same buffer, dehydrated in ethanol and critical-point dried in a Samdri 780A dryer. After mounting on a stub, the processed worms were dissected to open the uteri and then coated with gold. The surface ultrastructure of the uterine eggs was studied under a Hitachi S-450 scanning electron microscope.

RESULTS

The morphological features of *O. viverrini, P. bonnei* and *P. molenkampi* eggs are shown in Table 2 and Figs 1-4. Ninety-four percent of *P. bonnei* and 68% of *P. molenkampi* eggs showed a large brown mass at the posterior end of miracidium after staining with iodine (Fig 1A, B), whereas only 1% of *O. viverrini* eggs showed a much smaller brown mass at the same position. The stained

Table 1

Terminology used to describe the eggs recovered from both stood and uteri.

Terms	Description
Distinct operculum	well-defined edge at the opercular junction.
Indistinct operculum	junction of operculum and egg shell undefined.
Distinct shoulder	conspicuous rim at the opercular junction.
Indistinct shoulder	inconspicuous rim.
No shoulder	cannot detect opercular junction.
Smooth shell	no roughness detected.
Thin rough shell	indistinctly rough shell, requiring high power and fine adjustment for detection.
Thick rough shell	distinctly rough shell.
Ovoid	middle part wider than posterior and anterior part.
Pyriform	posterior part wider than middle and anterior part.

egg showed a more conspicuous mass than the unstained and so can be detected at low magnification (100x). Experienced examiners may detect the mass in unstained eggs (Fig 1D, E), but this requires a higher magnification (400x). This iodophilic body could not be observed in partially embryonated, unembryonated and malformed eggs which were found in a fewer number than embryonated eggs of the three (Fig 2A-H). All miracidia of *P. molenkampi* and *O. viverrini* were straight or substraight (Fig 1B, C, E, F), but 89% of *P. bonnei* miracidia were curved (Fig 1A, D).

The shell surface of *P. bonnei* and *P..molenkampi* was smooth (Fig 3B, C), whereas the rugose outer layer of *O. viverrini* egg shell was conspicuous under the scanning electron microscope (Fig 3A) or at a magnification of 1,000x using a light microscope (Fig 3D). This may be thick (64% of eggs, Fig 4A) or thin (36% of eggs, Fig 4B) rough shell but can, at 400x magnification, be distinguished from the smooth outer layer of *P. bonnei* and *P. molenkampi* eggs. The abopercular knob of *O. viverrini* eggs which varied in size, shape, and position (Fig 4A-F) was detected in 93%. It was observed in much higher frequency than that of *P. molenkampi* (11%) or *P. bonnei* egg (6%) which appeared as a smaller dot (Fig 2G).

The distinctive operculum (Fig 4A-F) was observed in most *O. viverrini* eggs (92%), some of *P. bonnei* (20%) and only rarely in *P. molenkampi* eggs (3%). The shoulder was found to vary from distinct (Fig 4B) to indistinct (Fig 4D) or absent (Fig 2C-E) in all three species, but a distinct shoulder was more commonly detected in *O.*

