

Stephanofilaria

(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 *Stephanofilaria* are transmitted by horn flies and cause exudative, sometime haemorrhagic, dermatitis in cattle.

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, Phasmidea) (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, produce larvated eggs or microfilariae)
Genus: *Stephanofilaria* (parasitic in skin of cattle)
Species: various species cause dermatitis in 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>Stephanofilaria</i> (7 spp.)	ungulates	0.2-1.4 cm (subcutis)	45-195 µm sheathed (skin)	-	flies	absent
<i>Parafilaria</i> (4 spp.)	ungulates	2-7 cm (subcutis)	40-58 x 23-33 µm larvated eggs (skin)	diurnal	flies	absent
Family Onchocercidae						
<i>Onchocerca</i> (35 spp.)	primates, carnivores, ungulates, rodents	1.5-80 cm (subcutis, ligaments)	105-440 µm unsheathed (skin)	-	flies, midges	present
<i>Mansonella</i> (29 spp.)	primates, carnivores, ungulates, rodents	3-8 cm (subcutis, serosa)	170-300 µm 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 µm 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 µm unsheathed (blood)	-	flies, fleas, lice, ticks	absent
<i>Wuchereria</i> (2 spp.)	primates	2.5-10 cm (lymphatics)	210-320 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
<i>Brugia</i> (10 spp.)	primates, carnivores, rodents	1-9 cm (lymphatics)	170-380 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
<i>Loa</i> (3 spp.)	primates, ungulates, rodents	2-7 cm (subcutis, eye)	250-300 µm sheathed (blood)	diurnal	flies	absent
Family Setariidae						
<i>Setaria</i> (42 spp.)	primates, ungulates, rodents, lagomorphs	4-19 cm (body cavities)	140-310 µm 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). *Stephanofilaria* infections in ruminants produce an exudative/haemorrhagic dermatitis which attracts dipteran (muscid) intermediate hosts.

<i>Stephanofilaria</i> spp.	Definitive Hosts (DH)	Location	Vectors/Intermediate Hosts (IH)	Distribution
<i>S. andamani</i> <i>sp. inq.</i>	Artiodactyla: bovid (water buffalo)	adults in skin		Andaman Islands
<i>S. boomkeri</i>	Artiodactyla: suid (pig)	adults in skin, eggs containing microfilariae in tissues		Africa
<i>S. dedoesi</i> (syn. <i>S. assamensis</i> , <i>S. kaeli</i> , <i>S. okinawaensis</i> , <i>S. rono</i>) (cascado, humpsore, Calcutta sores, Dum Dum sores, filarial sore, krian sore)	Artiodactyla: bovid (cattle, buffalo, goat); Proboscidea: elephantid (Indian elephant); Perissodactyla: rhinocerotid (Indian rhinoceros)	adults in skin, microfilariae in tissues	Diptera: muscid (<i>Musca conducens</i> , <i>Haematobia atripalpis</i> , <i>H. (Liperosia) irritans</i> , <i>H. (L.) titillans</i>)	Asia, India, southern Russia
<i>S. dinniki</i>	Perissodactyla: rhinocerotid (black rhinoceros)	adults in skin, microfilariae in tissues		Africa
<i>S. jaheeri</i>	Artiodactyla: bovid (cattle, buffalo, nilgai)	adults in skin		Asia
<i>S. srivastavai</i> <i>sp. inq.</i>	Proboscidea: elephantid (Indian elephant)	adults in skin		India
<i>S. stilesi</i>	Artiodactyla: bovid (cattle, goat)	skin (midventral line), eggs cont. mf	Diptera: muscid (horn fly) (<i>Haematobia (Liperosia) irritans</i> , <i>H. (L.) titillans</i>)	Americas, Eurasia, Australia
<i>S. thelazioides</i>	Artiodactyla: hippopotamid (hippopotamus)	adults in skin, microfilariae in tissues		Africa
<i>S. zaheeri</i> (ear sore)	Artiodactyla: bovid (cattle, buffalo, nilgai)	adults in skin (ears), eggs containing microfilariae in tissues	Diptera: muscid (<i>Musca autumnalis</i> , <i>M. planiceps</i>)	India, Asia

