

Parafilaria

(helminth: nematode)

Overview

Nematodes are triploblastic pseudocoelomate unsegmented worms that undergo protostomial embryonic cleavage and grow by cuticular moulting (ecdysis). Two groups identified by the presence/absence of sensory phasmids have partly been ratified by molecular studies recognising three subclasses: Enoplia and Dorylaimia (both without phasmids) and Chromadoria (most with phasmids). Many phasmodian parasites of vertebrates are grouped in the chromadorian order Rhabditida; including tylenchinids, rhabditinids and spirurinids. The latter contains the infraorder Spiruromorpha: an enigmatic clade linked by molecular characters, but all having indirect life-cycles involving one or more intermediate hosts, the first invariably being an arthropod. Most possess two trilobed lips (sometimes greatly reduced), a bipartite oesophagus (anterior muscular, posterior glandular) and non-bursate males with coiled tails and two dissimilar spicules. Several superfamilies are recognised: including filarioids (without lips) living in subcutaneous, intermuscular, vascular or lymphatic systems of mammals. Two main families include the oviparous filariids (lay eggs) and the ovoviviparous onchocercids (eggs hatch internally releasing pre-larvae called microfilariae). Infections by the filariid genus *Parafilaria* are transmitted by muscid and horn flies and cause bleeding lesions on the skin of cattle and horses.

Classification:

Domain: Eukaryota (membrane-bound nucleus)
Supergroup: Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Kingdom: Metazoa (multicellular eukaryotes, heterotrophs, notably animals)
Group: Protostomia (triploblastic, spiral cleavage)
Subgroup: Ecdysozoa (cuticle moulted = ecdysis)
Phylum: Nematoda (unsegmented, pseudocoelomate roundworms, tubular digestive tract, dioecious)
Class: Chromadorea (spiral amphids, three oesophageal glands, usually annulated bodies, free-living and parasitic)
Order: Rhabditida (Secernentea, Phasmodia) (secretors, with phasmids, bipartite oesophagus, single testis)
Suborder: Spirurina (mostly parasitic in vertebrate hosts)
Infraorder: Spiruromorpha (enigmatic clade linked by molecular characters, indirect cycles with IHs)
Superfamily: Filarioidea (tissue-dwelling filarial parasites, lack lips)
Family: Filariidae (numerous anterior papillae and cuticular ridges, lay eggs with L1 already fully formed)
Genus: *Parafilaria* (parasitic in connective tissues of horses/cattle)
Species: various species cause skin bleeding lesions in horses/cattle

Parasite biodiversity and host range: Most Metazoa are multicellular triploblastic animals with differentiated tissues, many being bilaterally symmetrical with a body cavity. Most invertebrate animals are protostomes as their embryonic development involves spiral determinate cleavage. Those that moult their external cuticles during their life-cycles (process known as ecdysis) are grouped together in the unique clade Ecdysozoa, including the nematodes (roundworms), onychophorans (velvet worms), tardigrades (water bears) and arthropods (myriapods, chelicerates, crustaceans and hexapods, all with jointed limbs). Nematodes (roundworms) are unsegmented tubular worms with a fluid-filled body cavity (pseudocoelom) that acts as a hydrostatic skeleton. They have longitudinal muscles and typically exhibit a sideways thrashing motion. They have well developed digestive tracts with various partitions: the foregut comprising the mouth (often with lips and papillae), buccal capsule (sometimes with ridges, rods, plates, spears, stylets or teeth) and oesophagus (glandular, muscular or both); the midgut (nonmuscular absorptive section); and hindgut (rectum) emptying through a subterminal anus (cloaca in males). Most nematodes are dioecious and form separate sexes. Male worms have a single testis (sometimes 2), an elongate vas deferens often equipped with a seminal vesicle and ejaculatory duct (glandular and/or muscular), 1-2 copulatory spicules (sometimes with an accessory gubernaculum), and bursate species with elaborate posterior claspers. Female worms are usually didelphic (some monodelphic or polydelphic) with 2 ovaries, 2 oviducts usually with spermatheca, 2 uteri opening into a common vagina and a vulva often equipped with a muscular ovejector. Female worms are oviparous or viviparous and produce numerous eggs or larvae, respectively. Larval stages undergo several moults (L1-L4) before maturing into adult worms. Some nematodes have direct life-cycles where eggs or larvae infect definitive hosts (per os or per cutaneous), but many have indirect cycles where larvae first develop in invertebrate intermediate hosts before infecting definitive hosts (by ingestion, injection or deposition). Many nematode species are free-living in terrestrial and aquatic habitats, while some species from diverse groups have become plant or animal parasites. Two nematode groups identified by the presence/absence of sensory phasmids have partly been ratified by molecular studies recognising three subclasses: Enoplia and Dorylaimia (both without phasmids) and Chromadoria (most with phasmids). Most Enoplia are free-living marine organisms but some are found in freshwater, and on land as plant parasites. The Dorylaimia comprise numerous freshwater and terrestrial species, including major groups of plant and animal parasites. The Chromadoria is represented by many marine groups as well as a terrestrial group of plant and animal parasites. The taxonomic ranks of many nematode assemblages vary considerably depending on which classification system has been followed. Molecular phylogenetic studies, however, have supported the separate