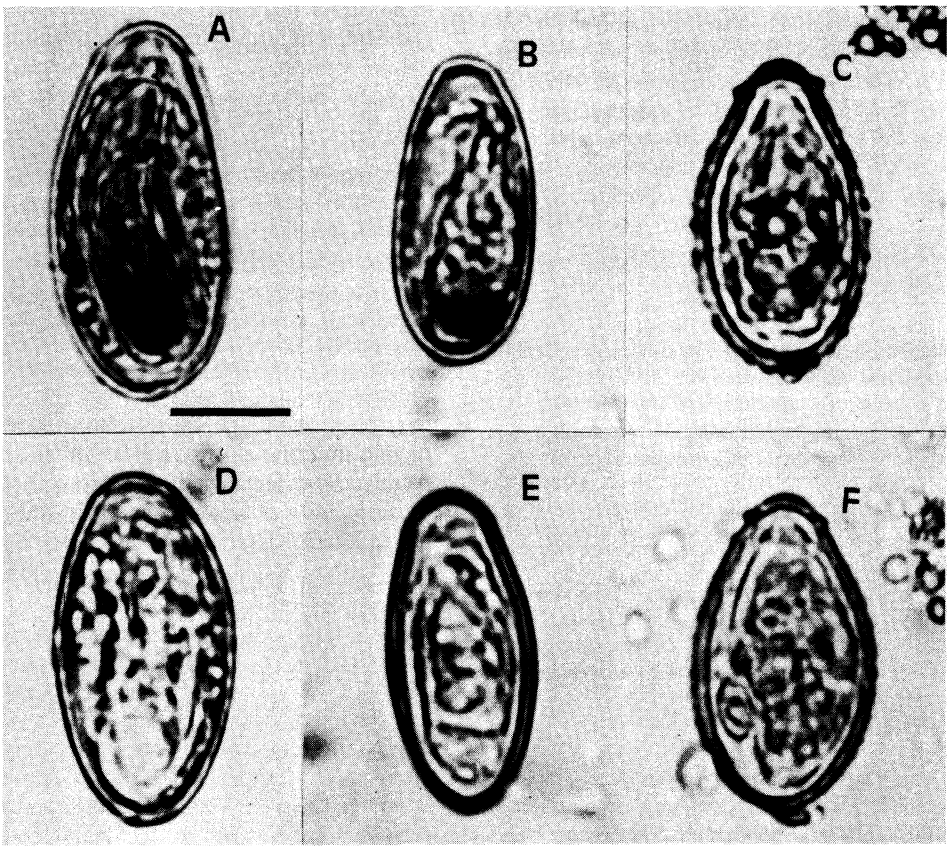

Fig 1—Comparative morphological features between the iodinestained and the unstained eggs of *P. bonnei* (A, D), *P. molenkampi* (B, E) and *O. viverrini* (C, F) in human feces. B-F have the same magnification as A, scale bar, 10 μm.

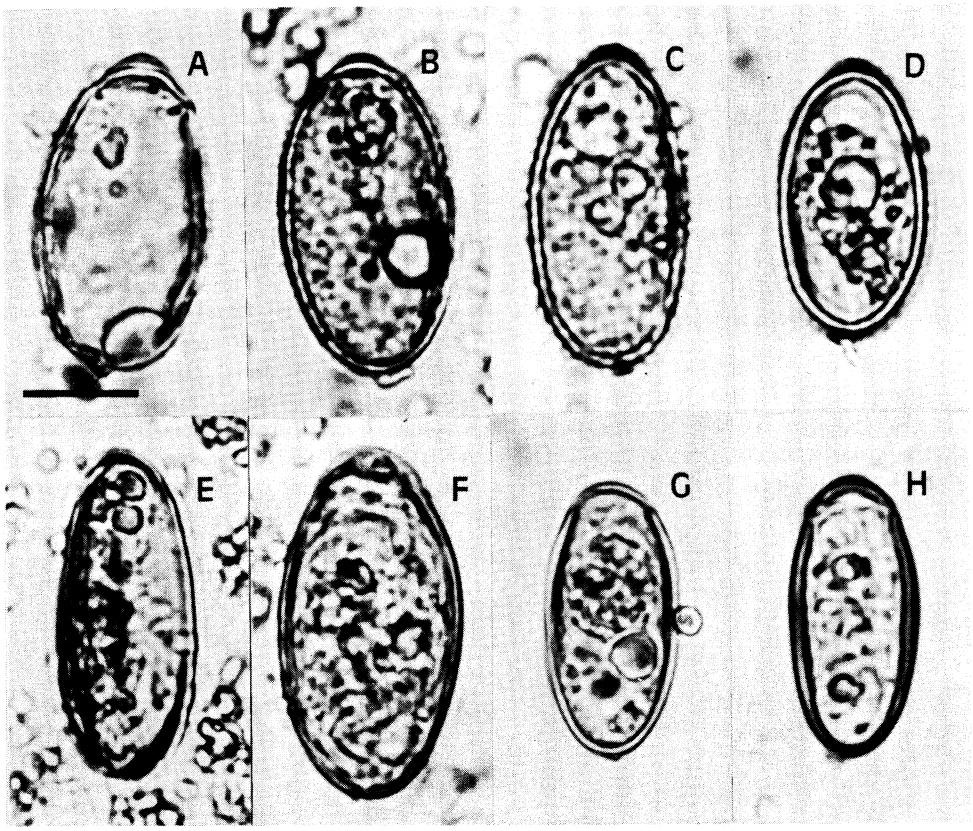

Fig 2—Unembryonated and malformed eggs of *O. viverrini* (A-D), *P. bonnei* (E, F), and *P. molenkampi* (G, H) in human feces. B-H have the same magnification as A, scale bar, 10 μm.

