EGGSHELL MORPHOLOGY OF THE SMALL EGGS OF HUMAN TREMATODES IN THAILAND

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Abstract. Light and scanning electron micrographs of *Opisthorchis viverrini, Haplorchis taichui, H. pumilio, A Phaneropsolus bonnei,* and *Prosthodendrium molenkampi* eggs were studied. Under light microscopy, *O. viverrini* eggs had rough eggshells and prominent shoulders. *H. taichui, H. pumilio* were similar in shape and had smooth eggshells and prominent shoulders. *H. pumilio* eggs were bigger than *H. taichui* eggs. *P. bonnei* and *P. molenkampi* eggs had smooth eggshells and indistinct shoulders. *P. bonnei* eggs were thinner and bigger than *P. molenkampi* eggs. Some deformed eggs of *O. viverrini* and *Haplorchis* sp. were found and they had no embryos and indistinct opercula. Under scanning electron microscopy, *O. viverrini* eggs looked like musk-melon skin; they had prominent shoulders and long knobs. *H. taichui* eggs had curly, thread-like ridges and prominent shoulders and knobs. *H. pumilio* eggs had streched ridges, like Chinese bitter mormodica, and prominent shoulders. *P. bonnei* and *P. molenkampi* eggs had smooth eggshells and small shoulders and knobs.

INTRODUCTION

Small eggs of human trematodes are commonly found upon fecal examination in Northeast Thailand, where people customarily eat raw or improperly cooked foods. These trematodes are *O. viverrini* (Preuksaraj, 1984), *H. taichui, H. yokogawai, H. pumilio, P. bonnei* and *P. molenkampi* (Manning *et al,* 1971; Radomyos *et al,* 1983). These eggs are similar in shape and size and are small, oval and possessed of opercula. Under a light microscope their morphology is difficult to determine. If the morphology is misidentified the measurements of prevalence and intensity will be incorrect.

This study intends to differentiate between the different small-size eggs of trematodes which are commonly found in Northeast Thailand by examining the eggshell structures under light microscopy and scanning electron microscopy.

MATERIALS AND METHODS

Adult liver flukes of *O. viverrini* and small adult intestinal flukes of the species *H. taichui, P. molenkampi* and *P. bonnei* were obtained from the small intestines of autopsy cases at Srinagarind Hospital, Khon Kaen University. *H. pumilio* from the small intestines of autopsy cases were obtained for measuring the sizes of eggs and studying the morphology under a light microscope while those to be studied under the scanning electron microscope were obtained from infected hamsters (Srisawangwonk *et al,* 1989). The worms were previously fixed in 10% formalin. The worms were morphologically identified under a light microscope by following Manning *et al* (1971), Pearson and Ow-Yang (1982) and Radomyos *et al* (1983). The worms from 6-10 autopsy cases were pooled and then sampled for 25-30 worms of each species. The worms were dissected for upper uteri under a dissecting microscope and then torn to obtain mature eggs. These eggs were pooled in separate vials for each species. One hundred eggs of each species were sampled for measurement and studying the morphology. For electron microscopic study the eggs were washed three times with phosphate buffer, pH 7.4, and then fixed with 1% osmium tetraoxide in phosphate buffer for 2 hours at 4°C. They were dehydrated in a graded series of ethanol and placed in amyl acetate. The specimens were dried at the critical point in solid carbon dioxide,

coated with gold and examined under a Hitachi S-450 scanning electron microscope at 15 kV.

