REVIEW ARTICLE

# A GUIDE TO IDENTIFY NEMATODES COMMONLY FOUND IN HUMAN TISSUE SECTIONS IN SRI LANKA

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## Introduction

Nematodes are round worms. They are found in almost all the habitats. Some are free living while others are parasitic on plants, animals or humans. Nematodes have an elongated non-segmented, bilaterally symmetrical, cylindrical body which taper towards the ends. They have separate sexes; male worms being smaller and shorter than females. Males have a curved posterior end which helps in easy identification of the sex. Nematodes are pseudocoelomate that distinguish them from platyhelminths (acoelomate). They possess a primitive central nervous system (circumesophageal ring of nerve ganglia, nerve trunks and branches), an excretory system, a complete digestive tract with oral and anal openings and a highly developed reproductive system which allow production of large number of eggs. Nematodes do not have a circulatory or respiratory system. Knowledge of these morphological and internal structures is useful in identification of nematodes in the laboratory.

Parasitic nematodes of humans reside either in the intestine or tissue. The tissue nematode infections can be caused by both human and non-human nematodes. Animal nematodes cannot complete the life cycle inside humans thus localizes mostly at the site of entry itself due to the host immune reaction. Nematode worm cross-sections may be encountered in tissue samples including biopsies removed at surgeries (especially from nodulectomies) or autopsies sent to histopathology laboratories. When worm cross-sections are detected in biopsy specimens histopathology slides and specimens are referred to parasitologists for species identification. Even though the therapeutic relevance of specific aetiological diagnosis is of limited value, it is important for epidemiological purposes. The knowledge derived will alert all relevant categories of doctors from clinical, diagnostic and preventive sectors to look for such infections or take necessary preventive measures. With the change of ecology and food habits even infections which were considered rare or uncommon in Sri Lanka could become more common (Eg: anisakiasis after eating shashimi). When literature is studied, it is noted that certain uncommon parasites have been recovered during examination of histological sections.

Nematode parasites found in human tissues include a wide variety such as Ascaroidida (Eg: *Ascaris lumbricoides, Toxocara* species), Oxyurida (Eg: *Enterobius vermicularis*), Strongylida (Eg: *Necator* and *Ancylostoma* species), Spirurida (superfamily filarioidea Eg: *Wuchereria bancrofti, Brugia malayi, Onchocerca volvulus, Loa loa, Mansonella* species, *Dracunculus medinensis, Dirofilaria species* etc.), Rhabditida (Eg: *Strongyloides species),* Enoplida (Eg: *Trichuris, Trichinella, Capillaria*) [1]. Both or either adult of larval stage can be found. Human is the natural host for some of these nematodes while some others who do not otherwise enter human tissue can invade accidentally (eg: *Dirofilaria* species).

Of the filarioidea, *Wuchereria bancrofti* and *Brugia malayi* has been detected in Sri Lanka[2]. Both of them occupy the lymphatic system of humans under usual circumstances thus the larva or adult can be seen in lymph node biopsies. Yet they have also been found in aberrant sites such as in subcutaneous tissue. Possible explanation is that the infective filariform larvae deposited on the skin by the mosquito vector were unable to enter their usual anatomical location as they were trapped in the subcutaneous tissues by the host immune response resulting in granuloma formation within which the filariform larva may develop further or may die in-situ. The commonest filarioidea detected in subcutaneous lumps is the zoonotic filarial worm of dogs *Dirofilaria repens* transmitted by mosquito vectors such as *Aedes aegypti, Armigeres subalbatus, Mansonia uniformis* and *Mansonia annulifera*[3]. Man being an unusual host, the animal filarial worm, is unable to complete its life cycle in the human body and thus get trapped by the human granulomatous reaction. The infection in humans is detected only by laboratory identification of *Dirofilaria* species in tissue sections as microfilaria is not formed within human body. *Dirofilaria* species has also seen located in the epididymis, spermatic cord, lung, breast, omentum and

under the conjunctiva[4]. In Sri Lanka over 170 cases of *Dirofilaria repens* were reported by 2002[5]. This includes several foreigners who have contracted the infection undoubtedly during their stay in Sri Lanka. Unlike in other affected countries dirofilariasis is frequently seen among Sri Lankan children and commonly affects the subcutaneous tissues of exposed parts of the body such as head and neck area. Another animal filarial worm *Brugia ceylonensis* of cats has also been recognized in Sri Lanka[5]. A study of dirofilariasis in a selected area in the Western Province in 2005 has reported that 45% of the screened dogs were infected with *Dirofilaria repens* or *Brugia ceylonensis*[6]. Abundance of vector mosquitoes and high prevalence of the parasites within the reservoir host makes the human population more vulnerable to these zoonotic filariasis infections.

There are also reports of tissue invading nematodes such as visceral larva migrans (Eg: *Toxocara* species), cat hookworm (*Ancylostoma tubaeforme*), *Parastrongylus* (*Angiostrongylus*), *Gnathostoma* and cestodes like subcutaneous sparganosis from Sri Lanka[6,7,8,9].

