

Morphological Studies of the Adult Female *Loa loa* (Nematoda : Filarioidea) and a Review of the Literature on Imported Human Loiasis in Japan

Hiroshi USHIROGAWA, Tetsuya OKINO, Ryo HATSUSHIKA
and Akio TABUCHI*

Department of Parasitology, Kawasaki Medical School,
Kurashiki 701-0192, Japan

*Department of Ophthalmology, Kawasaki Medical School,
Kurashiki 701-0192, Japan

Accepted for publication on September 16, 2003

ABSTRACT. The morphological features of the adult female *Loa loa* worm obtained from the right eye of a Nigerian male and a review of the literature on five cases of imported human loiasis in Japan are described. The morphological characteristics of the main portions of the adult female worm were as follows: 1) The worm body was threadlike, cylindrical in form and creamy-white in color, measuring about 5.0 cm in length and 0.6 mm in maximum diameter. 2) The body was widest at the base of the esophagus and tapered gradually to a lesser diameter posteriorly. 3) The body wall was approximately 108 μm thick, and numerous small bosses were recognized on the cuticular surface. 4) The cuticular bosses were round, wart-shaped structures that were about 20 μm in maximum diameter and 17.5 μm in height. 5) The bosses on the body surface began at a level posterior to the base of the esophagus and extended to the end of the tail. 6) The nerve ring was recognized about 0.2 mm from the cephalic end of the worm. 7) The esophagus was about 0.1 mm in length and 90 μm in maximum diameter, and the intestinal tube was about 200 μm in maximum diameter. 8) The vulva was situated posterior to the base of the esophagus in the ventral midline. 9) The uterine tube was about 170 μm in maximum diameter and contained numerous microfilariae. 10) The anal opening was located in the ventral region about 0.2 mm from the tip of the tail.

Detailed morphological informations about the adult female *Loa loa* worm from human body in Japan are given in this paper for an original report.

Key words : *Loa loa* — adult female — morphology — human loiasis — Nematoda

The human filarial parasite, *Loa loa* (Cobbold, 1864) Castellani *et* Chalmers, 1913 is distributed in the rain forests of West and Central Africa, and is commonly called the eye worm or the African eye worm. The definitive hosts of this parasite are man and African arboreal primates (drills, gorillas and monkeys). The main insect vectors are certain species of day-biting dipterous flies belonging to the genus *Chrysops* which live in

the rain forest and swamp forest areas of Africa. Only the adult female flies suck the blood of the definitive hosts.

The *Chrysops* species in the rain forests may derive their infection from primates and then transmit *L. loa* to other wild animals or man invading the forest.¹⁾ In Central Africa, the incidence of human infection with *L. loa* has been recorded to be approximately 3 million people²⁾ or as high as 50 to 90% among inhabitants.³⁾ The *L. loa* worms commonly migrate rapidly through the body and may be seen in the sub-conjunctival tissue of the eye or in thin-skinned areas of the infected patients.⁴⁾ They usually do not cause symptoms, but there may be very high eosinophil levels in the peripheral blood. The length of life of the adult worm in man has been variously reported to be from 4 to 17 years.¹⁾

Humans are infected when they are bitten by the infecting *Chrysops* species. The infective larvae enter the skin via the bite wound of the definitive host and migrate in the subcutaneous and deep connective tissues, and occasionally in the bulbar conjunctivae. After mating, the adult female worms begin to produce large numbers of sheathed microfilariae, which circulate in the peripheral blood of the host with strict diurnal periodicity.⁵⁾ The most common pathologic sequelae associated with *L. loa* infections are migratory pruritic swellings on the extremities or in the region of the orbit, so-called fugitive or Calabar swellings (angioedema).^{2,6)}

In Japan, morphological study of the *L. loa* worm is practically nonexistent in the literature, because only five cases of imported human loiasis have been reported to date. In the present paper, the morphological features of the adult female *L. loa* are briefly described together with some photomicrographs. In addition, a bibliographical review of the literature on imported human loiasis recorded in Japan is given.

MATERIALS AND METHODS

The adult female *Loa loa* worm which was studied was obtained from a conjunctival tumor of the right eye of a 29-year-old Nigerian male residing in Himeji City, Hyogo Prefecture, Japan. The worm removed from the patient had been preserved in 70% ethyl alcohol for three years, after a case report was finished by Yasuki *et al* (1998).⁷⁾ For new morphological observations of the worm, the specimen was cleared with lactophenol solution for several days and studied in detail with a light microscope.

