CERCARIAL EMERGENCE AND LONGEVITY OF *OPISTHORCHIS VIVERRINI* IN *BITHYNIA SIAMENSIS GONIOMPHALOS*

Jutharat Kulsantiwong[1], Sattrachai Prasopdee[2]

[1]Department of Biology, Faculty of Science, Udon Thani Rajabhat University, UdonThani, Thailand 41000

[2]Chulabhorn International College of Medicine, Thammasat University, Rangsit Campus, PathumThani, Thailand 12121

Jutharat_kulsantiwong@yahoo.com

ABSTRACT Amongst the snail species which are serving as hosts for parasites, *Bithynia siamensis goniomphalos*, a freshwater snail, plays a crucial role as an amplifier host of the carcinogenic liver fluke *Opisthorchis viverrini*. Numerous free swimming cercariae released from infected snail participate in *O.viverrini* transmission to humans. We investigated the cercarial emergence and longevity of *O. viverrini* infecting *B. siamensis goniomphalos*. Naturally infected snail samples were collected from a rice field in UdonThani Province, Thailand. The emergence of *O. viverrini* cercariae was monitored for 1-hour intervals in a day (24 hours). The survival time of released cercariae was also accounted as cercarial longevity. The result showed that cercarial emergence was peak during 3:00-8:00 PM, but not present during 12.00 PM – 5.00 AM. The average total cercarial output from all infected snail samples (n=4) was 767 with the maximum number of 1000 cercariae released from one snail. The released *viverrini* cercariae could survive for 48 hours, which implies the possibility of *O. viverrini* cercariae transmission.

1. INTRODUCTION

Trematode is medically important parasite which causes a health problem in humans and animals. Typically, the parasites require intermediate hosts to complete life cycle which include freshwater snails and freshwater fish as first and second intermediate hosts, respectively. Amongst trematodes, human liver fluke *Opisthorchis viverrini* infection was found to be a potential risk factor of cholangiocarcinogenesis in humans (Haswell- Elkins et al., 1992) and was classified into carcinogenic group 1 (IARC, 1994, 2011).The occurrence of cholangiocarcinoma (CCA) had a strongly positive correlation with the prevalence of *O. viverrini* infection (Srivatanakul et al., 1991; Sriamporn et al., 2004). The incidence of CCA in Udon Thani and Khon Kaen Provinces, northeast Thailand is the highest in the world (Vatanasapt et al., 1990; Parkin et al., 2002; Sriamporn et al., 2004; Khuhaprema, Srivatanakul, 2007). *Bithynia* spp., a freshwater snail, plays a crucial role as first intermediate host of the *O. viverrini*. Three taxa of *Bithynia* in Thailand have been as natural first intermediate hosts of the *O. viverrini* in different geographical habitats. There are *B. funiculata* in the north, *B. siamensis siamensis* in the central, north and *B. siamensis goniomphalos* in the northeast region (Wykoff et al., 1965; Brandt, 1974; Kulsantiwong et al.,2015). Theoverall of natural infection rates of *O. viverrini* in *Bithynia* snails varied from 0.083 to 1.6% (Wykoff et al., 1965; Upatham, Sukhapanth, 1980; Brockelman et al., 1986). Sri-aroon et al. (2005) reported that the natural infection rate of *O. viverrini* in *B. siamensis goniomphalos* varied from 0.61 to 1.3%. However, Kiatsopit et al. (2012) recently reported a higher prevalence of infection than any previous report with an average of 3.04% and a hot spot of 6.93% in Sakon Nakhon Province.

Cercaria, a free-swimming larval stage of parasites, is now in research focus to comprehend the biology of transmission. Several studies revealed cercarial emergence from infected snails, especially *Schistosoma* sp. (Ahmed et al., 2006, Wolmaransa et al., 2002). Moreover, Phongsasakulchoti et al. (2005) revealed that physiological and ecological factors influenced on cercarial emergence from infected snails. Mao et al. (1949) also reported that light, a physical factor, was an important manipulating the emergence of *S. japonicum* cercariae detcefni morf *Oncomelania hupensis* snail. In

addition, emergence pattern of *O. viverrini* cercariae from infected *B. siamensis goniomphalos* is in agreement with *S. japonicum* that numerous cercariae initially released when the snail exposed to the light (Phongsasakulchoti et al., 2005). However, a little number of cercariae can be found during the nighttime. Due tolittle knowledge on the biology of transmission of *O. viverrini* cercaria, this study aims to investigate the cercarial emergence and longevity. This biological information would be useful for surveillance and control of *O. viverrini* transmission.

