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TAXONOMICAL AND ECOLOGICAL STUDIES ON THE LUNG FLUKE, PARAGONIMUS IN THE PACIFIC AREA, WITH SPECIAL REFERENCE TO SOUTH-EAST ASIA

by

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August 1968

U. S. ARMY RESEARCH AND DEVELOPMENT GROUP
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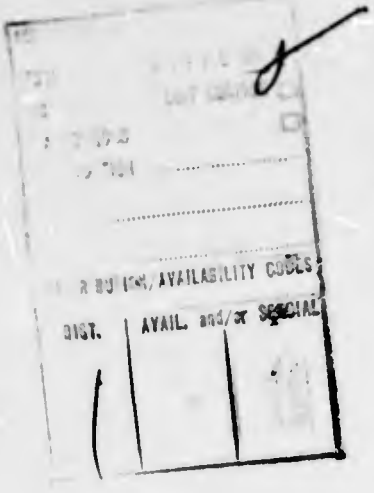

ABSTRACT

During past one year the following results were obtained: (1) As the host of P. miyazakii, a small snail, Bythinella nipponica akiyoshiensis was first determined by experiments. In addition, this snail was proved to be less susceptible to P. ohirai and non-susceptible to P. westermani. (2) The brown rat, Rattus norvegicus and the wild boar, Sus scrofa leucomystax were revealed to be the final host of P. miyazakii. (3) P. sadoensis was found as a new lung fluke on Sado Island, Niigata prefecture, which was proved to be able to mature in the albino rat, Rattus norvegicus, R. rattus, Mus molossinus and the dog. (4) Tricula minima was decided as the snail host of P. sadoensis. Its cercaria was first studied under the scanning electron microscope. (5) In Thailand, P. harinasutai was discovered as a new lung fluke, the metacercaria of which was abundantly parasitic in a crab, Potamon smithianus. Its egg was similar to that of P. westermani in morphology. (6) In Malaysia, two kinds of crab, Parathelphusa maculata and Potamicus cognatus were added to the list of the crab host for P. westermani. (7) In Indonesia, India and USSR, the existence of P. westermani was re-confirmed. (8) In Mexico, two species of Paragonimus were recognized, and one was most probably a new species and the other was similar to the new fluke found in Colombia.

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Section I

INTRODUCTION

The lung fluke, Paragonimus is one of harmful parasites, causing paragonimiasis among man and animals, but there are many confusions and unsettled questions in taxonomy and ecology. In Japan, for example, P. westermani (Kerbert, 1878) had long been believed to be the only species until 1939, when the author found the second species, P. ohirai Miyazaki, 1939 in Kyushu. Then, he first recognized another two species, P. iloktsuenensis Chen, 1940 and P. kellicotti Ward, 1908 also in Kyushu. But, the latter species was later proved to be a new species and named P. miyazakii by Kamo et al. (1961). Most recently the fifth species, P. sadoensis was found on Sado Island, Niigata prefecture by Miyazaki et al. (1968).

Outside Japan, on the other hand, the author is carrying on joint works on Paragonimus in foreign countries. In Taiwan, Miyazaki and Chiu (1962) first recognized P. iloktsuenensis; in the Philippines, Sumatra and Ceylon the author confirmed P. westermani, and approved the validity of P. compactus (Cobbold, 1859) in Ceylon. In Malaysia, Lee and Miyazaki (1965) confirmed P. westermani by finding its metacercaria in a crab, Potamicus johorensis for the first time. In Thailand, P. westermani was known as the only species. In 1965, Miyazaki and Wykoff discovered the second species, P. siamensis in North-east Thailand; then Miyazaki and Vajrasthira (1967 a) first recognized P. heterotremus Chen et Hsia, 1964 in the endemic area of human paragonimiasis near Bangkok. In addition, Miyazaki and Harinasuta (1966) demonstrated two young worms of the same species from a boy in the same area, and revealed for the first time the medical importance of this lung fluke. Miyazaki and Vajrasthira (1967 b) found the fourth species in the same endemic area, and named P. bangkokensis; then the same authors (1967 c) first recognized P. macrorchis Chen, 1962 in the same area as the fifth species. Thus, the member of Paragonimus in Thailand has rapidly increased, and a fresh water crab, Potamon smithianus was proved to be the most significant vector for human paragonimiasis.