classification of most groups, particularly at the level of superfamily. Collectively, species from at least 16 superfamilies are considered to pose serious threats to human and animal health as infectious diseases.

CLASSIFICATION* OF SUPERFAMILIES OF PARASITIC NEMATODES
Class: Enoplea (Aphasmidea, Adenophorea) (gland-bearers, cylindrical oesophagus, no phasmids, setae, two testes)
Subclass: Dorylaimia (five or more oesophageal glands, buccal stylet (odontostyle), free-living or parasitic)[clade I(2)]
Order: Trichinellida (Trichocephalida, Trichurida) (single spicule, stichosome oesophagus, L1 with buccal stylet)
Superfamily: Trichinelloidea (oesophagus with short anterior muscular and long posterior glandular portions)
Class: Chromadorea (spiral amphids, 3 oesophageal glands, usually annulated bodies, free-living and parasitic)
Order: Rhabditida (Secernentea, Phasmidea) (secretors, phasmids present, amphids anterior, bulbous oesophagus)
Suborder: Rhabditina (free-living or parasitic in invertebrates/lower vertebrates)[clade V(9)]
Infraorder: Rhabditomorpha ('rod-shaped' buccal cavity)
Superfamily: Rhabditoidea (open tube stoma, excretory system with lateral canals)
Superfamily: Strongyloidea (bursate males, prominent buccal capsules, parasites of mammals, birds, reptiles)
Suborder: Spirurina (animal parasites, many use invertebrate intermediate hosts (IH))[clade III(8)]
<i>Incertae sedis</i> Superfamily: Dracunculoidea (elongate parasites of vertebrate tissues, freshwater crustacean IH)
Infraorder: Ascaridomorpha (large roundworms, three large lips, numerous caudal papillae)
Superfamily: Ascaridoidea (ascarids, eggs thick-shelled, larvae may undertake hepato-pulmonary migration)
Superfamily: Heterakoidea (preanal sucker anterior to cloaca in males, direct cycle, infection by egg ingestion)
Infraorder: Gnathostomatomorpha ('jaw-mouthed' due to unique bulbous armed heads)
Superfamily: Gnathostomatoidea (first IH copepod, often use paratenic hosts)
Infraorder: Oxyuridomorpha (pinworms, pointed tails, oesophagus with terminal bulb, males with single spicule)
Superfamily: Oxyuroidea (common in mammals, birds, reptiles, amphibians)
Infraorder: Spiruromorpha (enigmatic clade linked by molecular characters, indirect cycles with IHs)
Superfamily: Acuarioidea (small parasites mostly of birds, with cephalic cordons, ptilina or serrated shields)
Superfamily: Camallanoidea (conspicuous phasmids, L1 with dorsal tooth, ovoviviparous, L1-L3 in copepod)
Superfamily: Filarioidea (tissue-dwelling filarial parasites, lack lips, infect tissues/vessels, arthropod IH)
Superfamily: Habronematoidea (unique head structures with small pseudolabia and median lips)
Superfamily: Physalopteroidea (stomach worms in mammals, insect IH)
Superfamily: Spiruroidea (pseudolabia, bipartite oesophagus, infect birds (crop/gizzard), arthropod IHs)
Superfamily: Thelazioidea (eye-worms of birds and mammals, transmitted by insects)
Suborder: Tylenchina (fungal, plant and animal parasites)[clade IV(10,11,12)]
Infraorder: Panagrolaimomorpha (free-living or parasitic (insects, reptiles, amphibians, mammals))
Superfamily: Strongyloidoidea (dauer stages, lip region without processes, striated cuticle)

*Contemporary genotypic classification schemes recognize strong monophyletic clades at the level of superfamily and infraorder, while previous phenotypic classification schemes had ranked many as separate orders.