2. EXPERIMENT

2.1 Snail collection

B. siamensis gonimphalos were collected in May and June 2015 from rice field (17°31'50.6"N, 103° 01'36.5"E) by either a wire-mesh scoops or hand-picked up. All snails were cleaned, air-dried, separately labeled and kept in pore plastic bags. The snail samples were brought back to the laboratory to be examined for trematode infection. The snails were identified based on shell morphology for species following available keys and descriptions (Brandt, 1974; Upatham et al., 1983; Chitramvong & Upatham, 1992; Kulsantiwong et al., 2013; Tesana et al., 2014).

2.2 Examination of trematode infection in snails

All snails were examined for trematode infection by cercarial shedding method.Prior to cercarial shedding, the snails were cleaned with 50 ml of dechlorinated tap water. The snails were placed in plastic containers with 50 ml of dechlorinated tap water, stimulated by 9W electric light bulbs for 2 hours for daytime shedding. For cercaria of that shed at night, black plastic covers were used to achieve total darkness and snails were allowed to shedding overnight at room temperature. The confirmation of *O. viverrini* cercariae used species-specific primer PCR (Wongratanacheewin et al., 2001).

2.3 Cercarial emergence patterns

Immediately after cercarial shedding, four infected snails were randomly selected and transferred individually to new plastic containers with 50 ml dechlorinated tap water at room temperature (29-33 °C). The infected snails were placed under artificial light for 12 hours, from 06:00 AM to 06:00 PM for the light period and 12 hours from 6:00 PM to 6:00 AM for dark period. The snails were fed with ivy leaves gourd. Every hour, each infected snail was transferred to a new plastic container filled with the same volume of dechlorinated tap water at room temperature. A number of released cercariae were counted under a stereoscope.

2.4 Longevity of *O. viverrine* cercaria

The infected snails were placed in the plastic containers with 50 ml dechlorinated tap water at room temperature. Released cercariae were checked under a stereoscope. When cercariae presented in the plastic containers, the snails were removed and transferred to new plastic containers. Survival of cercariae was observed every 5 min under a stereoscope. Under laboratory condition, the released cercariae were exposed to artificial light for day period (6:00 AM to 6:00 PM) and used black plastic covers for night period (6:00 PM to 6:00 AM). The survival rate of *O. viverrini* cercariaewas recorded.

3. ANALYSIS

3.1 Shell morphology of *B. siamensis goniomphalos*

Bithynia siamensis goniomphalos snails were sampled from anatural water reservoir in Nong Wua So district, Udon Thani Province. Shell morphology of the snail was identified based on available keys and descriptions. Briefly, the shell is dull, reddish-brown colored and a subovated conic. The whorls are a little rounded with horizontal and indented sutures. The surface sculpture consists of thick transverse raised lines and fine spiral incised lines. The outer part of the last whorl is quite straight. The basal lip of aperture is appeared sharply angled on the left side (as shown in Fig. 1).The average shell size of non-infected snails was 7.3 and 4.5 mm in length and width, respectively. The average shell size of infected snails was 6.8 and 4.3 mm in length and width, respectively.

3.2 Prevalence of natural infection

Out of 5,179 *B. siamensis goniomphalos*, 389 (7.5%) infected four different types of cercariae. The highest prevalence of natural infection was virgulate (6.28%) followed by armatae (0.62%), monostome (0.39%) and *O. viverrini* (0.19%), respectively (Table 1).

Table 1 Prevalence of naturally infected *B. siamensis goniomphalos* snail.

Type of cercariae	Number of infected snail	Prevalence
virgulate	325	6.28
armatae	32	0.62
monostome	20	0.39
O. viverrini	10	0.19
Total	389	7.6

3.3 Emergence of *O. viverrini* cercaria

Released *O. viverrini* cercariae from four infected *B. siamensis goniomphalos* in 24 hours was found during 6.00 AM-8.00 PM with a total number of 3,065 cercariae. The total number of released cercariae per each snail was 1000, 866, 752 and 447 cercariae, respectively. The cercarial emergence was peak during 3:00-8:00 PM. The average of total cercarial output was 767 cercaiae per snail per day (Fig. 2).

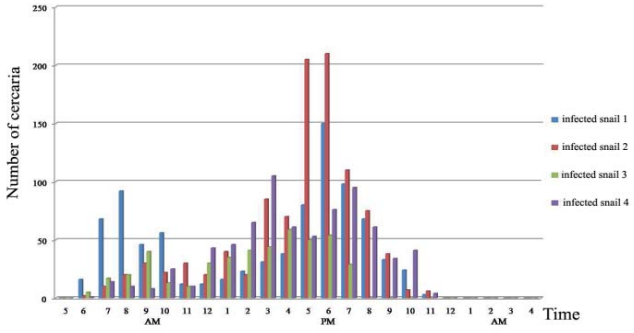

Fig. 2 Cercarial emergence of *O. viverrini* from naturally infected *B. siamensis goniomphalos*.

