Dirofilaria
(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 phasmidian 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 onchocercid genus *Dirofilaria* are transmitted by mosquitoes (in which L3 develop). One species (*D. immitis*) causes canine heartworm disease involving circulatory obstruction progressing to congestive heart failure, while another (*D. repens*) causes cutaneous disease with nodules formed in the skin and muscles of carnivores.

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:	Onchocercidae (adults loose in tissues or in nodules, viviparous (live birth of microfilariae))
Genus:	*Dirofilaria* (parasitic in heart of dogs/cats/humans)
Species:	*D. immitis* (cause heartworm disease in dogs)

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 species 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)]
Incertae sedis 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 spirurinid 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 ‘filarioids’ [Note: take care with terminology as the cognate family Filariidae (esp. genus *Filaria*) are known colloquially as ‘filariids’, 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: Filariidae and Onchocercidae infecting mammals, birds and amphibians; Setariidae infecting mammals; Aproctidae infecting birds; and Creagrocercidae, Drilonematidae, Homungellidae, Mesidionematidae, Scolecophilidae 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	*Wolbachia* symbiotes
Family Onchocercidae						
Dirofilaria (34 spp.)	primates, carnivores, ungulates, rodents, lagomorphs, marsupials	4-31 cm (blood vessels)	180-385 µm unsheathed (blood)	-	mosquitoes, flies	present
Dipetalonema, Acanthocheilonema (57 spp.)	primates, carnivores, ungulates, rodents, cingulates, marsupials	1-7 cm (subcutis, serosa)	85-300 µm unsheathed (blood)	-	flies, fleas, lice, ticks	absent
Onchocerca (35 spp.)	primates, carnivores, ungulates, rodents	1.5-80 cm (subcutis, ligaments)	105-440 µm unsheathed (skin)	-	flies, midges	present
Mansonella (29 spp.)	primates, carnivores, ungulates, rodents	3-8 cm (subcutis, serosa)	170-300 µm unsheathed (blood/skin)	-	midges, flies, mosquitoes	present
Wuchereria (2 spp.)	primates	2.5-10 cm (lymphatics)	210-320 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
Brugia (10 spp.)	primates, carnivores, rodents	1-9 cm (lymphatics)	170-380 µm sheathed (blood)	nocturnal, subperiodic	mosquitoes	present
Loa (3 spp.)	primates, ungulates, rodents	2-7 cm (subcutis, eye)	250-300 µm sheathed (blood)	diurnal	flies	absent
Family Filariidae						
Parafilaria (4 spp.)	ungulates	2-7 cm (subcutis)	40-58 x 23-33 µm larvated eggs (skin)	diurnal	flies	absent
Stephanofilaria (7 spp.)	ungulates	0.2-1.4 cm (subcutis)	45-195 µm sheathed (skin)	-	flies	absent
Family Setariidae						
Setaria (42 spp.)	primates, ungulates, rodents, lagomorphs	4-19 cm (body cavities)	140-310 µm sheathed (blood)	-	mosquitoes	absent

Members of the family Onchocercidae form adult worms that live loose in body cavities or in tissue nodules. Female worms release microfilariae which disperse into the blood or dermal connective tissues (unlike filariids which live in the skin close to where they deposit eggs or larvae). Some 88 onchocercid genera are divided into 7 subfamilies: Onchocercinae and Dirofilariinae (syn. Loainae) mostly in mammals but some in birds and reptiles, Waltonellinae and Icosiellinae in amphibians, Oswaldofilariinae in reptiles, Splendidofilariinae and Lemdaninae in birds, reptiles and mammals (former subfamily Setariinae in large mammals recently elevated to family status as Setariidae). Members of the subfamily Dirofilariinae are characterised as forming males with highly developed caudal alae (while members of the subfamily Onchocercinae form males with markedly dissimilar spicules and long tails lacking caudal alae). Some 16 genera are recognised in the subfamily Dirofilariinae: namely, *Bostrichodera*, *Dirofilaria*, *Dirofilariaeformia*, *Edesonfilaria*, *Eulimdana*, *Foleyellides*, *Heimnema*, *Loa* (syn. *Paraloa*), *Loaina, Macacanema*, *Madochotera*, *Ochoterenella*, *Skrjabinodera* and *Tawila* in mammals, *Pelecitus* (syn. *Spirofilaria*, *Eulimdana*) in birds, and *Foleyella* (syn. *Foleyellides*) in saurians.