In America, on the contrary, only two species are known: P. rudis (Diesing, 1850) was reported in South and Central America and P. kellicotti was in North America. Besides, it is unknown yet whether the two are different or not. Miyazaki (1964) and Ishii (1966) re-described the metacercaria and the adult worm of P. kellicotti from a new point of view. Mazzotti and Miyazaki (1965) found adult lung flukes from an opossum for the first time in Mexico. Human paragonimiasis is occurring in some parts of Central and South America, but the species of causative agent for the disease is unknown yet.

Section II

MATERIALS AND METHODS

For taxonomy of the genus Paragonimus, both adults and larvae, especially metacercariae must be exactly investigated.

1. Collection of adults. In Japan, wild boars, weasels and rats were examined for natural infection with the lung fluke. Outside Japan, tigers, wild cats, civet cats, wild boars, mongooses, bandicoots and rats were investigated. On the other hand, dogs, cats, albino rats and brown rats were experimentally fed with metacercariae of the lung fluke in order to get adult worms. Adults obtained were flattened in alcohol, stained and mounted in balsam, and morphologically studied in detail. In taxonomy of adult worms the following items were studied: 1) Arrangement of cuticular spines (single or grouped), 2) Branching and size of the ovary and testes, 3) Size ratio of the oral and the ventral sucker, 4) Size and shape of uterine eggs and thickness of egg-shell. Generally speaking, cuticular spines are inclined to split in case of old worms, especially on lateral and posterior part of the body surface, but they remain unchanged on anterior part of the body. Accordingly, the author examined the spines on antero-central part of the body for species differentiation; particularly in mounted specimens this part is most easily and clearly examined through a microscope.

2. Collection of metacercariae. Morphology of metacercariae is a good criterion for species differentiation, particularly fresh ones being much more useful for this purpose than preserved ones. Accordingly, fresh-water crabs such as Potamon dehaani and Eriocheir japonicus in Japan, Potamon smithianus in Thailand and Parathelphusa maculata, Potamicus johorensis, P. j. tahanensis, P. cognatus in Malaysia were examined for Paragonimus larvae. Living larvae obtained were partly observed morphologically and partly fed to experimental animals to get adult worms. For taxonomy of the genus, the following items were carefully investigated: 1) Number of cyst (one or two), 2) Shape and size of inner cyst (outer cyst being unsuitable because of its changeability and fragility), 3) Thickness of cyst walls, particularly that of inner cyst wall, 4) State of the larva (highly contracted or extended), 5) Existence of pinkish granules within the larval body, 6) Ratio of the oral and the ventral sucker, 7) Winding and ending of the intestines, and 8) Extent of the excretory bladder (to intestinal bifurcation or to the level of the ventral sucker).

3. Collection and experimental infection of snail hosts. Fresh-water snails such as Bythinella nipponica akiyoshiensis and Tricula minima were collected in mountainous streams and searched

for rediae and cercariae of Paragonimus. On the other hand, these snails were exposed to miracidia of the known species of Paragonimus and then kept in a special room in order to obtain rediae and cercariae. The larvae obtained were carefully studied, especially the cercariae of P. sadoensis were investigated by means of a scanning electron microscope.

Section III

RESULTS

1. In Japan

a) P. miyazakii Kamo, Nishida, Hatsushika et Tomimura, 1961: As the snail host of this fluke, Bythinella nipponica akiyoshiensis was reported without any experimental infection. Therefore, 700 snails were exposed to miracidia of this fluke and kept in an air-conditioned room at 22-24°C. The snails were examined from 24 hours to 80 days after exposure, and 35 (66.0 %) out of 53 snails examined were proved to be positive for Paragonimus larvae. Until 30 days after exposure only sporocysts were recognized in the snail. The first generation rediae were first obtained 35 days after exposure, and the second ones and cercariae were 70 days. The infection rate of the snails after 70 days was 85.7 %, viz. 6 of 7 snails were found to be infected (Hashiguchi and Miyazaki, 1968 a). On the other hand, the same snail host was collected at five mountainous areas in Fukuoka prefecture, and at three areas the snail was proved to be naturally infected with the larval lung fluke, showing wide distribution of P. miyazakii in the prefecture.

In order to clarify the susceptibility of the same snail to P. westermani (Kerbert, 1878), 1,250 snails were exposed to miracidia of this lung fluke. But, all of 984 snails examined were entirely negative for the larvae, showing non-susceptibility of the snail to P. westermani (Hashiguchi and Miyazaki, 1968 b). Furthermore, the same snail was exposed to miracidia of P. ohirai Miyazaki, 1939, and examined for the larval fluke 25 to 215 days after exposure. It was proved that 182 or 76.8 % out of 237 snails examined were positive for the larval fluke, but only a small number of the snails harbored the first generation rediae and other snails had only sporocysts. The second generation rediae and cercariae were not recognized throughout the experiment. This fact showed that P. ohirai could easily invade the snail host but could not mature even over 200 days postinfection (Hashiguchi and Miyazaki, 1968 c).