Molecular phylogenetic studies have grouped a variety of superfamilies into the infraorder Spiruromorpha whose members are parasites of vertebrates with indirect life-cycles involving larval development within invertebrate intermediate hosts. Most members were previously classified within the order Spirurida: either within the suborder Camallanina (worms with conspicuous phasmids, uninucleate oesophageal glands, larvae without cephalic hooks, usually with copepodid intermediate hosts); or the suborder Spirurina (worms with inconspicuous phasmids, multinucleate oesophageal glands, larvae with cephalic hooks or spines, usually with non-copepodid intermediate hosts). Ten spirurid superfamilies are recognised: Gnathostomatoidea and Physalopteroidea (buccal cavity weakly cuticularized, 2 large lateral pseudolabia); Habronematoidea and Acuarioidea (buccal cavity well cuticularized, 2 large lateral pseudolabia); Filarioidea, Rictularioidea, Aproctoidea and Diplotriaenoidea (buccal cavity well cuticularized, without pseudolabia); Thelazioidea (long cylindrical buccal cavity well cuticularized, body without caudal alae); and Spiruroidea (short buccal cavity well cuticularized, body with caudal alae).

The superfamily Filarioidea contains long thread-like nematodes which are predominantly tissue-dwelling parasites infecting the body cavities, subcutis, intermuscular tissues, blood vessels or lymphatic systems of terrestrial hosts. These worms are known colloquially as 'filariae', 'filarids' or 'filaroids' [Note: take care with terminology as the cognate family Filaridae (esp. genus *Filaria*) are known colloquially as 'filarids', and the unrelated metastrongyle (lungworm) family Filaroididae (genus *Filaroides*) are known colloquially as 'filaroids']. Adult filariae have a cylindroid pharynx with an anterior muscular portion and a posterior glandular portion. Males often have spirally coiled tails, well-developed alae and dissimilar spicules. Females of most species are ovoviviparous (eggs hatch within body of parent) releasing pre-larval stages known as microfilariae (sometimes sheathed). Filariae have indirect life-cycles whereby microfilariae are taken up by blood-sucking or tissue-feeding invertebrates (arthropods, esp. mosquitoes) which act as intermediate hosts for the development of infective L3 larvae. Ten families are recognised: Filaridae and Onchocercidae infecting mammals, birds, reptiles and amphibians; Setariidae infecting mammals; Aproctidae infecting birds; and Creagrocercidae, Drilonematidae, Homungellidae, Mesidionematidae, Scoleophilidae and Ungellidae infecting terrestrial annelids. Examples of filarioid genera covered in this resource are compared in the following table.

Genus	Definitive hosts	Adults (location)	Microfilariae (location)	Periodicity	Vectors	<i>Wolbachia</i> symbiotes
Family Filariidae						
<i>Parafilaria</i> (4 spp.)	ungulates	2-7 cm (subcutis)	40-58 x 23-33 μ m larvated eggs (skin)	diurnal	flies	absent
<i>Stephanofilaria</i> (7 spp.)	ungulates	0.2-1.4 cm (subcutis)	45-195 μ m sheathed (skin)	-	flies	absent
Family Onchocercidae						
<i>Onchocerca</i> (35 spp.)	primates, carnivores, ungulates, rodents	1.5-80 cm (subcutis, ligaments)	105-440 μ m unsheathed (skin)	-	flies, midges	present
<i>Mansonella</i> (29 spp.)	primates, carnivores, ungulates, rodents	3-8 cm (subcutis, serosa)	170-300 μ m unsheathed (blood/skin)	-	midges, flies, mosquitoes	present
<i>Dirofilaria</i> (34 spp.)	primates, carnivores, ungulates, rodents, lagomorphs, marsupials	4-31 cm (blood vessels)	180-385 μ m unsheathed (blood)	-	mosquitoes, flies	present
<i>Dipetalonema</i> , <i>Acanthocheilonema</i> (57 spp.)	primates, carnivores, ungulates, rodents, cingulates, marsupials	1-7 cm (subcutis, serosa)	85-300 μ m unsheathed (blood)	-	flies, fleas, lice, ticks	absent
<i>Wuchereria</i> (2 spp.)	primates	2.5-10 cm (lymphatics)	210-320 μ m sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
<i>Brugia</i> (10 spp.)	primates, carnivores, rodents	1-9 cm (lymphatics)	170-380 μ m sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
<i>Loa</i> (3 spp.)	primates, ungulates, rodents	2-7 cm (subcutis, eye)	250-300 μ m sheathed (blood)	diurnal	flies	absent
Family Setariidae						
<i>Setaria</i> (42 spp.)	primates, ungulates, rodents, lagomorphs	4-19 cm (body cavities)	140-310 μ m sheathed (blood)	-	mosquitoes	absent