The genus *Dirofilaria* is characterised by the formation of long tubular adult worms in the circulatory system or connective tissues of mammalian hosts. Mature female worms produce unsheathed microfilariae which undergo further larval development primarily in mosquito vectors. Some 34 *Dirofilaria* spp. have been described predominantly from carnivores and primates and allocated to 2 subgenera: *D.* (*Dirofilaria*) comprising long thin worms with smooth cuticles that infect the lungs, heart and blood-vessels; and *D.* (*Nochtiella*) being represented by shorter stout worms with cuticular ornamentations (longitudinal ridges with transverse striations) that infect subcutaneous and conjunctival tissues. Various *Dirofilaria* spp. are zoonotic and may infect humans, usually as accidental hosts, causing either subcutaneous or ocular dirofilariasis. The most common species infecting humans are *D. repens* mainly in Eurasia and *D. immitis* (syn. *D. lousianensis*, *D. magalhaesi*, *D. ochmanni*?) in the Americas. Other species occasionally infecting humans are *D. honkongensis* and *D. magnilarvatum* in Asia, and *D. tenuis*, *D. ursi*, *D. striata* and *D. spectans* in North America. The species *D. immitis* is quite notorious as the cause of canine heartworm disease and is transmitted by more than 70 different mosquito species. *D. immitis* has a world-wide distribution but it is found mainly in tropical and subtropical regions (roughly bounded by latitudes 43-48°N and 34°S). The geographical distribution of heartworm has expanded in recent years presumably due to greater translocations of infected dogs, anthropogenic changes favouring vector survival (e.g. irrigation, deforestation, global warming, increased urbanization), and paradoxically, adaptation of parasites to develop at lower temperatures.

***Dirofilaria* species**	**Definitive Hosts (DH)**	**Location**	**Vectors/Intermediate Hosts (IH)**	**Distribution**
Subgenus *Dirofilaria*				
D. ailure	Carnivora: ailurid (red panda)	heart		Asia
D. fausti (possible syn. of *D. immitis*)	Carnivora: otariid (California sea lion)	heart		North America
D. freitasi	Primates: bradypodid (pale-throated sloth)	abdominal cavity		South America
D. immitis (canine heartworm)	Carnivora: canid (dog, dingo, African wild dog, raccoon dog, golden jackal, wolf, Great Plains wolf, Northwestern wolf, red wolf, Florida black wolf, maned wolf, Gregory's wolf, Japanese wolf, coyote, dhole, crab-eating fox, gray fox, island fox, Bengal fox, red fox, American red fox, Iberian fox), felid (cat, bobcat, black-footed cat, Asian golden cat, tiger, lion, jaguar, jaguarundi, black panther, leopard, clouded leopard, snow leopard, ocelot), ursid (brown bear, Asian black bear, Florida black bear, polar bear), mustelid (wolverine, otter, giant otter, North American river otter, Eurasian otter, Japanese weasel, European polecat, ferret, American mink), ailurid (red panda), procyonid (white-nosed coati, raccoon), phocid (hooded seal, ringed seal, harbour seal), otariid (California sea lion, Stellar sea lion); Rodentia: cricetid (musk rat), castorid (American beaver); Lagomorpha: leporid (European rabbit, Japanese hare); Artiodactyla: cervid (sika deer); Perissodactyla: equid (horse); Primates: hylobatid (Lar gibbon), cercopithecid (rhesus macaque), hominid (human, orangutan); Sphenisciformes: spheniscid (Humboldt penguin)	heart (right ventricle, right atrium), lungs (pulmonary artery), vena cava, subcutaneous, intraocular tissues, abdominal cavity, microfilariae in blood (unsheathed, nonperiodic), exercise intolerance, chronic congestive heart failure, pulmonary emboli	Diptera: culicid (*Aedes aegypti, albopictus, canadensis, cantator, caspius, cinereus, detritus, echinus, edgari, excricians, fijiensis, gaumensis, geniculatus, infirmatus, koreicus, longitubus, melanimon, pandani, polynesiensis, pseudoscutellaris, punctor, rotumae, samoanus, scapularis, sierrensis, solicitans, stictius, stimulans, taeniorhynchus, togoi, triseriatus, trivittatus, vexans, Anopheles algeriensis, bradleyi, campestris, crucians, earlei, freeborni, hyracanus, lesteri, maculipennis, plumbeus, punctipennis, nigerrimus, quadrimaculatus, superpictus, walkeri, Armigeres malayi, Coquilletidia* (*Culex*) *richiardi, Culex annulirostris, declarator, erythrothorax, fatigans, modestus, nigripalpus, pipiens, quinquefasciatus, restuans, salinarius, saltanensis, sitiens, tarsalis, territans, theileri, torrentum, tritaeniorhynchus, Culiseta incidens, inornata,*	Americas, Europe, Indo-China, Asia, Australia