RESULTS

The adult female *L. loa* was threadlike, cylindrical in form and creamy-white in color when fixed, and bluntly tapered at both ends. The worm body measured about 5.0 cm in length and 0.6 mm in maximum diameter with a conical tail (Fig 1). The body was widest at the base of the esophagus and tapered gradually to a lesser diameter posteriorly. The body wall (including translucent cuticular, hypodermal and muscle layers) was approximately 108 μ m thick.

On external observation of the worm, the tough cuticle appeared smooth and numerous small bosses were clearly recognized on the cuticular surface of the entire body. The cuticular bosses were round, wart-shaped structures

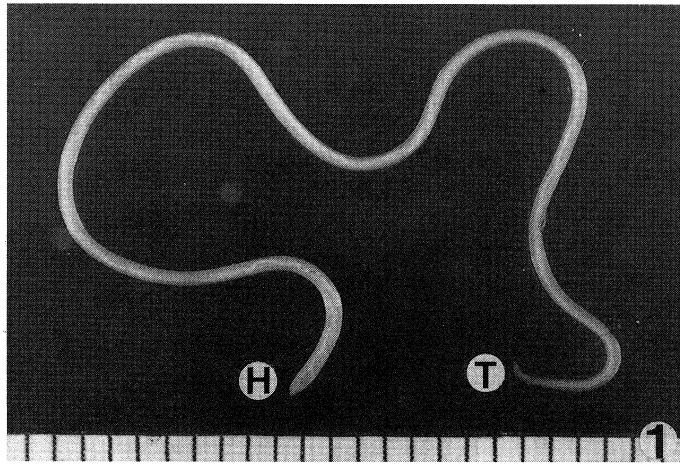


Fig 1. Whole body of a female *Loa loa* worm removed from a conjuncival tumor of the right eye of a Nigerian male, fixed specimen (Scale=1 mm)
H: head, T: tail

that were about $20\ \mu\text{m}$ in maximum diameter and $17.5\ \mu\text{m}$ in height. These bosses on the body surface began at a level posterior to the base of the esophagus, and irregularly extended to the end of the tail (Fig 2c-f).

The anterior end of the worm was bluntly rounded, having a small simple mouth without a buccal cavity and definite lips. The nerve ring was recognized about $0.2\ \text{mm}$ from the cephalic end of the worm (Fig 2a). The excretory pore, situated on the anterior portion of the worm, was very difficult to discern. The alimentary canal commenced at a funnel-shaped mouth as a slender straight esophagus, going on to an intestine and a short attenuated rectum. The esophagus was about $1.0\ \text{mm}$ in length, 1.7% of body length, and $90\ \mu\text{m}$ in base diameter. A portion of the esophageal-intestinal junction could not be illustrated clearly by a photomicrograph because the jointed portion was overlapped by the uterine tubes. The intestinal tube was a thin-walled structure about $200\ \mu\text{m}$ in maximum diameter throughout its length (Fig 2c-f).

The vulva was recognized somewhat posterior to the base of the esophagus in the ventral midline, about $2.0\ \text{mm}$ from the cephalic end of the worm (Fig 2b). The vagina was short, extended posteriorly without looping, and was joined to the bifurcated uterine tubes. Two sets of female reproductive organs (viz., uteri, oviducts and ovaries) were looped or coiled, and these genital organs were tapered posteriorly and filled the body cavity of the worm. The uterine tubes were thin-walled structures, measuring about $170\ \mu\text{m}$ in maximum diameter and contained numerous coiled microfilariae (Fig 3a, b).

The posterior end of the worm was very rounded, and a pair of small lateral papillae was recognized at the tip of the tail (Fig 2f). The anal region was slightly elevated from the cuticular surface, and the anal orifice was located in the ventral region about $0.2\ \text{mm}$ from the tip of the tail. The distance between the anal orifice and the caudal end was approximately 0.4% of body length.