viverrini eggs (79%) than in *P. bonnei* (12%) and *P. molenkampi* (3%). All *P. bonnei* and *P. molenkampi,* and 52% of *O. viverrini* eggs are ovoid (Fig 4E, F), whereas the pyriform eggs of *O. viverrini* were found in 48% (Fig 4A-D).

The size, length and width, and the opercular width (Table 2; Figs 5-6) of *P. bonnei* eggs were consistently larger than those of *O. viverrini* eggs, which, in turn, were larger than *P. molenkampi* eggs (*t*-test, $p < 0.001$, $n = 400$).

DISCUSSION

The difficulty in differentiating between *Opisthorchis* and MIF eggs is recognized among diagnostic parasitologists. This study has revealed that there are a number of distinctive features that aid in differentiation, namely the iodophilic body, the miracidial shape, the shell surface, the knob, the operculum, the shoulder, the shape and size. Among these, the outstanding features for distinguishing embryonated eggs of lecithodendriid flukes and *O. viverrini* are the iodophilic body, which is much smaller and very rarely found in *O. viverrini* eggs, the curved miracidium of *P. bonnei* and the smaller size of *P. molenkampi* eggs. Furthermore, the smooth shell, the infrequent observation of distinct opercular, shoulders and knobs, and the absence of pyriform eggs are also important supporting characteristics for differentiating all forms of lecithodendriid from *O. viverrini* eggs. *O. viverrini* eggs seem to have a consistent knob on every egg but this can not be demonstrated on a few eggs due to the variation in the position of the eggs and the knobs. Various features and positions of knobs can be detected, in contrast to the finding of Ditrich *et al* (1990) that knobs seem to