Members of the family Filariidae form adult worms bearing numerous anterior papillae and cuticular ridges which live under the surface of the skin near where they deposit eggs/larvae (unlike onchocercids which live deeper in tissues or cavities). Five filariid genera are recognised in 2 subfamilies: namely, Filariinae (syn. Pseudofilariinae) containing those which produce larvated eggs (*Parafilaria* of horses and cattle, *Suifilaria* of swine, *Pseudofilaria* of African antelopes, and *Filaria* (syn. *Hyracofilaria*) of carnivores and rodents); and Stephanofilariinae which produce microfilariae (*Stephanofilaria* of ruminants). *Parafilaria* infections in domestic animals (cattle and horses) induce bloody cutaneous lesions (referred to as ‘summer bleeding’) which attract dipteran (muscid) intermediate hosts.

<i>Parafilaria</i> species	Definitive Hosts (DH)	Location	Vectors/Intermediate Hosts (IH)	Distribution
<i>P. antipinni</i>	Artiodactyla: cervid (red deer), bovid (saiga antelope)	subcutaneous tissues		Eurasia
<i>P. bassoni</i>	Artiodactyla: bovid (springbok, African buffalo)	subcutaneous tissues, eyes		Africa
<i>P. bovicola</i> (syn. <i>P. sahaii</i>)	Artiodactyla: bovid (cattle, buffalo)	subcutaneous and intermuscular connective tissues	Diptera: muscid (face flies) (<i>Musca autumnalis</i> , <i>fasciata</i> , <i>lusoria</i> , <i>vitripennis</i> , <i>xanthomelis</i>)	Africa, Europe, India, Asia
<i>P. multipapillosa</i>	Perissodactyla: equid (horse, donkey, mule); Artiodactyla: bovid (cattle)	subcutaneous and intermuscular connective tissues	Diptera: muscid (horn flies) (<i>Haematobia atripalpis</i>)	North Africa, Europe, South America, Asia

Parasite morphology: *Parafilaria* spp. form 3 different developmental stages: adult worms (sexually dimorphic), eggs (larvated) and larvae (moulting through 4 stages designated L1-4). Worms are slender and white in colour, measuring from 20-70 mm in length by 0.4-0.5 mm in width. They are covered by a tough flexible cuticle which possesses anterior ornamentations in the form of numerous irregular transverse rugosities (wrinkles, ridges) or elliptical papilliform thickenings. They have a small mouth lacking lips, a short oesophagus and a tubular digestive system extending to the anus. Mature worms are sexually dimorphic with female worms being larger than males (40-70 x 0.42-0.44 mm cf. 20-40 x 0.26-0.28 mm). Males usually have short blunt slightly-coiled tails with caudal alae, large pedunculated precloacal and postcloacal papillae, a gubernaculum and 2 unequal spicules (one 500-750 µm long and the other 130-150 µm). Females have large ovaries and their uteri terminate in a vulva located very close to mouth (anterior to the nerve ring). Gravid females produce and lay thin-walled ellipsoidal eggs 40-58 x 23-33 µm. The eggs are embryonated when laid and contain a fully developed pre-larvae (microfilariae). Freed microfilariae are unshathed, measure 215-230 x 10 µm and have attenuated tails. They moult twice growing in size to form infective third-stage larvae (L3) measuring 1.6-4.3 mm with a short muscular oesophagus.

Site of infection: Adult *Parafilaria* worms infect subcutaneous and intermuscular connective tissues in their definitive hosts, mostly upper body parts and dorsal surfaces, especially the forequarters. Microfilariae hatch from eggs released onto the skin and are taken up by dipteran intermediate hosts in which larval development occurs in the body cavity and/or fat bodies.