			Mansonia annulata, bonneae, dives, indiana, titillans, uniformis, Psorophora columbiae, ferox, Wyeomyia bourrouli); reports of development in Phthiraptera: pulicid (*Ctenocephalides felis*) now attributed to *Acanthocheilonema reconditum*)	
D. indica (possible syn. of *D. immitis*)	Carnivora: canid (dog)	heart		India
D. louisianensis (possible syn. of *D. immitis*)	Primates: hominid (human)	heart		North America
D. lutrae	Carnivora: mustelid (North American river otter)	subcutaneous tissue, heart		North America
D. magalhaesi (possible syn. of *D. immitis*)	Primates: hominid (human)	heart		South America
D. nasuae (possible syn. of *D. immitis*)	Carnivora: procyonid (coati)	heart		South America
D. pongoi (possible syn. of *D. immitis*)	Primates: hylobatid (northern grey gibbon), hominid (orangutan)	heart		Asia
D. spectans	Carnivora: mustelid (tayra, giant otter, neotropical otter); Primates: hominid (human)	heart, pulmonary artery		Americas
D. spirocauda (syn. *Skrjabinaria*) (possible syn. of *D. immitis*)	Carnivora: phocid (hooded seal, harbour seal)	heart		
Subgenus *Nochtiella*				
D. acutiuscula (possible syn. of *D. repens p.p.*)	Carnivora: canid (Azara's fox), felid (Canada lynx); Rodentia: caviid (capybara); Artiodactyla: tayassuid (white-lipped peccary, collared peccary)	stomach wall, subcutaneous tissue, heart		South and North America
D. bonnei	Rodentia: murid (black rat)	body cavity		Asia, South America
D. cancrivori	Carnivora: procyonid (crab-eating raccoon)	subcutaneous tissue		South America
D. corynodes (syn. *D. aethiops*, *D. schoutedeni*)	Primates: cercopithecid (white-eyelid mangabey, sooty mangabey, mona monkey, Campbell's mona monkey, moustached monkey, grivet, greater spot-nosed monkey, patas monkey, black-and-white colobus, western red colobus, king colobus, rhesus macaque, steel-grey monkey)	subcutaneous and intermuscular connective tissues, mf in blood (nocturnal periodicity)	Diptera: culicid (*Aedes aegypti, pembaensis, Anopheles maculipennis*)	Africa, Asia
D. genettae (syn. *D. andersoni*)	Carnivora: felid (cat), viverrid (Cape genet), herpestid (banded mongoose)	connective tissue		Africa
D. hongkongensis	Carnivora: canid (dog); Primates: hominid (human)	subcutaneous and subconjunctival tissues		Hong Kong
D. incrassata (possible syn. of *D.*	Pilosa: brachypodid (brown-throated sloth, pale-throated sloth),	pleura, peritoneum		South and Central