RESULTS

Light microscopy

These small eggs of flukes were oval-shaped and operculated. All of them were embryonated eggs, except some did not have opercula and embryos (Fig 1). In order of their length, they were *H. pumilio* (Fig 2), *H. taichui* (Fig 3), *O. viverrini* (Fig 4), *P. bonnei* (Fig 5) and *P. molenkampi* (Fig 6). Measurements of 100 eggs were 31.74 ± 1.45, 29.39 ± 1.58, 27.7 ± 1.62, 26.36 ± 2.42 and 25.31 ± 1.58 μm, respectively (Table 1). Their sizes were significantly different ($p < 0.05$). The widths of *O. viverrini, H. pumilio, H. taichui, P. bonnei* and *P. molenkampi* were 15.33 ± 0.84, 15.04 ± 1.37, 13.89 ± 1.26, 12.78 ± 1.77 and 11.6 ± 0.73 μm, respectively. Almost of their widths were significantly different ($p < 0.05$), except that there were no statistically significant differences between *O. viverrini* and *H. pumilio* ($p > 0.05$). The diameters of the opercula of *O. viverrini, P. bonnei, P. molenkampi, H. pumilio, H. taichui* were 6.63 ± 0.65, 6.26 ± 0.93, 6.08 ± 0.56, 5.94 ± 0.67 and 5.21 ± 0.59 μm, respectively.

Under the light microscope, *O. viverrini* eggs showed prominent shoulders and *H. pumilio* and *H. taichui* eggs also had shoulders. But *H. taichui* shoulders were slightly broader than the others. *P. bonnei* and *P.molenkampi* eggs had indistinct shoulders. The outer surfaces of the eggshells of *O. viverrini* showed rough skins, but the rough surfaces could not be seen in *H. taichui* and *H. pumilio* eggs.

Scanning electron microscopy

The scanning electron micrographs of *O. viverrini* eggs showed musk-melon-like prominent ridges (Figs 7, 8, 9,). These ridges were detectable under a light microscope at high magnification (Fig 4). The ridges on the eggshells were the same as on the opercula. The shoulders showed prominent rims (Fig 7). Also the edges of the opercula had prominent rims but they were smaller than those of the egg shoulders. The complete knobs were long and curved (Fig 9) but some of them were absent.

H. taichui eggs showed flat, thread-like curly ridges on the surface of the eggshells and the opercula (Figs 10, 11, 12) which could not be seen under the light microscope. Shoulder rims nearly the same size as those of the opercula edges were prominent (Fig 10) and visible under the light microscope. The knobs were big and prominent (Fig 12).

H. pumilio eggs showed ridges like Chinese bitter mormodica on the eggshells and opercula (Fig 13, 14, 15). The ridges stretched along the length of the eggs and tubercles were present on both sides of the ridges. The shoulder rims and the edges of the opercula were prominent (Fig 13) and visible under the light microscope. The knobs could not be detected (Fig 15).

P. bonnei (Figs 16, 17, 18) and *P. molenkampi* (Figs 19, 20, 21) eggs had smooth surfaces on the eggshells and opercula. Their shoulders were not so prominent as in *O. viverrini, H. taichui* and *H. pumilio* (Figs 16, 19). The knobs had small protrusions (Figs 18, 21).

DISCUSSION

There were statistically significant differences between the sizes of the small eggs of these trematodes in this study ($p < 0.05$). In practice, it was difficult to determine the species by size by fecal examination under a light microscope, because the ranges of their lengths and widths overlapped. Detailed surfaces of the eggshells at high magnification under the scanning electron microscope would be a useful tool for explanation of what we could expect to see at low magnification under the light microscope.

The ridges on *O. viverrini* eggs, which were musk-melon-like, were similar to those found in the study of Ditrich *et al* (1990), were similar to the ridges on *Clonorchis sinensis* eggs reported by Suzuki (1983) and Zaman (1987) and were also similar to the *Opisthorchis felineus* eggs reported by Ditrich *et al* (1990).