As the final host of P. miyazakii, weasels, martens, dogs, cats and albino rats were recorded. This time the brown rat, Rattus n. norvegicus was experimentally proved to be a suitable

host of this fluke (Hashiguchi, Takei and Miyazaki, 1968), and the wild boar, Sus scrofa leucomystax was revealed to be a natural host of this fluke.

b) P. sadoensis Miyazaki, Kawashima, Hamajima et Otsuru, 1968*: Kawashima et al. (1967) found a new type of Paragonimus metacercaria from a crab Potamon dehaani on Sado Island, Niigata prefecture, Honshu, which is situated about 50 km to the northwest of Niigata city. According to them, 132 (28.9 %) out of 457 crabs examined were infected with the new metacercaria, but were not infected with larvae of P. westermani and P. miyazakii, which frequently parasitize the crab of the same species in Japan. On the other hand, Hamajima, Kawashima and Miyazaki (1968) reported a small snail, Tricula minima as the first intermediate host of the new fluke. They exposed 45 snails to 25 miracidia each, and proved that 11 (52.3 %) out of 21 snails examined 35 to 106 days after exposure harbored rediae and cercariae of the fluke. Thereafter, 1,337 snails of the same species were examined at the infested locality, of which two (0.15 %) were revealed to be naturally infected with the fluke. Morphologically, the metacercariae were similar to those of P. ohirai, but more spherical than the latter and pinkish granules were hardly recognized in the body of P. sadoensis. The larvae developed to adult worms in albino rats, brown rats (Rattus norvegicus), roof rats (R. rattus), mice (Mus molossinus) and dogs in one month after experimental feeding. Natural infection of the fluke was found in the weasel, Mustela sibirica itatsi. The adult worms were also similar to those of P. ohirai in all respects, except that the body was stout as compared with the latter. Cercariae of the new species were also close to those of P. ohirai, but the flame cell pattern was different from each other. Under scanning electron microscope, the body surface of cercariae of the new species was observed, and cilia-like hairs and minute spines were clearly recognized on the ventro-posterior part and on the whole surface, respectively.

2. Outside Japan

a) In Thailand: The author has been carrying on joint works with staffs of the Bangkok School of Tropical Medicine in the endemic area of human paragonimiasis in Nakorn-nayok province situated about 90 km from Bangkok. They have already identified the following five species in that area: P. westermani, P. siamensis, P. heterotremus, P. bangkokensis, and P. macrorchis. Moreover, the author found another new species in the same endemic area, and proposed the following name for Prof. Chamlong Harinasuta, Dean of the Bangkok School of Tropical Medicine:

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* This new species will appear on the Japanese Journal of Parasitology, Vol. 17, No. 2, April, 1968.

Paragonimus harinasutai Miyazaki et Vajrasthira, 1968**: The metacercaria was found in a crab, Potamon smithianus, the most important vector for human paragonimiasis in Thailand. It was spherical in shape and provided with the outer and the inner cyst, and the latter measured 533 to 666 by 513 to 649 (aver. 601 by 579) μ in diameter, being the biggest metacercaria the author has ever investigated. The larva usually contained a lot of pinkish granules within the body, showing very beautiful appearance. The larva experimentally developed into mature adult in two cats and a dog, but the natural host of the fluke is unknown yet. The adult worm was provided with single spines, a moderately branched ovary and less divided testes. The egg was characteristic to some extent, because the shell of some eggs was gradually thickened at the non-operculated end, just like that of P. westermani. In addition, there are some possibility of human infection with the new species, as the crab host is frequently eaten uncooked by inhabitants of the endemic area. Accordingly, if the egg of the above-mentioned character appears from humans, medical attention should be paid not only to P. westermani, but also to the new species.