macrodemos p.p.)	choloepodid (Hoffman's two-toed sloth); Carnivora: procyonid (white-nosed coati, South American coati			America
D. linstowi	Primates: cercopithecid (Toque macaque, gray langur, purple-faced leaf monkey)	subcutaneous tissues		Sri Lanka
D. macacae	Primates: cercopithecid (Assam macaque, northern pig-tailed macaque, stump-tailed monkey, iris monkey)	subcutaneous tissues		Indochina
D. macrodemos	Pilosa: brachypodid (brown-throated three-toed sloth, pale-throated sloth)	subserosa		South America
D. magnilarvatum	Primates: hylobatid (silvery gibbon), cercopithecid (iris monkey, rhesus macaque, silvered leaf monkey, dusky leaf monkey, Sumatran surili), hominid (human)	subcutaneous tissues, large microfilariae in blood (nonperiodic)	Diptera: culicid (*Mansonia longipalpis*, *uniformis*)	Asia
D. minor	Carnivora: felid (leopard cat)	subcutaneous tissues		Asia
D. pagumae	Carnivora: viverrid (masked palm civet)	subcutaneous tissues		Indochina
D. panamensis	Pilosa: choloepodid (Hoffman's two-toed sloth)	subserosa		South America
D. repens (syn. *D. conjunctivae, Loa extraocularis*)	Carnivora: canid (dog, wolf, golden jackal, red fox, crab-eating fox, Bengal fox, Azara's fox), felid (cat, wildcat, Asian golden cat, fishing cat, Canada lynx, lion), viverrid (Cape genet), procyonid (South American coati), mustelid (giant otter); Primates: hominid (human)	subcutaneous, subconjunctival and intermuscular tissues, cutaneous dirofilariosis (skin nodules, pruritus)	Diptera: culicid (*Aedes aegypti, albopictus, caspius, detritus, fasciata, geniculatus, mariae, pembaensis, vexans, Anopheles atroparvus, claviger, daciae, maculipennis, petragnanii, sinensis, stephensi, Armigeres obturbans, Culex pipiens, Mansonia africanus, annulifera, uniformis*); tabanid (*Haematopota variegata*); Phthiraptera: pulicid (*Ctenocephalides canis*)	Eurasia, Africa, North America
D. sachsi	Artiodactyla: bovid (hartebeest, blue wildebeest)	subcutaneous and intermuscular connective tissue		Africa
D. striata	Carnivora: felid (Florida panther, long-tailed tiger cat, ocelot, bobcat, margay, cougar, tiger), canid (dog); Artiodactyla: tayassuid (white-lipped peccary); Primates: hominid (human)	subcutaneous and intermuscular connective tissues; microfilariae in blood	Diptera: culicid (*Anopheles quadrimaculatus*)	Americas
D. subdermata (syn. *D. hystrix*, *D. spinosa*, *D. subcutanea*)	Rodentia: erethizontid (North American porcupine); Carnivora: mustelid (polecat)	peritoneal and subcutaneous tissues		North America, Africa
D. sudanensis (syn. *D. granulosa*)	Carnivora: felid (lion, leopard), hyaenid (hyaena)	subcutaneous tissue		Africa, Asia
D. tawila	Primates: cercopithecid (western red colobus)	subcutaneous tissues		Africa
D. tenuis	Carnivora: procyonid (raccoon); Primates: hominid (human)	subcutaneous, submucosal and intermuscular tissues, microfilariae in blood, nodules, ophthalmic	Diptera: culicid (*Aedes taeniorhynchus, sollicitans, Anopheles quadrimaculatus, Psorophora confinnis*)	North America

		dirofilariasis		
D. ursi (syn. *D. desportesi*)	Carnivora: ursid (American black bear, Japanese black bear, Asian black bear, brown bear, Kamchatka brown bear), felid (tiger); Primates: hominid (human)	subcutaneous, perirenal and peritracheal tissues, abdominal cavity, microfilariae in blood	Diptera: simuliid (*Simulium venustum*)	Asia, North America
Species inquirenda				
D. ochmanni (syn. *Microfilaria*)	Carnivora: canid (dog, red fox); Primates: hominid (human)	no adults described, mf in blood		Africa
D. timidi (syn. *Brugia*)	Lagomorpha: leporid (mountain hare)	abdominal cavity		Siberia
Species transferred to other genera				
D. digitata (syn. *Dipetalonema*, *Dirofilariaeformia*) (now *Sandnema*)	Artiodactyla: bovid (cattle); Primates: hylobatid (hoolock gibbon, Lar gibbon, northern white-cheeked gibbon), cercopithecid (dusky leaf monkey, stump-tailed macaque)	abdominal cavity		Indochina
D. kuelzii (syn. *D. asymmetrica*) (now *Skrjabinodera*)	Artiodactyla: bovid (duiker, bay duiker, Maxwell's duiker)	subcutaneous and intermuscular connective tissue		Africa
D. pauliani (now *Paulianfilaria*)	Primates: lepilemurid (weasel sportive lemur), indriid (Verreaux's sifaka)	pleural cavity		Madagascar
D. roemeri (syn. *Filaria, F. websteri, Acanthocheilonema, Dirofilaria, D. websteri, Agamofilaria tabanicola*) (now *Pelecitus*)	Diprotodontia: macropodid (agile wallaby, antilopine wallaby, black-striped wallaby, whiptail wallaby, western grey kangaroo, eastern grey kangaroo, common wallaroo, red-necked wallaby, red kangaroo, bridled nailtail wallaby, swamp wallaby)	subcutaneous and intermuscular connective tissues, limb joints, mf in blood (loose sheath)	Diptera: tabanid (*Dasybasis acutipalpis, circumdata, dubiosa, hebes, moretonensis, neobasalis, oculata, Mesomyia fuliginosa, Scaptia testaceomaculata, Tabanus australicus, pallipennis, particaecus, parvicallus, strangmenii, townsvilli*)	Australia
D. scapiceps (syn. *Loaina*) (now *Pelecitus*)	Lagomorpha: leporid (European rabbit, cottontail rabbit, eastern cottontail, marsh rabbit, snowshoe hare, Cape hare, prairie hare)	limb joints, mf in blood (loose sheath)	Diptera: culicid (*Aedes aegypti, canadensis, euedes, excrucians, provocans, punctor, stimulans, vexans, Mansonia perturbans*)	Africa, Europe, North America
D. uniformis (now *Loaina*)	Lagomorpha: leporid (European rabbit, eastern cottontail, marsh rabbit)	subcutaneous tissue, microfilariae in blood (sheathed)	Diptera: culicid (*Anopheles quadrimaculatus*)	North America

