

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 28 Studies on Opisthorchis viverrini in Thailand -
Morphology of the cercaria

Project No. 3A 025601 A 811 Military Medical Research Program
S. E. Asia.

Task 01: Military Medical Research Program
S. E. Asia.

Subtask 01: Military Medical Research Program
SEASIA (Thailand)

Reporting Installation: US Army-SEATO Medical Research Laboratory,
APO 146 San Francisco, California.

 Division of Medical Research Laboratories
 Department of Medical Zoology

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Major Dale E. Wykoff, MSC

Associate Investigator: Miss Kobkul Ariyaprakai

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

ABSTRACT

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The purpose of this investigation was to carry out a morphological study of the cercarial stage of O. viverrini. It is described in detail. The most striking difference between it and O. felineus is the flame cell pattern which was found to be 2 (3+3)+(3+3+3) for O. viverrini as compared to 2 (5+5)+(5+5) for O. felineus.

BODY OF REPORT

SEATO Medic Study No. 23 Studies on Opisthorchis viverrini in Thailand
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Final Report

Objective: The purpose of this investigation was to identify and study the morphological characteristics of the cercarial stage of O. viverrini.

Description: The snail Bithynia goniomphalus shed 10 types of cercariae, eight of which did not resemble the cercariae of O. felineus or Clonorchis sinensis. The remaining two types 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 parallel to the bottom of the container. The body of the later 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 of 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 eyespots were still visible. The lesser-active or spade-body type cercaria produced metacercariae which did not resembled 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 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 hosts. The trematodes were removed from the liver, stained and identified as being Opisthorchis viverrini. The cercaria of O. viverrini had thus been identified for the first time.

Progress: 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). The length of the body averaged 154 u (range 140 to 183). The width of the body varied from 61 to 96 u (mean 73 u), while that of the tail was 26 u. The cercaria assumes the characteristic pipe-form, resting on the bottom or briefly hanging in the water its head down. This behavior is similar to that of Clonorchis sinensis (Komiya and Tajimi, 1940), and O. felineus (Vogel, 1934). The cercariae of O. viverrini are both geo- and phototropic. The surface of the body is covered with minute spines. A conspicuous pair of eyespots 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 3.6-3.7 by 3.4-5.1 u (mean 3.6 to 4.3). It is protrusible with the opening somewhat ventral to the anterior and 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 cercaria is filled by five pairs of finely granulated secretory (penetration) glands. Each cell possesses a rather distinct round nucleus. The cephalic secretory gland ducts from each side proceed to the anterior portion of the oral sucker where they separate, some following the outer lateral margins of the oral sucker, others proceeding along the inner aspects of this organ. These ducts open in minute hollow spines on the sides of the oral aperture. 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 rough 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 midsection of the body. Here, each divides into an anterior and posterior collecting tube. The anterior tube divides into two tertiary collecting tubules, one terminating with 3 flame cells anterior to each eyespot, the other ending with 3 flame cells located posterior to the eyespot. 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 authors are indebted to Dr. Yoshitaka Komiya, NIH Tokyo, Japan, for his assistance in determining the flame cell pattern). The secondary and tertiary collecting tubules can be recognized only in young cercariae, for in older specimens the scattered pigment tends to obscure them. The cytogeneus glands are found dorso-laterally on both sides of the body. A nerve commissure is located just posterior to the pharynx. The genital anlage 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 350-437u (mean 392u) 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.

Summary: The cercaria of O. viverrini has been identified by a series of experimental exposures which resulted in the infection of definitive hosts. The cercaria has been found to be similar in many ways to that of O. felineus.

Conclusion: The cercaria of O. viverrini can be definitely differentiated from that of O. felineus on the basis of the flame cell pattern, 2 (3+3)+(3+3+3) for the former, and 2 (5+5)+(5+5+5) for the latter.

List of Publications: This report is being incorporated into the following paper which is now in manuscript and which will be submitted for publication in May, 1964: Wykoff, D. E., Harinasuta, C., Juttijudata, P., and Winn, M. M. Studies on Opisthorchis viverrini in Thailand - Notes on the life cycle and comparison with O. felineus.