

Identification of *Edesonfilaria malayensis* from Cynomolgus Monkeys (*Macaca fascicularis*), and Description of the Microfilariae

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Edesonfilaria malayensis Yeh, 1960 (ONCHOCERCIDAE: DIROFILARIINAЕ) is a filarial parasite of cynomolgus monkeys (*Macaca fascicularis*) from Malaya and Thailand (Yamaguti and Hayama, 1961; Yeh, 1960). Yeh (1960) was the first to describe the major morphological characteristics of the worm, and Yamaguti and Hayama (1961) presented more detailed features; subsequent parasitological studies have been rare (Chitwood, 1970; Reardon and Rininger, 1968). Consequently, although cynomolgus monkeys have been used in a variety of biomedical studies in recent years, the existence of *E. malayensis* has been almost unknown, and in particular, the morphological features of the microfilaria have never been described. Recently, we have identified filarial parasites, from cynomolgus monkeys imported from Indonesia, as *E. malayensis* based on their structure. Here we describe for the first time the morphology of the microfilariae stained with Giemsa solution; comparison is made with the microfilariae of *Macacanema formosana* Schad and Anderson, 1963 (Schad and Anderson, 1963), because we observed that *E. malayensis*

microfilariae are similar to those of *M. formosana* in body length.

Materials and Methods

Animals

Thirty six adult cynomolgus monkeys (18 males and 18 females), caught in a forest in Java, Indonesia, and imported into Japan from April, 1980 through April, 1981, were examined for parasites between December, 1981 and February, 1982. In a 4-month quarantine in Japan, tests for *Entamoeba histolytica*, *Shigella* spp., *Salmonella*, spp., helminth eggs in feces, and tuberculosis by tuberculin test were negative.

Parasitological examinations

One female and three intact adult male worms from three different monkeys were fixed in 3% formaline-saline solution and cleared in lactophenol solution to prepare them for a *camera lucida* drawing and measurement. A drop of fresh blood, collected between 9:00 am and 11:00 am, from the femoral vein of the four infected monkeys was smeared on glass slides, dried at room temperature, fixed with methyl alcohol, and stained with Giemsa solution. Thirty six microfilariae from seven stain-

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ed preparations were photographed with color film, and morphometric measurements were made from 1:7600-magnified colored prints using Morphometry® (type MOP-AM-03, Contron Co.).

Results

Morphology of Adult Worms

Adult filarial parasites were seen in six of the 18 female monkeys, but none of the 18 males (16.7% positive). Although slight anemia and eosinophilia were seen in the infected animals by hematological tests, no clinical abnormalities were observed. The parasites were located in the connective tissue under the serous membrane around the kidneys, the cardiac orifice of the stomach, the pancreas, the thymus, the radix of mesentery, or the mediastinal pleura; worms were not found free in body cavities, nor in the organ parenchyma.

Male: The following measurements were based on three intact male worms. The specimens were filiform, 127–135 mm in length by 0.27–0.30 mm in width at the nerve ring, 0.50–0.62 mm at the posterior extremity of the esophagus (maximum width), and 0.10–0.12 mm at the cloaca, with three loose spiral turns at the tapering posterior extremity. The mouth was without lips, and the head was blunt. The cuticula was finely striated just under the smooth surface. The nerve ring was situated 0.18–0.22 mm from the head end. The esophagus was divided into the anterior and the posterior portions, which were 0.45–0.63 mm and 41–53 mm long, respectively. The anterior esophagus consisted of a thick wall of longitudinal muscle fibers and a cuticular lining. The anterior part of the anterior esophagus was free, but the remaining part attached to the posterior esophagus; the parietal muscle fibers of the anterior esophagus continued caudad along the posterior esophagus. The posterior esophagus was a

simple tube with a thin membranous wall; it contained granular ingesta. The tail was blunt and only 0.05–0.07 mm long. There was a pair of well developed, symmetrically arranged, 0.18–0.26 mm long, caudal alae. There were about 30 pairs of caudal papillae. The posterior nine pairs were pedunculate and close to each other, whereas the other papillae were sessile and gradually became more widely spaced anteriorly; the anterior most papilla was about 11.5–12.7 mm from the tip of the tail. The wedge-shaped short right spicule was 0.11–0.16 mm long; an exceptionally long, narrow left spicule measured 5.5–8.4 mm. The slender tubular testis arose at the point where the esophagus jointed the intestine (Figs. 1, 2, 3).