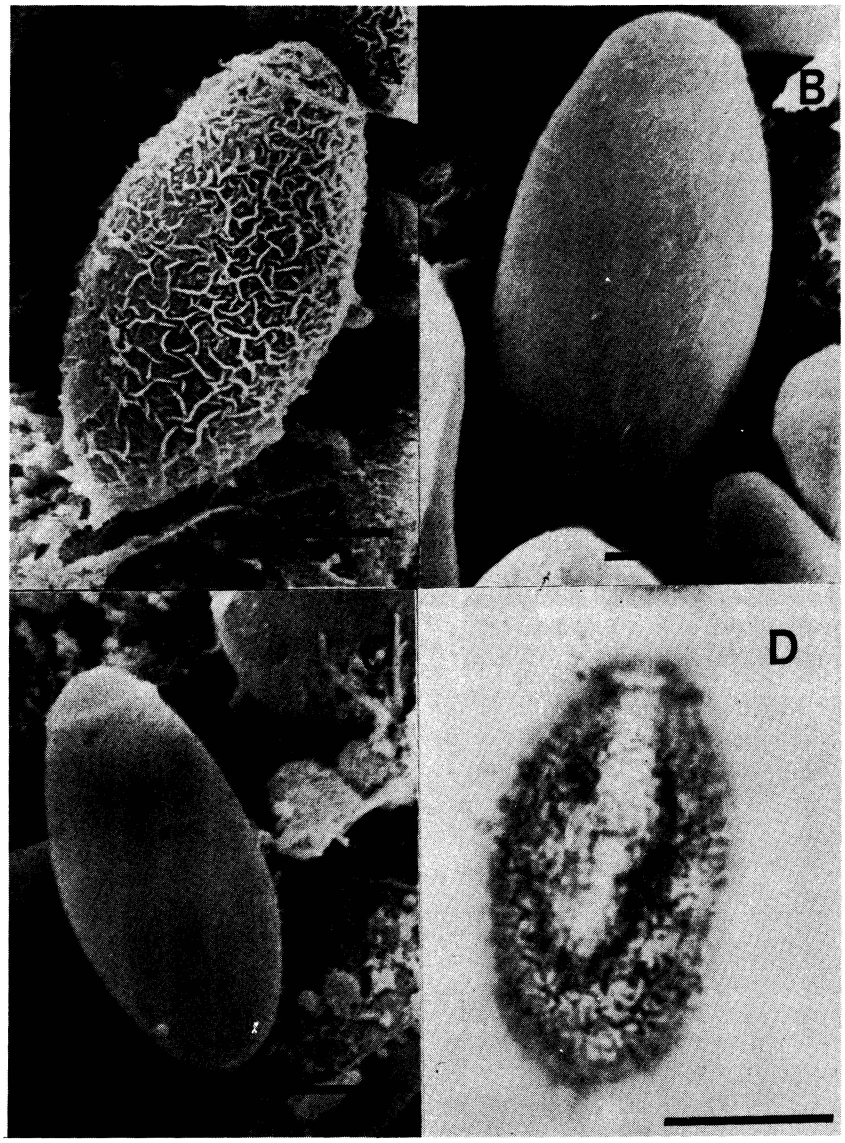

Fig 3—Surface ultrastructure of the uterine egg shell of *O. viverrini* (A), *P. bonnei* (B), *P. molenkampi* (C) and the surface structure of *O. viverrini* egg under the magnification of 1,000x using light microscopy (D), scale bar, 10 μm.

occur only in the perpendicular position.

The morphology of *P. molenkampi* unembryonated eggs containing a large round mass inside are frequently found in the feces (Fig 2G). The reason why unembryonated eggs of *P. molenkampi* are found in feces more than those of *O. viverrini* or *P. bonnei* is unknown. *P. molenkampi* might break more easily in human intestine or this worm could lay both embryonated and unembryonated eggs.

The differentiation between *O. viverrini* and heterophyid eggs using light microscopy is of practical importance. In this study, we found only 2 cases of light heterophyid infection in 84 cases of lecithodendriid infection. The specimens were not

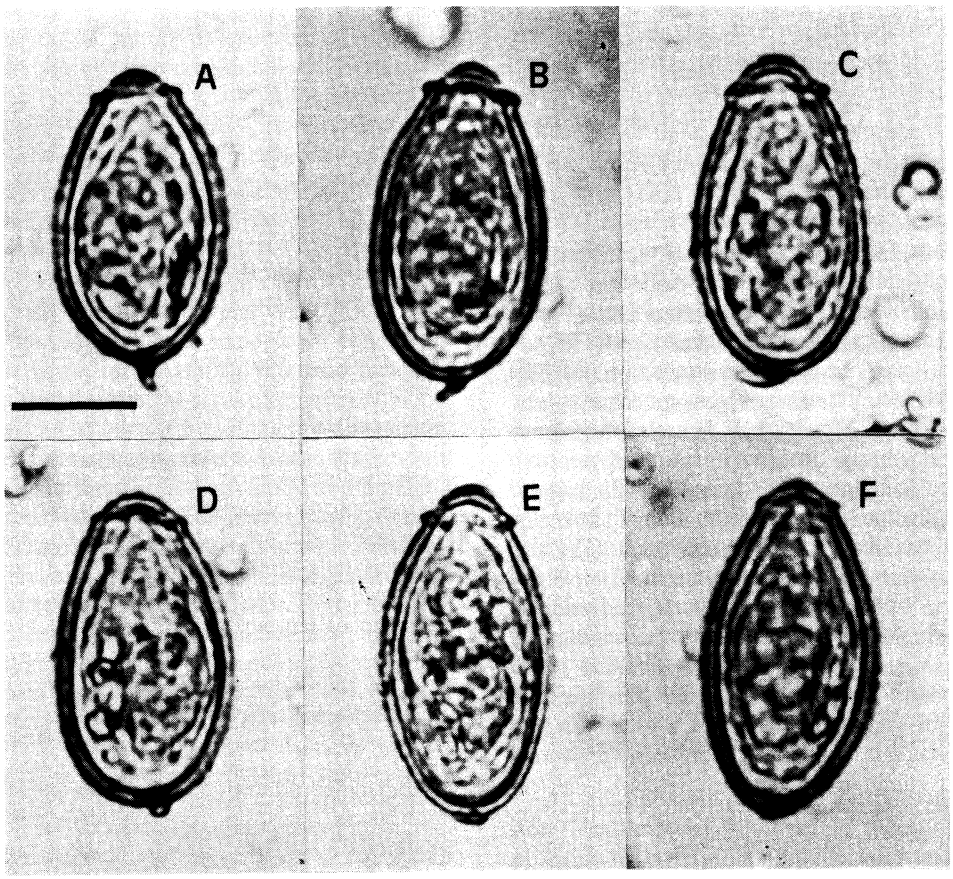

Fig 4—Various morphological features and positions of abopercular knobs detected on *O. viverrini* eggs in human feces. B-F have the same magnification as A, scale bar, 10 μm.