Pathogenesis: *Parafilaria* infections are not highly pathogenic for livestock, but subcutaneous lesions may result in haemorrhagic dermatitis (known as summer bleeding, summer sores, bloody sweat, verminous nodules or parafilariasis) which predispose to secondary bacterial infections. The lesions may interfere with harnessing working animals and economic losses may be incurred following slaughter due to carcase trimming, rejection or condemnation as well as hide degradation. Carcasses may contain numerous pea-sized nodules that are usually superficial but may involve underlying musculature resulting in irregular oedematous green-yellow lesions (resembling bruising). The lesions are caused by gravid female worms which penetrate the skin to lay larvated eggs. The perforated nodules bleed through a small pore which may ooze for several hours running in streaks on hairs before clotting and drying to brown crusts and mats. Although bleeding is transient, it may be profuse before resolving and occasionally nodules suppurate and become exudative. Further haemorrhagic nodules develop as the worms moves to different site. The bleeding spots attract flies which feed on exudates and they often demonstrate diurnal periodicity as sunlight appears necessary for lesions to bleed. The lesions are also markedly seasonal, occurring in spring and summer and disappearing during the winter leaving distinctive scars.

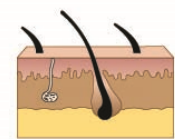
Developmental cycle and mode of transmission: These filariid worms have indirect life-cycles where adults develop in mammalian definitive hosts and larval developmental stages occur in blood-feeding dipteran intermediate hosts. Female worms in subcutaneous nodules pierce the skin causing transient focal haemorrhages into which they lay larvated eggs. The bleeding spots attract flies which feed on host blood and parasite eggs. Ingested eggs hatch almost immediately in suitable intermediate hosts releasing their contained microfilariae. Larval development has been observed in the body cavity and/or fat body of various muscid flies, notably face flies (*Musca* spp.) and horn flies (*Haematobia* spp.). Infective third-stage larvae (L3) develop within 10-15 days and move to the fly mouthparts where they infect new mammalian hosts when feeding on skin wounds, lachrymal secretions (tears) or other exudates. Larvae penetrate to subcutaneous tissues and migrate to various body parts (especially the neck, shoulders, rump and loins) where they complete development to adults in 5-7 months. Nodules and bleeding spots then develop shortly thereafter and the prepatent period (time from infection to first eggs shed) ranges from 7-12 months. Infections are most pronounced during

summer and their prevalence and intensity varies considerably by region but depends largely on the distribution and abundance of suitable fly vectors. Infections have been found in wild and domesticated animals, in free-range animals (especially in African savannas) and penned animals (notably European herds turned out to pasture in spring after winter housing).

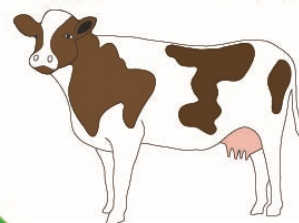
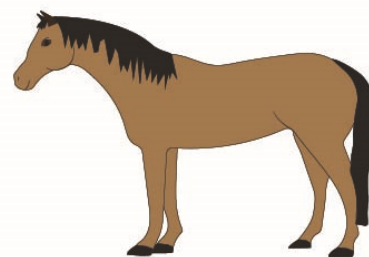
Differential diagnosis: Infections may be suspected by the occurrence of characteristic nodules and bleeding spots, although similar traumatic lesions may be caused by thorns, wire, ticks or biting insects. Diagnosis may be confirmed by the microscopic examination of fresh or dried blood mixed with water for the presence of characteristic larvated eggs or microfilariae, before or after sedimentation by mild centrifugation. Nodules may be biopsied, incubated in warm saline and examined for adult worms but the recovery rate is very low due to their sparse distribution, migrating behaviour and cryptic colouration (same as host tissues). Carcase lesions may be differentiated from bruising by the occurrence of numerous eosinophils in impression smears, and some maintain that affected tissues have a disagreeable metallic smell. More recently, an enzyme immunoassay has been developed to detect host antibodies against *P. bovicola*, but the long persistence of many antibodies makes it difficult to discriminate between recent infection and previous exposure.

Treatment and control: The long life-cycle of the parasite presents a challenge to effective treatment and control. While many conventional anthelmintic drugs have proven ineffective, several macrocyclic lactones, including the avermectins (doramectin, eprinomectin, ivermectin) and milbemycin (moxidectin), as well as the broad spectrum halogenated monophenol (nitroxylnil) have been found to kill adult worms, but they do not completely eliminate migrating larvae so new nodules may appear following treatment. Animals are usually treated 70–90 days prior to slaughter to allow sufficient time for lesions to resolve, but should not exceed 120 days as new nodules may form. The control of fly vectors is a sensible precaution to reduce infections, but muscid flies are difficult to manage as they are ubiquitous, able to feed on many other hosts or substrates (such as manure or other organic wastes), and may rapidly develop resistance to a range of insecticides. Nevertheless, improved sanitation in livestock facilities by removing manure can help to reduce fly populations and the use of long-lasting residual pyrethroid insecticides (in dips or impregnated ear tags) has effectively reduced the prevalence and transmission of infections in several countries.