Female: The following measurements were based on one intact specimen. It was longer than the male specimens: 305 mm in length and 1.0 mm in width at the posterior extremity of the esophagus (maximum width). Two close-parallel columns of brown pigment granules were observed in each lateral field of the body. The nerve ring was located 0.2 mm from the head extremity. The esophagus, the posterior portion of which commenced 0.26 mm behind the head end, was 92 mm long. The short rounded, slightly curved ventrad tail was 0.06 mm long. The vulva was situated 0.54 mm from the cephalic end, 0.24 mm behind the anterior end of the posterior esophagus. The vagina, 4.0 mm long, was covered with outer longitudinal muscle fibers with twisted inner cuticular nodules, which was then led into the unpaired uterus. The uterus occupied the greater part of body length along with the esophagus and intestine (Figs. 4, 5).

All these features of the adult worms were identical with those of *E. malayensis* reported by Yeh (1960), and Yamaguti and Hayama (1961).

Morphology of the Microfilariae

Sixteen unstained microfilariae in the uterus had no sheath, and measured 124 μm in average length. Microfilariae were detected in the peripheral blood of five of six infested female monkeys. Absolute and relative measurements of 36 Giemsa stained microfilariae are given in Table 1 together with those of *Macacanema formosana* (Schad and Anderson, 1963). Microfilariae had no sheath, and a fine striation on the cuticula. They were narrow sinuous parasites, that were frequently crooked L or V shape near the center of the body. A swelling at the mid point between the head and nerve ring, the point of maximum body width, was characteristic. The body gradually narrowed to the last tail nucleus, beyond which it tapered rapidly as a transparent thin tube. The nuclei were stained purplish-red by Giemsa, and were closely packed and overlapping. The cephalic tip was well rounded. The nerve ring, excretory pore, and anal pore were easily seen. The excretory cell, situated close to the excretory pore, and the first genital cell were often difficult to distinguish in Giemsa-stained preparations. Three-parallel rows of nuclei between the head tip and the excretory pore, two-parallel rows of nuclei between the excretory pore and anal pore, and a single row of nuclei in the tail were counted. Two-parallel nuclei at the cephalic portion arranged like lips stood out clearly in about 80% of microfilariae. The tail, with a pointed end, was devoid of nuclei (Fig. 6).

Discussion

E. malayensis is the sole species of the genus *Edesonfilaria* (Anderson and Bain, 1979; Yamaguti and Hayama, 1961). According to Flynn (1973), the following five species of filarial parasites infect macaque monkeys: *E. malayensis*, *M. formosana*, *Brugia malayi*, *Dirofilaria magnilarvatum*,

and *Dipetalonema digitatum*. *E. malayensis* we found are differentiated from *M. formosana* (Schad and Anderson, 1963), the most closely related worm from the Formosan rhesus monkeys (*Macaca cyclopsis*) of Taiwan (Anderson and Bain, 1979), by having (1) a simpler and longer left spicule, (2) a convoluted vagina, and (2) symmetrically arranged caudal papillae in the male. The remaining three species are readily distinguished from *E. malayensis* by dimensions and morphology (Faust *et al.*, 1975; Price, 1959; Webber and Hawking, 1955).

E. malayensis has been found in monkeys from Asian countries (Reardon and Rininger, 1968), Malaya and Thailand (Yeh, 1960), and Thailand (Yamaguti and Hayama, 1961). We are the first to report the parasite in Indonesian cynomolgus monkeys. The prevalence of infestations of *E. malayensis* is still obscure; Reardon and Rininger (1968) found the worm in only one of 303 (0.3%) cynomolgus monkeys from Asian countries. On the contrary, we detected the parasites in six of 36 (16.7%) monkeys. Although wider surveys are needed, this high prevalence may reflect ecological changes, such as large numbers of development of an unknown hemophagous vector of this parasite in the areas where the monkeys were captured.