b) In Malaysia: As mentioned previously, only Potamicus johorensis was known as the crab host of P. westermani in this country. Miyazaki, Kawashima and Tan (1968) found a kind of Paragonimus metacercaria from another crab, Parathelphusa maculata collected at Ulu Langat near Kuala Lumpur, and brought to Japan in an ice-box. Forty metacercariae were still alive, and ten were fed to each of two albino rats and two domestic cats. Six fully mature adults and two juvenile ones were obtained from one of the cats 330 days after feeding. All worms were identified as P. westermani, and this crab was added to the list of the second intermediate host of this lung fluke. At the beginning of May of this year, the author and the staffs of the George W. Hooper Foundation, University of California Medical Center made a research trip to Baling, Kedah, situated near the northern border of the country. They collected a kind of crab, Potamicus cognatus in a jungle brook near Baling, and found the metacercaria of P. westermani from 27 or 44.2 per cent out of 61 crabs examined. Some larvae were studied morphologically and others were fed to two cats. The animals will be autopsied about one month later. On the other hand, seven civet cat (Paradoxurus hermaphroditus), four wild pig (Sus cristatus), five mongooses (Herpestes auropunctatus), one water rat (Rattus muelleri) and one wild cat were examined for Paragonimus, but all animals were non-infected.

** This new species will soon appear on the Annals of Tropical Medicine and Parasitology, Vol. 62, 1968.

c) In Indonesia: Kwo and Miyazaki (1968) examined the lungs of ten tigers for the lung fluke, of which eight were shot in the Kisaran district, 125 miles to the east of Medan city, North Sumatra, and the other two came from Tandjung Pura jungle, 40 miles to the west of the city. All of these tigers were infected with the lung fluke, and the number of worms per host was 1,596, 870, 776, 552, 452, 330, 208, 182, 74 and 56, totaling 5,096 in all. The worms obtained were carefully studied, and all of them were identified as P. westermani. At the same time, the lungs of 26 wild cats, Felis bengalensis were examined in the suburbs of Medan city, but no lung flukes were found.

d) In India: By the courtesy of Prof. Kr. Suresh Singh of the Postgraduate College of Animal Sciences, Indian Veterinary Research Institute in Izatnagar, the author examined five adult specimens from a tiger and six ones from a wild cat caught in Pantnagar, and identified all of them as P. westermani.

e) In Ryukyus: At the end of May of this year the author collected some fresh-water crabs on Ishigaki-jima, Yaeyama Islands, and among them he found for the first time a kind of crab, Potamon miyazakii. This crab was discovered in the northern part of Taiwan and determined as the second intermediate host of P. iloktsuenensis and P. westermani, but no Paragonimus metacercariae were recognized in Ryukyus.

f) In USSR: By the courtesy of Prof. A. V. Maslov of the Medical Institute of Khabarovsk, the author examined three adult specimens from a tiger, Felis tigris longipilis and 12 ones from a raccoon dog, Nyctereutes procyonoides, and identified all of them as P. westermani.

g) In America: According to a private communication from Dr. M. D. Little of the Tulane University, School of Public Health and Tropical Medicine, USA, he recently found a new species of Paragonimus in Colombia. On the other hand, Miyazaki and Ishii (1968 ?) carefully studied 27 adult specimens of Paragonimus obtained by Dr. L. Mazzotti from two opossums, Didelphis marsupialis captured in Colima, Mexico, and compared them with 47 specimens of P. kellicotti, which were experimentally and naturally obtained in the United States and Canada. The authors obtained the following results: 1) Among the Mexican lung flukes, two species (A and B) were clearly distinguished from each other. Species A was represented by 26 specimens, species B by only one. 2) Species A was covered with single spines like P. kellicotti, but its ovary was generally more delicately branched than the latter. 3) In species A the oral sucker was larger than the ventral in 17 of 23 specimens examined, while in P. kellicotti only eight of 28 specimens examined had a larger oral sucker than ventral sucker. 4) The egg was significantly larger, and the egg shell thicker in P. kellicotti than in species A. 5) Species B was also provided with single integumentary spines, but with a far simpler branched ovary and testes, being easily differentiated from the above-mentioned two species. Its eggs were apparently smaller and egg

shells thinner than those of P. kellicotti. Ultimately, two species of Paragonimus occur in Mexico at present, and they are certainly different from P. kellicotti. Speices A is most probably a new species, and species B is closest to a new lung fluke recently found in Colombia by Dr. Little. By the courtesy of Dr. V. E. Thatcher the authors examined ten adult specimens from Panama, of which nine were probably species A and the remaining one species B of the Mexican lung flukes. Dr. M. Martínez Báez kindly lent to the authors many sections of the lung tissue excised from a Mexican male patient of 35 years old, which contained a lot of Paragonimus eggs. It was proved that these eggs belonged neither to P. kellicotti nor to P. westermani. It seems that they belong to the above-mentioned species A of the Mexican lung flukes.