3.4 Longevity of *O. viverrini*cercaria

Thirteen *O. viverrini* cercariae were observed for survival under a stereoscope. The result showed that maximum survival time of *O. viverrini* cercariae was 48 hours 55 min and minimum survival time was 21 hours 4 min. The average survival time was 31hours 31 min (Table 3).

Table 3 Longevity of *O. viverrini* cercariae released from infected *B. siamensis goniomphalos* snail (room temperature; 25 °C).

Number of cercariae	Longevity (hours)
1	47.35
2	24.1
3	46.5
4	46.2
5	48.35
6	45.25
7	45.35
8	21.4
9	26.15
10	28.5
11	28.1
12	29.25
13	48.55

4. CONCLUSION AND DISCUSSION

The emergence of *O. viverrini* cercariae was monitored 1-hour intervals in a day. The survival time of released cercariae was also accounted as cercarial longevity. The study showed that cercarial emergence was peak during 3:00-8:00 PM, but not present during 12.00 PM – 5.00 AM which is in agreement with previous reports (Phongsasakulchoti et al., 2005; Kaitsopit et al., 2014). *B. siamensis goniomphalos* snail normally lives in shallow water or the edge of the water reservoir (Brockelman et al., 1986; Chitramvong & Uptham, 1992). Thus, it is possible that numerous *O. viverrini* cercariae released in the evening would help increase transmission to next intermediate host, cyprinid fishthat normally inhabits shallow water at that time. The released *O. viverrini* cercariae could survive more than21 hours and up to 48 hours, which implies the possibility of *O. viverrini* cercariae transmission. The results of this study would prove beneficial for parasitologist, malacologist and government to set strategies for prevention and control of *O. viverrini* infection.

5. ACKNOWLEDGEMENTS

This work wassupported by the Research and Development Institute Udon Thani Rajabhat University. We also acknowledge the Faculty of Science, Department of Biology, Udon Thani Rajabhat University and Chulabhorn International College of Medicine, Thammasat University.

6. REFERENCES

Ahmed, AAM., Ibrahim, NA., Idris, MA. Laboratory studies on the prevalence and cercarial rhythms of trematodes from *Bulinus truncates* and *Biomphalaria Pfeifferi* snails from Khartoum state, Sudan. *Sultan Qaboos University Medical Journal*, vol. 6, pp. 65-69, 2006.

Brandt, RAM. The non-marine aquatic Mollusca of Thailand. *Arch Mollusken*, vol 105, pp. 1-423, 1974.

Brockelman, WY., Upatham, ES., Viyanant, V., Ardsungnoen, S., Chantanawat, R. Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini*, in northeast Thailand: population changes of the snail intermediate host. *Int J Parasitol*, vol. 16,pp. 545-552. 1986.

Chitramvong, YP., Uptham, ES. The Bithyniidae (Gastropoda: Prosobanchia) of Thailand: comparative external morphology. *Malacol Rev*, vol. 25, pp. 21-38. 1992.

Haswell-Elkins, MR., Sithithaworn, P., Elkins, D. *Opithorchis viverrini* and cholangiocarcinoma in Northeast Thailand. *Parasitol Today*, vol. 8, pp. 86-89. 1992.

Internationalagency for research on cancer (IARC). Infection with liver flukes (*Opisthorchis viverrini, Opisthorchis felineus*and *Clonorchis sinensis*). *IARC Monogr Eval Carcinog Risks Hum*, vol. 61, pp. 121-175. 1994.

Internationalagency for research on cancer (IARC). A review of human carcinogens: Biological agents, *Opisthorchis viverrini* and *Clonorchis sinensis*. *IARC Monogr Eval Carcinog Risks Hum*, vol. 100B, pp. 351-376. 2011.

Kiatsopit, N., Sithithaworn, P., Boonmars, T., Tesana, S., Chanawong, A., Saijuntha, W., Petney, TN., Andrews, RH. Exceptionally high prevalence of infection of Bithynia siamensis goniomphalos with *Opisthorchis viverrini* cercariae in different wetlands in Thailand and Lao PDR. *Am J Trop Med Hyg*, vol. 86, pp. 464-9. 2012.

Kiatsopit, N., Sithithaworn, P., Kopolrat, K., Andrews, RH., Petney, TN.Seasonal cercarial emergence patterns of *Opisthorchis viverrini* infecting *Bithynia*

siamensis goniomphalos from Vientiane Province, Lao PDR. *Parasites & Vectors*, vol. 7, pp. 551. 2014.

Khuhaprema, T., Srivatanakul P., Sriplung H, Wiangnon S, Sumitsawan Y, Attasara P, eds. (Cancer in Thailand Vol. IV, 1998-2000. Bangkok, Thailand:Bangkok Medical Publisher, 2007.