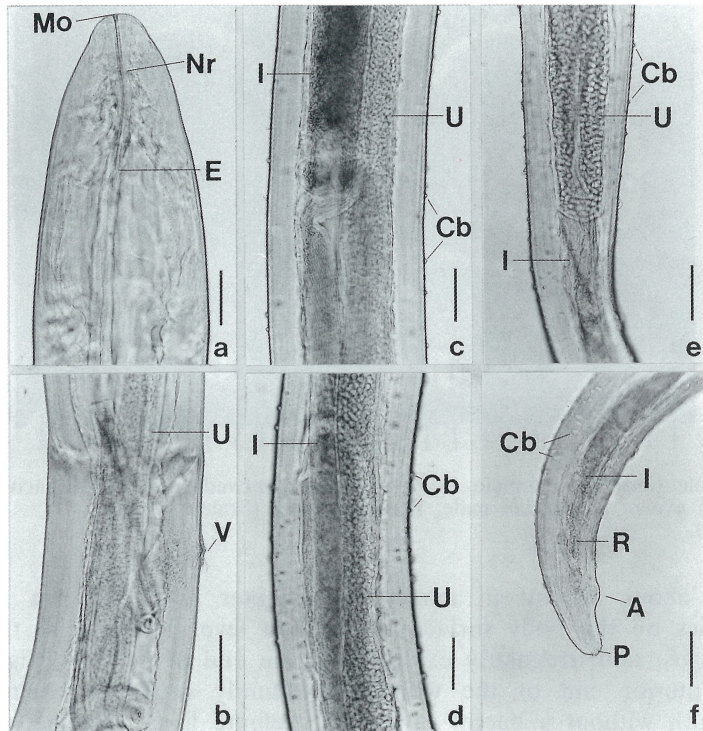


Fig 2. General features of different regions of the worm—(a) anterior portion, (b) about 2.0 mm (lateral view), (c) about 3.0 mm from anterior end and (d) about 4.5 mm, (e) about 1.2 mm from posterior end, and (f) posterior portion (lateral view), respectively, — of the worm (Scale bars=0.2 mm)
 A: anus, Cb: cutaneous bosses, E: esophagus, I: intestine, Mo: mouth opening, Nr: nerve ring, P: papillae, R: rectum, U: uterus, V: vulva

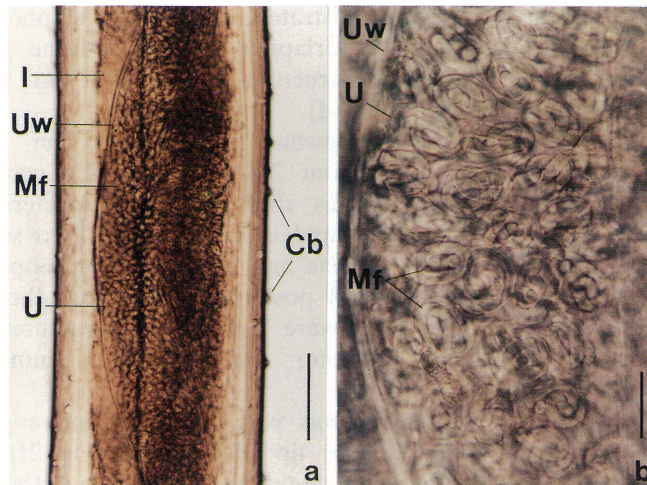


Fig 3. Portion of midbody of the worm (a) (Scale bar = 0.2 mm), and high-magnification of Fig 3a, showing numerous coiled microfilariae in the uterus (b) (Scale bar=0.05 mm)
 Cb: cutaneous bosses, I: intestine, Mf: microfilariae, U: uterus, Uw: uterine wall

DISCUSSION AND HUMAN LOIASIS IN JAPAN

As mentioned in the introduction, no other detailed study has been reported on the morphological informations of the adult *L. loa* worm from human body in Japan.

The morphological features of the adult worms of *L. loa* have been reported on by two investigators.^{8,9)} Looss (1904)⁸⁾ schematically illustrated the morphological characteristics of the male and female worms of *L. loa*, and this is still evaluated by many workers as a valuable drawing. He described the female and male adult worms to be 5.2 cm in length and 0.5 mm in maximum diameter and 3.3 cm in length and 0.4 mm in maximum diameter, respectively. In the female worm, the vulva and excretory pore were respectively situated about 2.4 mm and 0.75 mm from the anterior end. The esophagus of the female worm was about 1.1 mm in length and 70 μ m in diameter. The cuticular bosses on the body surface of the female worm were distributed between the area posterior to the vulva and the caudal end of the worm. Thus the morphological features of the present worm closely resembled those of the adult female worms of *L. loa* described by Looss (1904).⁸⁾