Section IV

DISCUSSION AND CONCLUSION

By the experimental studies in the author's laboratory, the snail, Bythinella nipponica akiyoshiensis was first determined as the host of P. miyazakii. It was also revealed experimentally that this snail was less susceptible to P. ohirai and non-susceptible to P. westermani. In the vicinity of Fukuoka city, on the other hand, this snail was much more widely distributed and more densely infected with P. miyazakii than the author had imagined. This fact is very convenient to study this lung fluke in the author's laboratory. In Japan, a new species, P. sadoensis is going to appear in literature as the fifth species of the genus, and as the snail host of this fluke Tricula minima was definitely decided in the author's laboratory. This snail lives in quite different environment from Assiminea parasitologica, the snail host of P. ohirai. Furthermore, the crab hosts of both lung flukes live also in quite different environment from each other. In addition to morphological differences between P. sadoensis and P. ohirai, these ecological dissimilarities of the intermediate hosts of the two lung flukes were the reason why the author regarded P. sadoensis as a valid species. The susceptibility of Tricula minima to other lung flukes such as P. westermani and P. ohirai is now under observation. The cercaria of P. sadoensis was first studied by means of the scanning electron microscope. This method seems to be very useful for differential morphology of Paragonimus cercariae.

Among foreign countries, Thailand is most interesting at present: there are six species, including three new species, in one endemic area near Bangkok city, of which five species were discovered or first recognized by the author. The newest species, P. harinasutai is going to appear in literature as the sixth species in Thailand. The only species which was proved to infect

humans in this country is P. heterotremus, but there are some possibility of human infection with P. bangkokensis and P. harinasutai, because the three flukes are commonly parasitize the crab, Potamon smithianus that is frequently eaten uncooked by inhabitants. Malaysia is also interesting for Paragonimus study. The author confirmed two kinds of crabs, Parathelphusa maculata and Potamicus cognatus, as the host of P. westermani. He is searching for another crab host, the snail host and another kind of the lung fluke. From medical point of view, Nepal is significant, because many human paragonimiasis are occurring in Tansen district (Iwamura, 1965), but no worms have been confirmed in this country. Nepal needs an extensive parasitological study on the causative agent. There are 17 species of Paragonimus in Asia, of which P. westermani is most widely distributed in Indonesia, Malaysia, Thailand, India, Ceylon, Philippines, China, Taiwan, Ryukyus, Japan, Korea, and USSR, which were confirmed by the author. On the contrary, the author does not think that P. westermani occurs in America, although some people reported this lung fluke in the United States.

In America, on the other hand, only two species were reported, viz. P. rudis and P. kellicotti. But, the former is regarded as Nomen dubium, since its characteristic features are unknown as yet. Meanwhile, two new species of Paragonimus are going to appear in literature from Colombia and Mexico. Both or one of them may be the causative agent of the human paragonimiasis in South and Central America. Taxonomical and ecological studies on Paragonimus are quite necessary in that area.

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13. ABSTRACT

During past one year the following results were obtained: (1) As the host of P. miyazakii, a small snail, Bythinella nipponica akiyoshiensis was first determined by experiments. In addition, this snail was proved to be less susceptible to P. ohirai and non-susceptible to P. westermani. (2) The brown rat, Rattus norvegicus and the wild boar, Sus scrofa leucomystax were revealed to be the final host of P. miyazakii. (3) P. sadoensis was found as a new lung fluke on Sado Island, Niigata prefecture, which was proved to be able to mature in the albino rat, Rattus norvegicus R. rattus, Mus molossinus and the dog. (4) Tricula minima was decided as the snail host of P. sadoensis. Its cercaria was first studied under the scanning electron microscope. (5) In Thailand, P. harinasutai was discovered as a new lung fluke, the metacercaria of which was abundantly parasitic in a crab, Potamon smithianus. Its egg was similar to that of P. westermani in morphology. (6) In Malaysia, two kinds of crab, Parathelphusa maculata and Potamicus cognatus were added to the list of the crab host for P. westermani. (7) In Indonesia, India and USSR, the existence of P. westermani was re-confirmed. (8) In Mexico, two species of Paragonimus were recognized, and one was most probably a new species and the other was similar to the new fluke found in Colombia. (Author)

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14. KEY WORDS	LINK A		LINK B		LINK C	
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Identification						
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