The ridges on *H. taichui* and *H. pumilio* eggs were similar to those found in the study of Fujino *et al* (1989) but the ridges on the eggshells were the same as on the operculum in this study. Therefore, the ridges on these eggs could not be detected

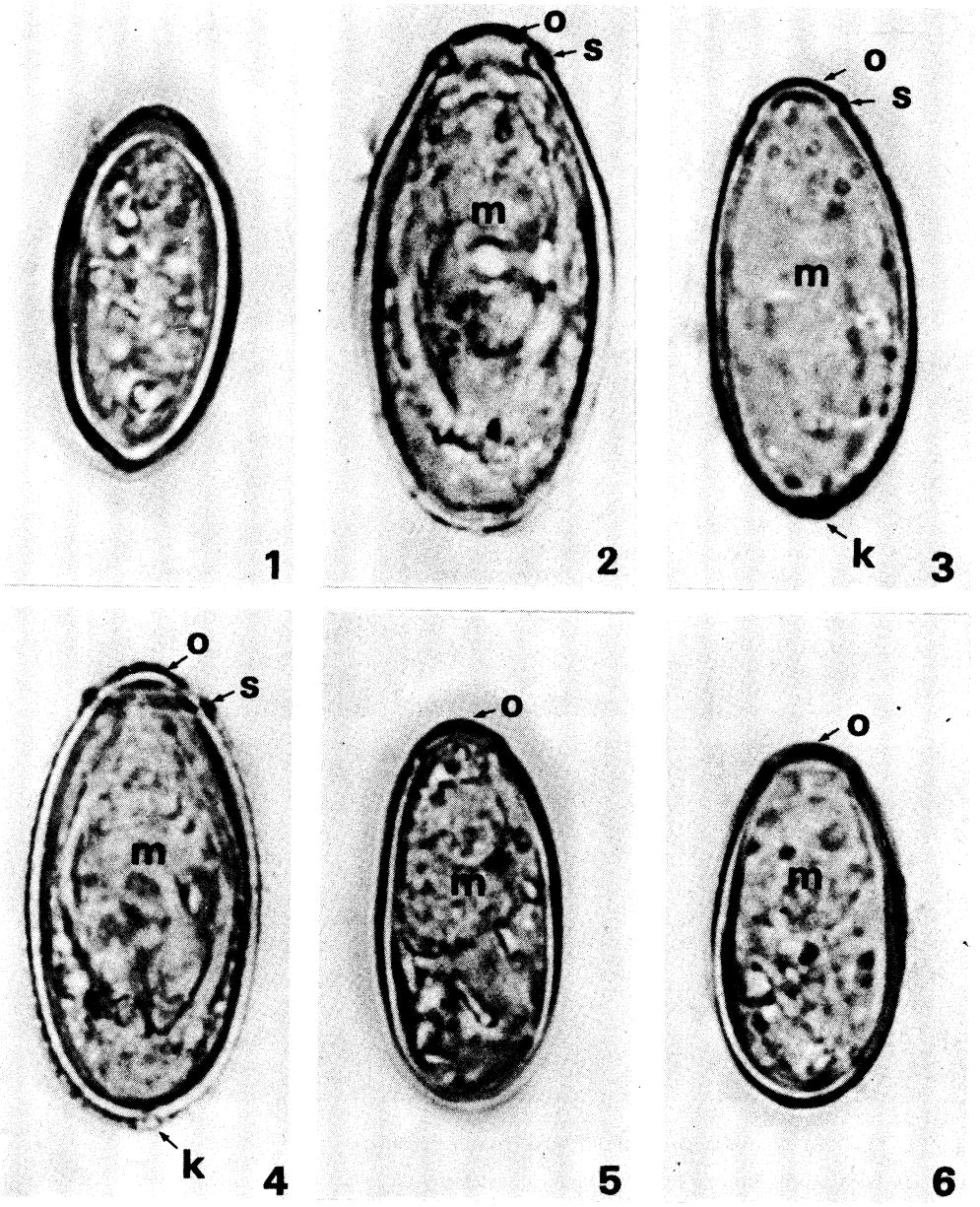

Fig 1-6—Light micrographs of small eggs of trematodes (1,047 X)