Since the brief description of the microfilariae in the uterus of the adult female *E. malayensis* by Yamaguti and Hayama (1961), there have been no subsequent reports. Our detailed examinations of the microfilariae confirm the findings of Yamaguti and Hayama, and we have added to their description by presenting a Giemsa-stained drawing and close measurements. Although *E. malayensis* microfilariae are similar to those of *M. formosana* (Schad and Anderson, 1963) in body length, they can be distinguished by two features; (1) the most outstanding character of *E. malayensis* is the presence of a two-parallel nuclei arranged like lips at the cephalic

Table 1 Measurements of microfilariae of *Edesonfilaria malayensis* in comparison with those of *Macacanema formosana*

| | <i>E. malayensis</i> (present authors) | <i>M. formosana</i> (Schad and Anderson, 1963) |
|--|---|---|
| No. measurements | 36 | —* |
| Body length (μm) | 99.7 (85.8—123.4) † | 96 (85—102) |
| Maximum body width (μm) | 4.1 (3.5—5.2) | 3.0 |
| Body width at excretory pore (μm) | 3.2 (2.5—4.1) | — |
| Body width at anal pore (μm) | 2.5 (1.6—3.5) | — |
| Fixed points: ‡ | | |
| cephalic space | 1.1 (0.3—1.8) | 3 (2—4) |
| nerve ring | 19.6 (16.2—22.4) | 22 (20—23) |
| excretory pore | 29.0 (24.9—31.8) | 32 (31—34) |
| excretory cell | 31.9 (27.9—35.2) | 36 (33—38) |
| first genital cell | 62.0 (56.1—67.2) | 67 (65—69) |
| anal pore | 77.0 (74.9—78.9) | 81 (79—82) |
| last tail nucleus | 98.9 (96.7—99.7) | — |

*: Not described

†: Mean (range)

‡: Percent distances from anterior end

end, and (2) *E. malayensis* possesses a swelling on the center between the head and nerve ring, the point of maximum width, which is obvious on a rapid survey of a slide. There are other important differences between the two microfilariae in distance from the anterior tips of the microfilariae to internal anatomic markers relative to total body length, including differences in position of such key structures as the cephalic space, nerve ring, excretory pore, excretory cell, first genital cell, anal pore, and the last tail nucleus (Table 1). All these distances of *E. malayensis* microfilariae are smaller than those of *M. formosana* (Schad and Anderson, 1963). We are able to readily separate *E. malayensis* microfilariae from those of *M. formosana* by these differences, and by considering host species and differences in geographic distribution. The precise identification of a microfilaria from an animal is usually of great importance for a clinical diagnosis in veterinary parasitology. The morphological observations presented here, may contribute to subsequent studies of *E. malayensis*.

Summary

Filarial parasites found in six of 36 adult cynomolgus monkeys (*Macaca fascicularis*) imported from Indonesia, were identified and the microfilariae are described. One female and three intact adult male worms were taken from the connective tissues of the body cavities of three different monkeys. The male worms were 127–135 mm long by 0.5–0.62 mm wide; the female worm was 305 mm long by 1.0 mm wide. The worms had the following morphological characters: the esophagus consisting of a muscular anterior portion and an unusually long, simple, tubular, glandular posterior portion; the well-developed caudal alae with nine pairs of large papillae in males; and a short right spicule and an exceptionally long left spicule in males. These features were identical with those of *Edesonfilaria malayensis* Yeh, 1960. Microfilariae in the peripheral blood of four infested monkeys had no sheath, and averaged 99.7 μm in length by 4.1 μm in maximum width, which occurred at a peculiar swelling between the head and nerve ring. Two-parallel nuclei arranged like lips at

the head portion were noticeable as well. Morphological differential points between the microfilariae of *E. malayensis* and those of *Macacacnema formosana* are discussed.