Parasite morphology: These filariid parasites form 3 different stages in their developmental cycles: eggs (releasing microfilariae), larvae (four stages designated L1-4) and adult worms. Female worms are ovoviviparous and produce thin-shelled ovoid eggs measuring 60-70 μm *in utero* which embryonate internally to contain elongate microfilariae (they are less well developed than typical nematode first-stage larvae (L1) so are considered advanced embryos or pre-larvae). The microfilariae vary in size depending on species: most being small ranging from 45-60 μm in length (e.g. *S. stilesi*), although some are larger measuring 100-120 μm (e.g. *S. kaeli*) or 160-195 μm (e.g. *S. assamensis*). Most have a blunt anterior end with a digitiform process, long conical tail and are usually surrounded by a highly-specialised semi-rigid fluid-filled vitelline membrane (sheath) that is elongate-oval in shape (enclosed microfilariae cannot migrate). Microfilariae mature and moult twice to form infective third-stage larvae (L3) ranging in size from 0.6-1.3 x 0.04-0.05 mm with narrow ends, the head bearing a single ventral lateral spine or small crown-like projections with numerous spines, a short undivided oesophagus, a rounded tail with delicate ventro-terminal serrations, and genital primordia mid-body. In the definitive host, infective L3 grow in the dermis to 0.9-1.2 mm long, moult and grow to L4 measuring 1.7-2.2 mm long which subsequently moult to form adults. Mature worms are small and slender, measuring from 2-14 x 0.1-0.2 mm, and covered with a tough flexible cuticle with numerous anterior transverse striations/thickenings. The head possesses a characteristic protruding cuticular ring with numerous small denticles or spines around the mouth (4-24 cephalic spines, 14-19 peribuccal spines). The digestive system is tubular, the oesophagus is bipartite with an anterior muscular and a posterior glandular section, and female worms lack an anus. Worms are sexually dimorphic whereby females are larger than males (6-14 mm cf. 2-6 mm). Males have slightly curved tails with a gubernaculum and 2 elongate spicules that are unequal in size (right spicule smaller). Females have larger ovaries and their uteri terminate in a parabuccal vulva (located anterior to the nerve ring).

Site of infection: Adult *Stephanofilaria* worms infect the dermis of their mammalian definitive hosts, often being located just beneath the epidermis particularly along the mid-ventral line between the navel and brisket, and sometimes around the hump and ears. Microfilariae may be found in skin lesions and in surrounding dermal papillae. Larvae develop in the haemocoel of their dipteran intermediate hosts and infective L3 move to the fly mouthparts.

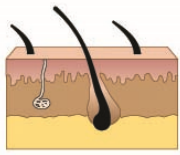
Pathogenesis: *Stephanofilaria* infections are not highly pathogenic, but worms in the skin damage hair follicles and glands and may cause unsightly dermatitis. The disease is called stephanofilariasis, known variously as filarial sores, cascado sores, hump sores, Calcutta sores, Dum Dum sores, krian sores or ear sores. Infections cause a circumscribed haemorrhagic and/or exudative dermatitis, often along the belly but with repeated exposure, lesions may spread to the teats, scrotum, flanks neck and ears. Active lesions may be raw and bloody or covered with a serous exudate, and may persist for as long as 1-3 years. Lesions begin as small papules but later coalesce to become 3-15 cm in diameter. The epidermis demonstrates hyperkeratosis and parakeratosis and affected areas become intensely inflamed, pruritic (itchy) and show partial hair loss (alopecia). As exudates dry, they form thick flaky or crumbly scabs that often split to expose blood-tinged fluids. Lesions are highly attractive to flies and secondary bacterial infections may ensue. Severe infections may cause significant stress to animals which may undertake erratic behaviours in relieve the intense irritation (laying, rocking, sliding, rubbing, rolling, etc). Infections in dairy cattle may hamper manual or mechanical milking, as well as compromise milk hygiene. Chronic lesions may heal over after several months to years but they often leave a dry thickened scar devoid of hair. At slaughter, hides may be so damaged that they are downgraded or rejected. Infections of cattle by *S. stilesi* were found to be more prevalent on yearlings and mature animals, though this may probably reflect horn fly vector preferences for older animals.

Developmental cycle and mode of transmission: Like other filarioid parasites, *Stephanofilaria* spp. have indirect heteroxenous life-cycles involving vector-borne transmission between adult worms in mammalian definitive hosts and larval stages in blood-feeding dipteran intermediate hosts. Gravid females living in dermal beds release microfilariae into tissues and bleeding lesions where they are taken up by flies which are attracted to wounds and feed on host exudates. Suitable intermediate hosts include the horn fly or buffalo fly *Haematobia irritans*, particularly in Europe, America and Australia, but numerous other muscid flies (stable flies, face flies, house flies) act as intermediate hosts elsewhere. Most of these flies flourish in rangelands or irrigated pastures where manure and other organic waste is abundant, but are scarcer in well-maintained intensive production systems (feed-lots and barns) where manure is frequently disturbed, removed or scattered. Ingested microfilariae penetrate to the body cavity (haemocoel), sometimes the fat body, of the fly and moult to L2 within 4-10 days and again to L3 within 15-25 days. The infective L3 migrate to the head and proboscis and are deposited on the skin of new definitive hosts when the flies feed again. Transmission appears to be mechanical and contaminative rather than inoculative. The larvae develop, moult and grow in the dermis of the mammalian host and form adults which mature and mate. The ovoviviparous female worms produce eggs which hatch internally releasing microfilariae. The prepatent period (time from infection to first shedding of microfilariae) is around 6-8 weeks. It is not known how long adult worms survive in hosts nor how long they continue producing microfilariae. The incidence of infection is partly seasonal (spring and autumn) and attuned to periods of peak fly activity (e.g. horn flies prefer temperatures ranging from 22-28°C and seek shade under hosts when temperatures become too warm). In endemic areas, infections may afflict most animals in a herd.