Fig 1—Deformed eggs of *Haplorchis taichui*

Fig 2—*H. pumilio* egg

Fig 3—*H. taichui* egg

Fig 4—*O. viverrini* egg

Fig 5—*P. bonnei* egg

Fig 6—*P. molenkampi* egg

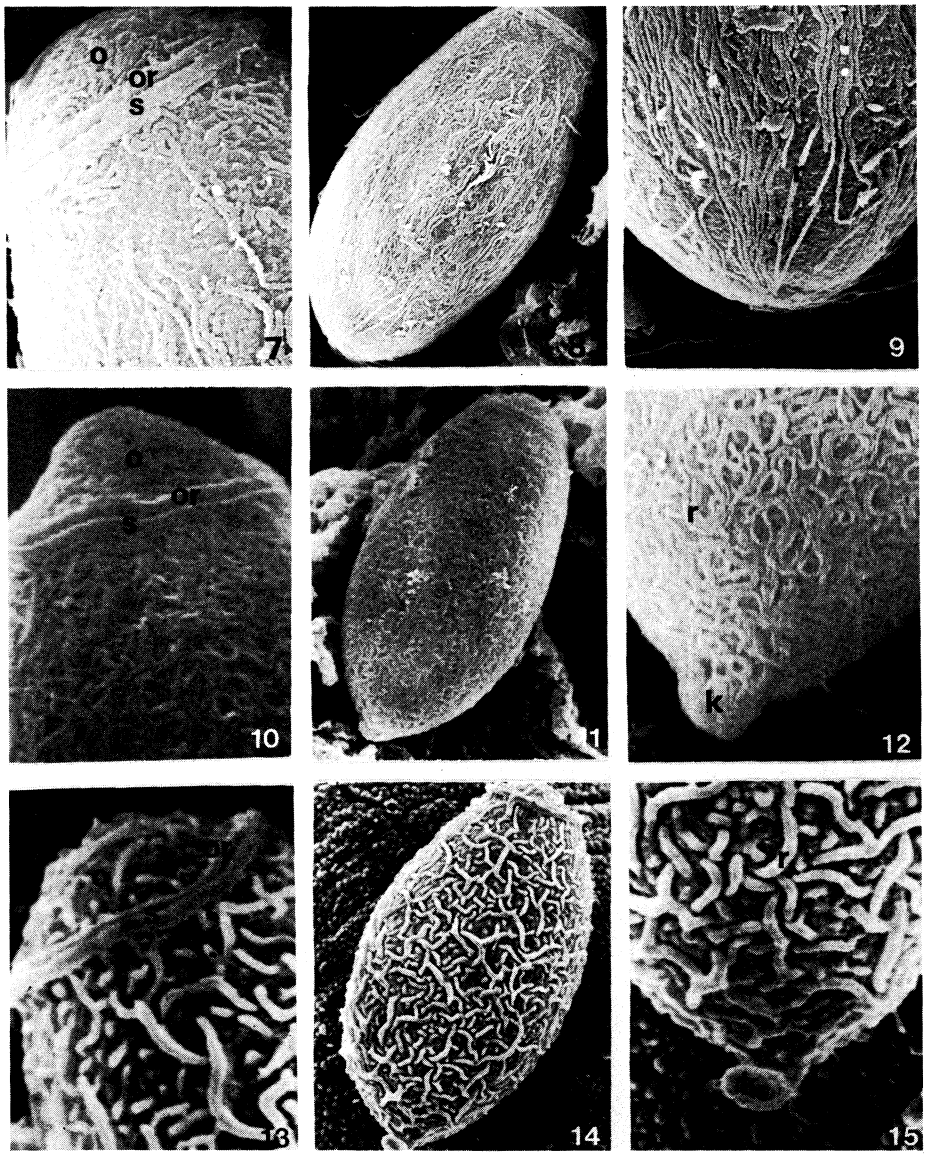

Fig 7-21—Scanning electron micrographs of small eggs of trematodes.

Fig 7—*H. pumilio* egg, showing the operculum, shoulder and the ridges on the surface (10,000 X).

Fig 8—*H. pumilio* egg, showing the whole egg (3,500 X).

Fig 9—*H. pumilio* egg, showing posterior end (8,800 X).