Kulsantiwong, J., Prasopdee, S., Ruangsittichai, J., Ruangjirachuporn, W., Boonmars, T., Viyanant, V., Pierossi, P., Hebert, PDN., Tesana, S. DNA Barcode Identification of Freshwater Snails in the Family Bithyniidae from Thailand. *PLOS ONE*, vol. 8, pp. e79144. 2013.

Mao, CP., Li, L., Wu, CC. Studies on the emergence of cercariae of *Schistosoma japonicum* from their Chinese snail host, *Oncomelania*. *Am J Trop Med*, vol. 24, pp. 937-944. 1949.

Parkin, DM., Whelan, S.L., Ferlay, J., Teppo, L., Thomas, DB. *Cancer Incidence in Five Continents Vol.VIII*. International Agency for Research on Cancer. Lyon: France, 2002.

Phongsasakulchoti, P., Sri-aroon, P., Kerdpuech, Y. Emergence of *Opisthorchis viverrini* cercariae from *goniomphalos*. *Southeast Asian J Trop Med Public Health*, vol. 36, pp. 189-191. 2005.

Sriamporn, S., Pisani, P., Pipitgool, V., Suwanrungruang, K., Kamsa-ard, S., Parkin, DM. Prevalence of *Opisthorchis viverrini* infection and incidence of cholangiocarcinoma in Khon Kaen, Northeast Thailand. *Tropical Medicine and International Health*, vol. 9, pp. 588–594. 2004.

Sri-aroon, P., Butraporn, P., Limsomboon, J., Kerdpuech, Y., Kaewpoolsri, M., Kiatsiri, S. Freshwater mollusks of medical importance in Kalasin province, Northeast Thailand. *Southeast Asian J Trop Med Public Health*, vol. 36, pp. 653-657. 2005.

Srivatanakul, P., Parkin, DM., Jiang, YZ., Khlat, M., Kao-lan, UT., Sontipong, S., Wild, C. The role of infection by *Opisthorchis viverrini*, hepatitis B virus, and aflatoxin exposure in the etiology of liver cancer in Thailand. A correlation study. Cancer, vol. 68, pp. 2411–2417. 1991.

Tesana, S., Thabsripair, P., Suwannatrai, A., Haruay, S., Piratae, S., Khampoosa, P., Thammasiri, C., Prasopdee, S., Kulsantiwong, J., Chalorkpunrut, P., Jones MK. Parasite surveys and environmental management for prevention of parasitic infection in cultivated *Barbonymus gonionotus* (Cyprinidae) in fishponds, in an opisthorchiasis endemic area of northeast Thailand. *Aquaculture*, vol. 428-429, pp. 54-60. 2014.

Upatham, ES., Sukhapanth, N. Field studies on the bionomics of *Bithynia siamensis siamensis* and the transmission of *Opisthorchis viverrini* in Bangna, Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 1980; 11: 355-358.

Upatham, ES., Sornmani, S., Kitikoon, V., Lohachit, C., Burch, JB. Identification key for the fresh-andbrackish-water snails of Thailand. *Malacol Rev*, vol. 16,1983.

Vatanasapt,V., Tangvoraphonkchai, V., Titapant, V., Pipitgool, V., Viriyapap, D. & Sriamporn, S. A high incidence of liver cancer in Khon Kaen province, Thailand. *Southeast Asian J Trop Med Public Health*, vol. 21, pp. 489-494. 1990.

Wolmaransa, CT., de Kocka, KN., Straussa, HD., Bornmana, M. Daily emergence of *Schistosoma mansoni* and *S. haematobium* cercariae from naturally infected snails under field conditions. *Journal of Helminthology*, vol. 76, pp. 273-277. 2002.

Wongratanacheewin, S., Pumidonming, W., Sermswan, RW., Maleewong, W. Development of a PCR-baxed method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. *J Parasitol*, vol. 122, pp. 175-180. 2001.

Wykoff, DE., Harinasuta, C., Juttijudata, P., Winn, MM. *Opisthorchis viverrini* in Thailand- the life cycle and comparison with *O. felineus*. *J Parasitol*, vol. 51, pp. 107-132. 1965.

Jutharat Kulsantiwong received the B.E. (1988) degrees in Biology, (2005) degrees in Biotechnology, Ph.D.(2012) degrees in Parasitology from Khon Kaen University.
She is a lecturer, Department of Biology,Udon Thani Rajabhat University, Thailand. Her current interests include DNA barcode and trematode infection of intermediate of parasite.

Sattrachai Prasopdee received the D.V.M. (2008) degree and the Ph.D.(2014) degree in Parasitology from Khon Kaen University.
He is a lecturer, Department of preclinical science, Chulabhorn international college of medicine, Thammasat University. His current interests include host-parasite interaction and modulation of host's immune response.