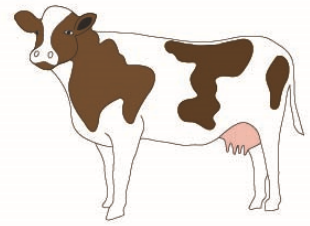
Differential diagnosis: Infections are usually detected on the basis of symptomatology, involving the detection of characteristic wounds and animal rubbing/scratching behaviors, and confirmed by the microscopic detection of worms, worm fragments or microfilariae in skin scrapings after maceration and incubation in warm isotonic saline. Nonetheless, the detection of parasites is often difficult due to their scattered distribution, cryptic appearance and possible confusion with microfilariae of other filarioid nematodes (e.g. *Onchocerca*, *Setaria* and *Pelodera* spp.). Histological sections of skin biopsies may reveal sections of adult worms in hair follicles with microfilariae and eosinophils present in the dermis.

Treatment and control: Few countries have approved anthelmintic products for the treatment of stephanofilariasis, but several studies have shown that macrocyclic lactones, including the avermectins (ivermectin, eprinomectin) and milbemycin (moxidectin), were effective against infections in livestock, including lactating cattle. Other studies suggest that the injectable imidazothiazole (levamisole) may help control infections, though multiple applications appeared necessary. Several preventive strategies have been designed to minimise transmission by reducing vector (fly) populations. The topical application of organophosphates (trichlorfon) and long-lasting residual pyrethroids (in dips or impregnated ear tags) have been found to reduce populations of biting horn flies in several endemic regions, but had little impact on other non-biting muscid flies breeding in manure and other organic wastes. In Asia, it was noticed that many infections required a pre-existing wound for infection to take place, so simple wound prevention and treatment with ointments containing insecticides was recommended to reduce the risk of disease. In regions where horn flies are endemic (such as Europe, America and Australia), it was found that keeping livestock indoors during the peak fly season reduced the incidence of infections (horn flies do not attack stock indoors). In regions where the main vectors are non-biting flies (such as face flies and house flies), population numbers may be reduced by improving environmental hygiene and sanitation (cleaning stalls, manure and waste removal).

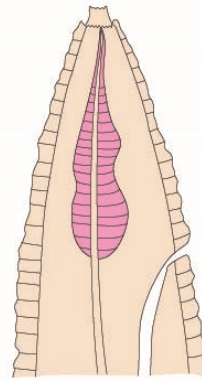
Stephanofilaria



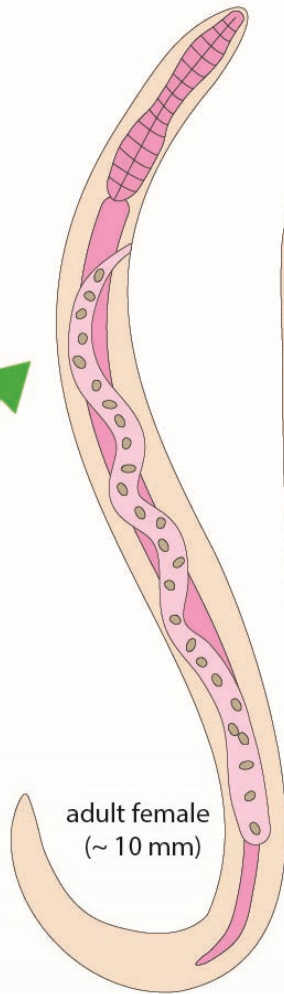
skin
(exudative or
haemorrhagic
dermatitis)



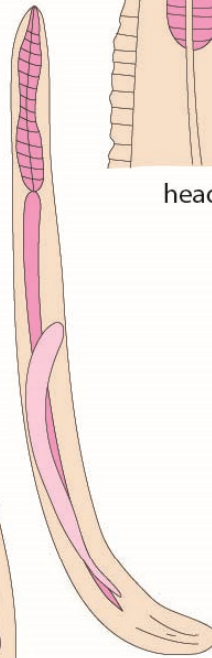
Definitive Hosts
(ungulates)



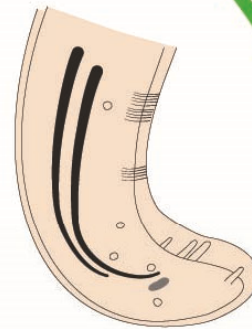
head



adult female
(~ 10 mm)



adult male
(~ 5 mm)

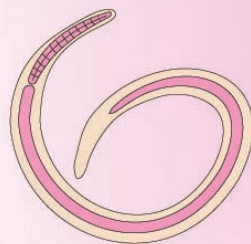


male tail
(lateral)

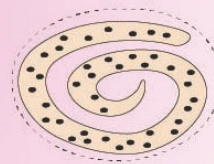
L3
deposited
on skin



Intermediate Hosts (IH)
(horn/buffalo flies)
(haemocoel, then mouthparts)



third-stage larvae
(L3) (~ 1 mm)



microfilaria (mf) (~ 100 μ m)
(thin egg membrane)

mf released into skin lesions

mf
ingested

vector-borne transmission



Stephanofilaria adult worm



NCVP

Stephanofilaria lesion on cow