Intestinal nematodes prevalent in Sri Lanka include *Ascaris lumbricoides, Necator americanus* and *Strongyloides stercoralis* inhabiting small intestine and *Enterobius vermicularis* and *Trichuris trichuira* in large intestine. Of those intestinal nematodes *Ascaris, Trichuris* and *Enterobius* are also found in tissue sections.

### General morphological features assisting identification of nematodes

Nematodes have a well-developed body wall covered with a cuticle. Cuticle may bear surface modification such as transverse or longitudinal marking (striations/annulations), lateral alae, blister-like inflations (bosses/plaques), spines, combs, spiral rings. Thickness of the cuticle varies in different species. Thin hypodermis/epidermis which is mostly syncytial (cellular in *Trichuris*) projects into pseudocoelom to form dorsal, ventral, lateral chords (lateral chords are more prominent). Well-developed longitudinally oriented elongated spindle-shaped smooth muscles separated by chords into quadrants. Body cavity (pseudocoelom), contains digestive and reproductive tracts. Digestive tract possesses a triradiate esophagus. Reproductive tract consists of tubular gonads (male or female). One or two gonads open at the vulva of females and into the rectum of males. Tubular male organ is divided into three parts, testes, seminal vesicles and vas differens which enters rectum to form cloaca. Spicules (which enter to cloaca) have cuticular lining and yellow to dark brown or black. On the tail, caudal alae, rays (caudal papillae) may be present. Female: usually have two ovaries which are long and highly coiled.

### Muscle cell types seen in nematodes

In platymyarin muscle cells, basal contractile portion of the cell is wide and shallow and lies close to the hypodermis. In ceolomyarin muscle cells, basal contractile portion of the cell is U shaped and extends upwards along the sides of the cell. In polymyarin muscle cells, all somatic muscle cells are arranged in a row beneath the hypodermis and are parallel to and overlapping each other. When those numbers of rows of muscle cells are more than five they are called polymyarin. Two or less are holomyarin and two to five are meromyarin. Typically polymyarin are ceolomyarin and holomyarin and meromyarin are platymyarin.

### Esophageal variations seen in nematodes

Rhabitidiform esophagus is divided into three parts as corpus, isthmus and bulb. Cylindroid form has an anterior muscular and posterior glandular (Eg:filariae) portions. Hookworm has a clavate or club shaped esophagus. In trichuris esophagus is tubular and embedded in a row of block-shaped cells (Stichocytes) forming a stichosome. Its lumen is not triradiate but tubular.

### Tissue habitats of adult and larvae of nematodes commonly encountered in Sri Lanka

Adults of *Ascaris lumbricoides, Necator americanus, Enterobius vermicularis* and both adult and larvae of *Strongyloides stercoralis* are encountered in small intestines and appendix. Larvae of *Ascaris lumbricoides* and *Necator americanus* are seen in lung tissues. If an adult worm is seen in large intestinal tissue, it could mostly likely be *Enterobius vermicularis* or *Trichuris trichuira. Toxocara canis and Toxocara cati* are usually found in tissues of liver, gall bladder and eye. Immature and mature adults of human filarial parasites *Wuchereria bancrofti* and *Brugia malayi* are commonly seen in lymph nodes, lymphatics and subcutaneous tissues. Meanwhile animal filarial parasite *Dirofilaria repens* is usually encountered in subcutaneous tissues and eyes. In addition to these usual sites, all these nematodes can also be found in certain secondary and rare sites.

### Morphological characteristics useful in identification of adult nematodes in histology slides

First clue to the diagnosis of *Ascaris lumbricoides* is the large sizes of the cut sections of the worm. *Ascaris lumbricoides* has a thick, multi-layered cuticle with a smooth surface. Its fibrous-looking hypodermis is thin but widens to form dorsal, ventral and lateral chords. Large lateral excretory canals are embedded in lateral chords. The well-developed ceolomyarin type muscles are tall and slender. Digestive tract consist of a short muscular

esophagus and an intestine with irregular contorted lumen that is lined by tall slender columnar cells with nucleus in the base. Female *Ascaris lumbricoides* has paired extensively coiled genital tubes containing developing eggs and paired uteri containing mature eggs. Male reproductive tract consists of a highly coiled tube.

*Necator americanus* adults are only less frequently seen in tissues but may present occasionally. It has a thick cuticle. Its hypodermis is thin but widens to form dorsal, ventral and lateral chords. Lateral chords are prominent. Muscle cells are of platymyarin type and 3-4 cells per quadrant. Esophagus is club shaped and has a triradiate lumen. Intestine has few multinucleated cells. Reproductive tract is confined to the posterior two third of the body.

*Trichuris trichuira*'s thick cuticle has cuticular annulations. Hypodermis has a cellular appearance. Bacillary band (formed by cuticle and hypodermis together) is composed of tall columnar cells. Muscle cells are of ceolomyarin type. Esophagus is tubular and embedded in a row of block-shaped cells (Stichocytes) forming a stichosome.