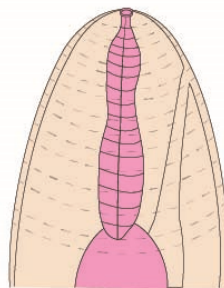
Parafilaria



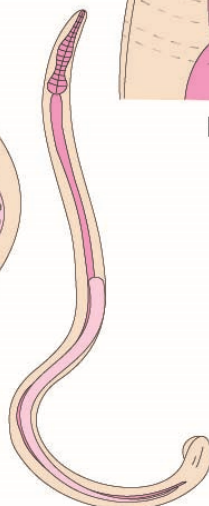
skin
(haemorrhagic dermatitis,
verminous nodules)



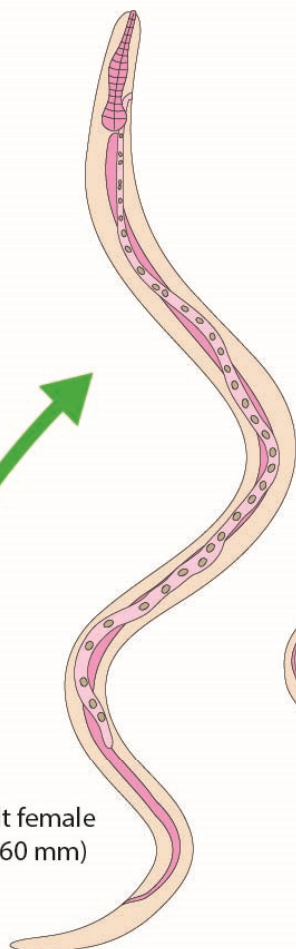
Definitive Hosts
(ungulates)



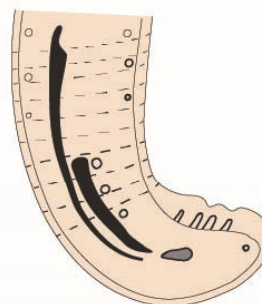
head



adult male
(~ 30 mm)



adult female
(~ 60 mm)



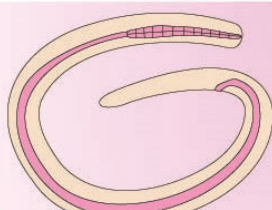
male tail
(lateral)

larval
migration

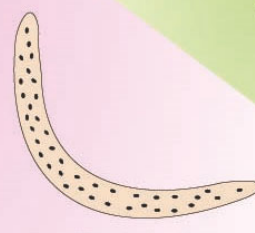
L3
deposited
on skin



Intermediate Hosts (IH)
(muscid flies) (body cavity,
fat bodies, then mouthparts)



third-stage larvae
(L3) (~ 2 mm)



microfilaria (mf)
(~ 220 µm)



eggs
(~ 50 µm)

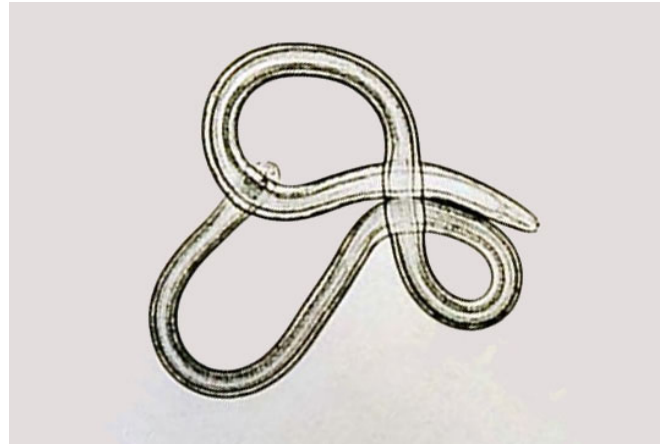
eggs
released
onto skin

eggs
ingested

vector-borne transmission



Parafilaria adult worm



Parafilaria adult worm



Parafilaria larvated egg



Parafilaria larvae