Fig 10—*H. taichui* egg, showing the operculum, shoulder and the ridges on the surface (10,000 X).

Fig 11—*H. taichui* egg, showing the whole egg (3,700 X).

Fig 12—*H. taichui* egg, showing knob (8,800 X).

Fig 13—*O. viverrini* egg, showing operculum, shoulder and ridges on the surface (10,000 X).

Fig 14—*O. viverrini* egg, showing the whole egg (3,500 X).

Fig 15—*O. viverrini* egg, showing knob (8,800 X).

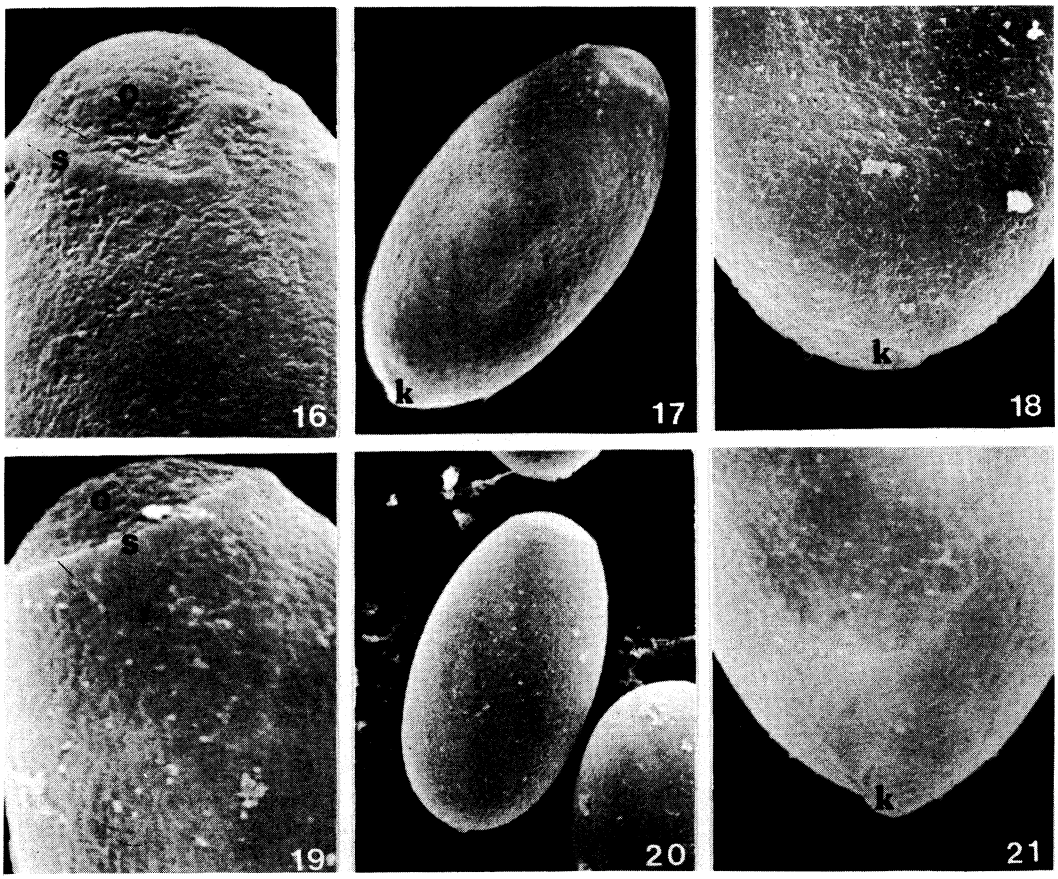

Fig 16—*Phaneropsolus bonnei* egg, showing operculum and shoulder (10,000 x).

Fig 17—*P. bonnei* egg, showing the whole egg (3,500 X).

Fig 18—*P. bonnei* egg, showing posterior end (8,800 X).

Fig 19—*Prosthodendrium molenkampi* egg, showing operculum and shoulder (10,000 X).