*Enterobius vermicularis*'s cuticle is thin but when dead swollen and thicker and presence of lateral alae aids the identification. Hypodermis is thin. Prominent lateral chords have a highly vacuolated appearance. Muscle cells are of platymyarin or meromyarin type and 2-3 cells per each quadrant. Esophagus is rhabditoid. Intestine may be tubular or narrow and irregular. Female reproductive tract is didelphic and amphideliphic, confined to middle half of the body. Typical eggs may be seen. Male worm has a single tube in posterior two third of the body. When found from ectopic sites, worm is usually female.

Filarial worms generally lie in coiled position thus many cross sections can be seen in tissue sections. Features of the body wall are the most important for identification of *Wuchereria bancrofti*. Cuticle is thin but thickened at lateral sides. It appears smooth but fine transverse striations are present. Hypodermis is thin except at prominent but short lateral chords which occupy two fifth of body circumference. Hypodermal nuclei are typically seen at the base. Muscle cells are of ceolomyarin type with voluminous cytoplasmic portion. Height of cells depends on the fullness of the pseudoceolom. Muscle cells of male worms tend to be taller. Anterior part of esophagus is muscular while posterior is glandular. Intestine is a simple cylindrical tube and has no histological diagnostic value. Reproductive tract does not provide clue to species identification either. It indicates only the state of maturity and fertility. Females have paired uterine tubes at most levels and coiled ovaries at the posterior end. Reproductive tube of male is the testis found in anterior end and remainder or reproductive tract is straight and cylindrical.

Cuticle of *Brugia malayi* is thin (2µm) but thickened (twice the size) at lateral sides. Surface is smooth. Hypodermis is extremely thin but expands to form prominent lateral chords that are tall in the extremities and flatter in rest of areas. In the male, at the level of the tail, it is conoid in shape.

Lateral chords occupy one third of the circumference in middle two thirds of the body. Ventral and dorsal chords are not apparent at most levels but when present, they are tall and slender. Muscle cells are eolomyarin and tallest at extremities, shorter when pseudoceolom is full with organs. There are 4-5 cells per quadrant. In males in tail area, contractile portion is strong and cytoplasmic portion is almost non-existent. Esophagus is long, two areas as anterior muscular and posterior glandular. Triradiate lumen is always seen. Intestine is a small simple tube lined by low cuboidal cells. Intestine is mostly pushed to the wall by other oragns. Vagina of female is moderately long and coiled and placed anteriorly. There are two parallel uterine tubes running most of the body length. Typically they are filled with developing eggs and microfilaria or unsegmented eggs if not inseminated or infertile. Ovaries are long and highly coiled. Male testis is located in anterior part. There is a single genital tube. Both sexes have a head bulb. There are no alae. In males at all levels cross sections show only two tubes (reproductive tube and thin walled intestine). Differentiation of *B. malayi* from *W.bancrofti* is due to its smaller size, thicker cuticle and prominent thickening of the cuticle in the lateral fields.

*Dirofilaria repens* has a thick, multilayered (layers are oblique to body axis) cuticle with longitudinal cuticular ridges giving cog-wheel like appearance in cross sections. Thomas C. Orihel and Mark L. Eberhard[10] states that cuticular ridges are 7.5-11um apart from each other. The space between the longitudinal ridges are separated by a space equal or greater than the width of the ridge itself; 95-105 ridges on the circumference of the body. The worm measures up to 660 im in maximum diameter. Further they also reports that all of the described species of *Dirofilaria* inhabiting subcutaneous tissues of their natural hosts, with the exception of *Dirofilaria lutrae*, have longitudinal ridges on the surface of the cuticle. Most dirofilarias recovered from human subcutaneous tissues have also shown these cuticular features. At lateral margins, internal cuticular ridges are present. Hypodermis is thin but expands to form prominent lateral chords. Ventral and dorsal chords are

inconspicuous. Muscle cells are typical Ceolomyarin in type. Males show only two tubes (reproductive tract and intestine) in cross sections while females have two or more reproductive tracts (paired uteri and ovaries). In dead worms, muscles, hypodermis, intestine, reproductive tracts degenerate quickly. Cuticle is the most resistant to degeneration; it swells up and shows the multilayers, cuticular ridges and internal lateral cuticular ridges well.

### A guide to identify larval stages of nematode infections prevalent in Sri Lanka

*Ascaris lumbricoides* 2nd and 3rd larval stages may be encountered occasionally in tissue sections. Ascaris L2 is 14-16 × 300 µm in size and has a patent gut that is composed of three cells. It has prominent lateral alae, a small well-defined excretory column which helps in identification. In comparison, Ascaris L3 is 26-50 × 1600 µm in size and has a patent gut of which the lumen is lined with microvilli. It also has prominent lateral alae, a large excretory column of which the width is equal or larger than the intestine.

Another larvae that may come across is Toxocara larvae. It is 17-21 × 400 µm in size and has a non-patent gut that is composed of 7 cells. Toxocara larva also has prominent lateral alae, a large excretory column of which the width is larger than the intestine.

The details given in this article are only about the nematodes prevalent in Sri Lanka. This gives an easy guide for routine diagnosis. However, it is vital to observe the features with open mind and then refer the guide for confirmation. Otherwise one trying to fit the features seen only to the ones given here will miss an important or rare case or an infection which has never been reported from Sri Lanka.

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