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References

- 1) Anderson, R. C. and Bain, O. (1979): Subfamily Dirofilarinae. In CIH Key to the Nematode Parasites of Vertebrates, Part 3, No. 3, ed. by R. C. Anderson, A. G. Chabaud, S. Willmott, Commonwealth Agricultural Bureau, Farnham Royal, Bucks, England, 84-89.
- 2) Chitwood, M. (1970): Comparative relationships of some parasites of man and old and new world subhuman primates. Lab. Animal Care, 20, 389-394.
- 3) Faust, E. C., Beaver, P. C. and Jung, R. C. (1975): Animal Agents and Vectors of Human Disease, 4th ed., Lea and Febiger, Philadelphia, 292-324.
- 4) Flynn, R. J. (1973): Parasites of Laboratory Animals, Iowa State University Press, Ames Iowa, 203-320.
- 5) Price, D. L. (1959): *Dirofilaria magnilarvatum* n. sp. (NEMATODA: FILARIOIDEA) from *Macaca irus* Cuvier. I. Description of the adult filarial worms. J. Parasitol., 45, 499-504.
- 6) Reardon, L. V. and Rininger, B. F. (1968): A survey of parasites in laboratory primates. Lab. Animal Care, 18, 577-580.
- 7) Schad, G. A. and Anderson, R. C. (1963): *Macacacnema formosana* n. g., n. sp. (ONCHOCERCIDAE: DIROFILARIINAE) from *Macaca cyclopsis* of Formosa. Can. J. Zool., 41, 797-800.
- 8) Webber, W. A. F. and Hawking, F. (1955): The filarial worms *Dipetalonema digitatum* and *Dipetalonema gracile* in monkeys. Parasitology, 45, 401-408.
- 9) Yamaguti, S. and Hayama, S. (1961): A re-description of *Edesonfilaria malayensis* Yeh, 1960, with remarks on its systematic position. Proc. Helminthol. Soc. Wash., D. C., 28, 83-86.
- 10) Yeh, L. S. (1960): On a new filarioid worm, *Edesonfilaria malayensis* gen. et sp. nov. from the long-tailed macaque (*Macaca irus*). J. Helminthol., 34, 125-128.

カニクイザルから検出された *Edesonfilaria malayensis* 成虫の同定 およびそのマイクロフィラリアの記載

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インドネシア産カニクイザル (*Macaca fascicularis*) 36頭中6頭 (16.7%) の胸腔および腹腔の結合組織内にフィラリア成虫の寄生を認めた。採取した雄3匹および雌1匹の成虫を、その形態学的特徴から *Edesonfilaria malayensis* Yeh, 1960, と同定し、さらに感染サル末梢血ギムザ染色標本のマイクロフィラリアの形態

を記載した。マイクロフィラリアは無鞘で、36匹の平均体長は 99.7 μm 、最大体幅 (4.1 μm) は頭端から神経輪のほぼ中央にあり、特徴的に膨大していた。また、頭域における口唇様に配列した2個の核が特徴的であった。

Explanation of Figures

Figs. 1-6 *Camera lucida* drawings of *Edesonfilaria malayensis* and its microfilaria from cynomolgus monkey.

- Fig. 1 Anterior extremity of male, ventral view.
 Fig. 2 Esophago-intestinal region of male, lateral view.
 Fig. 3 Posterior extremity of male, ventral view.
 Fig. 4 Anterior extremity of female, lateral view.
 Fig. 5 Posterior extremity of female, lateral view.
 Fig. 6 Microfilaria.

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|-------------------------|-------------------------|
| A: Anus | LTN: Last tail nucleus |
| AE: Anterior esophagus | NR: Nerve ring |
| AP: Anal pore | O: Ovary |
| CA: Caudal alae | P: Papilla |
| CL: Cloaca | PE: Posterior esophagus |
| EC: Excretory cell | RS: Right spicule |
| EP: Excretory pore | T: Testis |
| PGC: First genital cell | V: Vulva |
| I: Intestine | VA: Vagina |
| LS: Left spicule | |