Fig 20—*P. molenkampi* egg, showing the whole egg (3,500 X).

Fig 21—*P. molenkampi* egg, showing posterior end (8,800 X).

Abbreviation

k-knob, m-miracidium, o-operculum, or-opercular rim, r-ridge, s-shoulder

under a light microscope because they were not as high and prominent as the ridges of *O. viverrini* eggs. The shoulders of these eggs and the edges of the opercula were prominent, thick and wide in light micrographs. These two kinds of eggs were difficult to distinguish by species under the light microscope except by means of their sizes and *H. taicui* had prominent knobs, but they differed from *O. viverrini* by the ridges which were visible under the light microscope.

The scanning electron micrographs of *P. bonnei* and *P. molenkampi* showed smooth egg surfaces and less prominent shoulders, which were difficult to detect under the light microscope. Differentiation can be accomplished by size and shape, with

Table 1

Mean size of *Opisthorchis viverrini* eggs and small intestinal fluke eggs commonly found in Thailand.

Measurement	Size (μm)				
	Pb	Pm	Ht	Hp	Ov
Length					
Mean	26.36	25.31	29.39	31.74	27.70
SD	2.42	1.58	1.58	1.45	1.62
Range	23.75	22.50	26.25	27.50	22.50
	-33.75	-28.75	-33.75	-37.50	-30.00
Width					
Mean	12.78	11.60	13.89	15.04	15.33
SD	1.77	0.73	1.26	1.37	0.84
Range	10.00	10.00	12.50	12.50	13.75
	-17.50	-12.50	-16.25	-17.50	-17.50
Diam. of operculum					
Mean	6.26	6.08	5.21	5.94	6.63
SD	0.93	0.56	0.59	0.67	0.65
Range	5.00	5.00	3.75	5.00	5.00
	-8.75	-7.50	-7.50	-7.50	-7.50

P. bonnei eggs being bigger and thinner than *P. molenkampi* eggs.

The deformed eggs of *H. taichui* showed the indistinct opercula which are also found in the other species. They were probably deformed during the formation of the eggshells or they were unfertilized eggs, resulting in lack of embryos inside. This study should be continued to determine further characteristics of these kinds of eggs.

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REFERENCES

Ditrich O, Giboda M, Sterba J. Species determination of eggs of opisthorchiid and heterophyid flukes using scanning electron microscopy. *Angew Parasitol* 1990; 31 : 3-9.

Fujino T, Higo H, Ishii Y, *et al.* Comparative studies on two similar species of *Haplorchis* and *Metagonimus* (Trematoda : Heterophyidae) surface ultrastructure of adults and eggs. *Proc Helminthol Soc Wash* 1989; 56 : 35-41.

Manning GS, Lertprasert P, Watanasirmkit K, Chetty C. A description of newly discovered intestinal parasites endemic to northeastern Thailand. *J Med Assoc Thai* 1971; 54 : 466-75.

Pearson JC, Ow-Yang CK. New species of *Haplorchis* from Southeast Asia, together with keys to the *Haplorchis*-group of heterophyid trematodes of the region. *Southeast Asian J Trop Med Public Health* 1982; 13 : 35-60.

Preuksaraj S. Public health aspects of opisthorchiasis in Thailand. *Arzneimittel Forschung* 1984; 34 : 1119-20.

Radomyos P, Bunnag D, Harinasuta T. *Haplorchis pumilio* (Looss) infection in man in northeastern Thailand. *Southeast Asian J Trop Med Public Health* 1983; 14 : 223-7.

Srisawangwonk T, Kanla P, Tesana S, Arunyanart C. Scanning electron microscopy of the tegumental surface of adult *Haplorchis pumilio* (Looss). *J Helminthol* 1989; 63 : 141-7.

Suzuki N. Color atlas of human helminth eggs. Tokyo : Kikuya Shobo 1983; 95 pp.

Zaman V. Scanning electron microscopy of helminth ova. *Southeast Asian J Trop Med Public Health* 1987; 18 : 1-13.