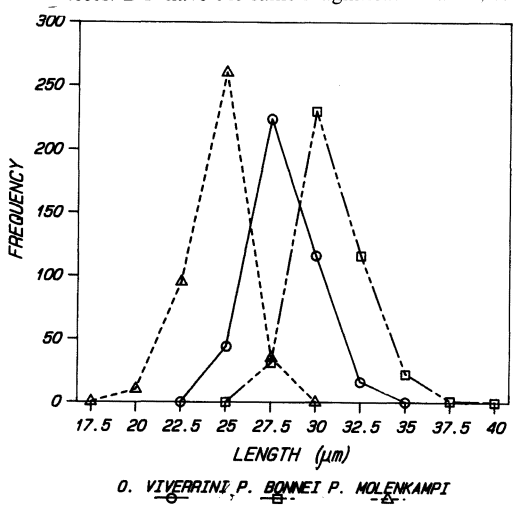

Fig 5—Frequency distribution of the length of *O. viverrini*, *P. bonnei* and *P. molenkampi* eggs recovered in human feces.

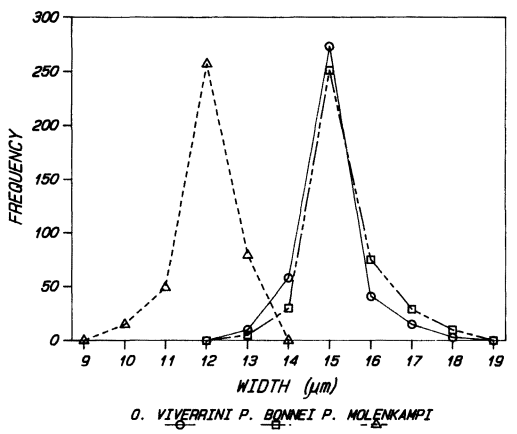

Fig 6—Frequency distribution of the width of *O. viverrini*, *P. bonnei* and *P. molenkampi* eggs recovered in human feces.

Table 2

Comparative observations and measurements of morphological features and size of *Opisthorchis viverrini* and lecithodendriid *(Phaneropsolus bonnei* and *Prosthodendrium molenkampi)* eggs. (Measurements are in micrometers and presented with mean ± standard deviation. Others are presented as percentages).

Characters of the egg	*O. viverrini* (n = 400)	*P. bonnei* (n = 400)	*P. molenkampi* (n = 400)
Iodophilic body	1 (small)	94 (large)	68 (large)
Embryonated egg	92	94	68
Curved miracidium	0	89	0
Smooth shell	0	100	100
Thick rough shell	64	0	0
Thin rough shell	36	0	0
Knob	93	6	11
Distinct operculum	92	20	3
Distinct shoulder	79	12	3
Ovoid	52	100	100
Pyriform	48	0	0
Length	26.8 ± 1.5	30.4 ± 1.6	23.9 ± 1.3
Width	14.9 ± 0.7	15.1 ± 0.7	11.5 ± 0.7
Opercular width	6.1 ± 0.8	7.3 ± 0.8	5.5 ± 0.6

sufficient to study the egg morphology because heterophyid trematodes produced a small number of eggs in the uteri and could not be detected in feces. The ultrastructure to the shell surface of heterophyid eggs as described by Ishii (1972), Fujino *et al* (1989), and Ditrich *et al* (1990) appears to differ from that of *O. viverrini* egg shell. However, these differences are not detectable under the light microscope.

O. viverrini and MIF eggs, which are similar in both size and general morphology, cannot be differentiated using only one characteristic because of the presence of many forms of eggs in stools. Thus, knowledge of the different characteristics and experience in examination are essential.

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