Eberhard and Orihel (1981),⁹⁾ on the other hand, reported on the morphological characteristics of the larval and adult stages of *L. loa* which they obtained from experimentally infected primate hosts (patas monkeys or baboons). They stated that the mature female and male worms were respectively 6.4 cm in maximum length and 0.51 mm in average diameter and 2.7 cm in maximum length and 0.34 mm in average diameter. The average thickness of the body walls in the mature female worms was 105 μ m and that in the males was 85 μ m. In female worms, the cuticular bosses began at a level posterior to the base of the esophagus, and extended to the end of the tail, whereas in male worms they terminated 1 to 2 mm from the tip of the tail. The cuticular bosses were round, dome-shaped structures that were 6 to 8 μ m high by 20 to 38 μ m in diameter in the females and 3 to 5 μ m high by 15 to 20 μ m in diameter in the males. They were more numerous in females and often appeared clumped in small groups, whereas in males they were relatively scant. The intestine of the female worms was a thin-walled tube about 50 μ m in diameter throughout its length. The morphological features of the female worm in the present study were consistent with those of the mature female worms of *L. loa* reported by Eberhard and Orihel (1981),⁹⁾ except for the height of the cuticular bosses and the diameter of the intestinal tube.

As mentioned above, human loiasis is found only in Africa. It occurs in tropical Africa, from Senegal in the west to Sudan and Uganda in the east and to Angola in the south,³⁾ and the disease is most prevalent in Nigeria, the Cameroon Republic, and Zaire.⁵⁾ The characteristic symptoms of the patient infected with *L. loa* are referred to as fugitive or Calabar swellings. The Calabar swellings may be found on the extremities of the infected patient, occasionally in the bulbar conjunctivae. These inflammatory swellings are thought to be allergic reactions, and they eventually subside.¹⁰⁾ It has generally been considered that human loiasis is clinically less serious than bancroftian and malayan filariasis and

onchocerciasis.

The human cases of *L. loa* infection reported in Japan are summarized in Table 1. As shown in Table 1, all of the victims discovered in Japan have been imported cases of this disorder. The first case of *L. loa* infection, a 33-year-old German male who visited Japan in 1981, was reported by Tani *et al* (1982, 1985).^{11,12)} Since then, four other authentic cases have been recorded by several investigators.^{7,13-17)} Of the past five victims infected with *L. loa* in Japan, two each were Japanese and African, and one was German. As indicated in Table 1, they ranged in age from 28 to 58 years old, and all of them were male. The localities of infection of those five patients were confined to tropical Africa. The two Japanese patients (Case nos. 2 and 3) occurred after brief stays in the endemic areas of West Africa¹³⁾ and Zaire,^{14,15)} respectively. These patients were bitten on the skin surface by the infected *Chrysops* during their stays in tropical Africa, although they did not notice any symptoms at the time.

It is well known that an infected patient exhibits edema of the eyelids and conjunctiva, as well as itchy swellings on various parts of the body.¹⁰⁾ In general, the established diagnosis of human loiasis is based on the morphology of the sheathed microfilariae in the peripheral blood taken during daytime hours, and adult worms removed from the orbit may be identified on the basis of their morphological features, particularly small bosses on the body surface of the adult worm. A presumptive diagnosis, on the other hand, may be made on the basis of Calabar swellings, a high eosinophilia count, and residence, even for a short period, in an endemic area for loiasis.⁶⁾ Otherwise, an immunological examination, such as an intradermal skin test or a complement fixation test can also be useful in making a tentative diagnosis of human loiasis, although they are not species specific.¹⁰⁾

The lesion sites of the *L. loa* worm in the previous five victims found in Japan were the eye^{7,14-17)} on three cases, and the right forearm^{11,12)} and a lower extremity¹³⁾ in one case each, as shown in Table 1.

TABLE 1. Human cases of infection with *Loa loa* reported in Japan

Case nos.	Patients			Localities infected	Lesion sites	Authors (year)
	ages	sexes	nationalities			
1*	33	M	German	Cameroon	Right forearm	[Tani <i>et al</i> (1982) ¹¹⁾ Tani <i>et al</i> (1985) ¹²⁾
2**	58	M	Japanese	West Africa	Lower extremity	Yoneda <i>et al</i> (1984) ¹³⁾
3§	53	M	Japanese	Zaire	Right eye	[Sugiyama <i>et al</i> (1988) ¹⁴⁾ Ohtaki <i>et al</i> (1988) ¹⁵⁾
4*	28	M	Gabonese	Gabon	Right eye	[Yara <i>et al</i> (1997) ¹⁶⁾ Yara <i>et al</i> (2001) ¹⁷⁾
5§§	29	M	Nigerian	Nigeria	Right eye	Yasuki <i>et al</i> (1998) ⁷⁾

※ Diagnosed by the morphology of microfilariae in the blood

※※ Diagnosed by immunological examination

§ Diagnosed by the morphology of an adult female and the microfilariae

§ § Diagnosed by the morphology of an adult female

M= male

Taking into account the increasing phenomena of people returning from visits to tropical Africa and visitors from endemic areas of Africa, a continuous increase in infection by the *L. loa* worm can be anticipated in Japan in the near future. The diagnosis of the external ophthalmopathy and Calabar or fugitive swellings caused by the *L. loa* worm ordinarily offer little or no difficulty, if Japanese physicians have some preliminary knowledge of this disease. Therefore, special attention should constantly be paid to this type of imported parasitosis from tropical Africa.

ACKNOWLEDGMENTS

The authors are grateful to Misses Kumiko Matoba and Noriko Furukawa of the Parasitology Laboratory for their technical assistance.

REFERENCES

- 1) Belding DL: Textbook of Parasitology. 3rd ed, New York, Appleton-Century-Crofts. 1965, pp 542-544
- 2) Garcia LS, Bruckner DA: Diagnostic Medical Parasitology. 3rd ed, Washington DC, ASM Press. 1977, pp 288-291
- 3) Miyazaki I: An Illustrated Book of Helminthic Zoonoses. Tokyo, International Medical Foundation of Japan. 1991, pp 432-434
- 4) Crewe W: A Guide to Human Parasitology for Medical Practitioners. 10th ed, London, HK Lewis. 1977, pp 151-152
- 5) Grove DI: Filariasis. In Handbook Series in Zoonoses (Sect. C, Vol. II), eds by Steele JH and Schultz MG. Florida, CRC Press. 1982, pp 123-146
- 6) Orihel TC: Cutaneous filariasis and dracontiasis. In Infectious Diseases: A Treatise of Infectious Processes. 5th ed, eds by Hoeprich PD, Jordan MC and Ronald AR. Philadelphia, JB Lippincott. 1994, pp 1073-1078
- 7) Yasuki K, Miyazaki S, Tabuchi A, Hatsushika R, Okino T, Akamatsu T: A case of loiasis in a Nigerian in Japan. *Folia Ophthalmol Jpn* **49**: 620-623, 1998 (in Japanese with English summary)
- 8) Looss A: Zur Kenntniss des Baues der *Filaria loa* Guyot. *Zool Jahab Abt Syst* **20**: 549-574, 1904 (in German)
- 9) Eberhard ML, Orihel TC: Development and larval morphology of *Loa loa* in experimental primate hosts. *J Parasitol* **67**: 556-564, 1981
- 10) Najarian HH: Textbook of Medical Parasitology. Baltimore, Williams & Wilkins. 1967, p 132
- 11) Tani S, Shibuya T, Beisler GK, Tanaka H: A case report of loiasis. *Jpn J Trop Med Hyg* **10**: 178, 1982
- 12) Tani S, Shibuya T, Beisler GK, Tanaka H: An imported case of human infection with *Loa loa*. *Jpn J Exp Med* **55**: 71-74, 1985
- 13) Yoneda K, Fukushima N, Mori S, Ohtomo H, Hioki A: A case of loiasis. *Jpn J Dermatol* **94**: 1085, 1984 (in Japanese)
- 14) Sugiyama E, Shinonaga S, Tsukidate S, Fujita K, Nawata H, Sasaki N, Kamikawadoko S, Ohtaki N, Oka K: A case of loiasis. *J J A Inf D* **62**: 490-494, 1988 (in Japanese with English summary)
- 15) Ohtaki N, Oka K, Kato T, Sugiyama E, Shinonaga S, Kamikawadoko S, Nawata H, Sasaki N: A human case of *Loa loa* infection. *rinsho derma* **30**: 1063-1067, 1988 (in Japanese)
- 16) Yara S, Higa F, Arakaki N, Ishimine T, Tateyama M, Kusano N, Saito A: A case of *Loa loa* infection. *J J A Inf D* **71**: 843, 1997 (in Japanese)
- 17) Yara S, Higa F, Arakaki N, Ishimine T, Shinzato T, Tateyama M, Toma H, Saito A: A case of loiasis. *J J A Inf D* **75**: 151-154, 2001 (in Japanese with English summary)