Parasite morphology: *Dirofilaria* spp. form 3 different morphotypic stages in their developmental cycles: adult worms; pre-larvae (microfilariae); and 4 sequential larval stages (designated L1-4). Female worms are ovoviviparous and do not lay eggs but produce live microfilariae in their mammalian (definitive) hosts. Even though microfilariae are often called first-stage larvae (L1), they are not as well developed as typical nematode L1 and may also be referred to as advanced embryos or pre-larvae. Microfilariae occur in the blood and are tubular in shape and elongate in size ranging from 180-385 x 3.0-8.3 µm depending on species; e.g. *D. repens* 207-385 µm, *D. immitis* 180-340 µm, *D. corynodes* 250-290 µm, *D. ursi* 185-290 µm, *D. tenuis* 220-250 µm, and *D. striata* 230-240 µm. They are typically unsheathed, as opposed to those of other onchocercid filarial worms, such as *Wuchereria, Brugia,* and *Loa,* whose microfilariae are ensheathed by vitelline eggshell membranes. *Dirofilaria* microfilariae have straight bodies with pointed tapering heads and pointed filamentous tails (compared to *Dipetalonema* and *Acanthocheilonema* microfilariae which have curved bodies with hooked tails). The internal nuclear column (formed by the primordia or anlage of developing organs) also does not extend to the end of the tail inside *Dirofilaria* microfilariae (unlike that of other filarial taxa). In insect (intermediate) hosts, microfilariae mature to L1 and then moult twice to form infective third-stage larvae (L3) measuring from 0.7-1.3 mm in length and possessing a cigar-shaped tail with one terminal and 2 subventral papillae. At this stage, different sexes may be discerned by the position of genital primordia, especially spicule primordia in males. Following transmission back to definitive hosts, infective L3 moult to fourth-stage larvae (L4) measuring from 30-110 mm long and then to sub-adults (effectively L5). Adult worms may grow up to 310 mm in length and 2 subgenera are recognised primarily on the basis of their girth and cuticle morphology: *D.* (*Dirofilaria*) comprising long thin worms (< 0.1 mm in diameter) with smooth cuticles; and *D.* (*Nochtiella*) containing shorter stout worms (0.2-0.5 mm diameter) with cuticles adorned with longitudinal ridges and transverse striations). Worms of both subgenera have a rounded head, a small buccal cavity without lips, a mouth surrounded by 8 small inconspicuous cephalic papillae and 2 lateral amphids, an oesophagus indistinctly divided into anterior and posterior regions, and a short tail (straight in females, spirally-coiled in males). Adult worms are sexually dimorphic in many characters, especially in size with females being larger than males (e.g. *D. immitis* females 210-310 x 1.0-1.3 mm, males 110-200 x 0.6-0.9 mm; *D. repens* females 84-170 x 0.4-0.7 mm, males 39-70 x 0.3-0.5 mm; *D. tenuis* females 80-130 x 0.3-0.4 mm, males 40-50 x 0.2-0.3 mm; *D. ursi* females 180-230 x 0.6-0.8 mm, males 63-93 x 0.3-0.6 mm). In addition to their short spirally-coiled tails, males also have prominent caudal alae, 5 or more large pedunculate precloacal papillae (compared to less than 5 in *Dipetalonema* males), and 2 unequal dissimilar spicules (long left spicule with a cylindrical handle and a membranous lamina, and a short blunt right spicule). Female worms have 2 ovaries each with an oviduct and uterus terminating in a common vagina that opens into an anterior vulva (located postoesophageal). Gravid females produce and release unsheathed microfilariae.

Site of infection: Adult *Dirofilaria* worms are tissue-dwelling parasites that live in the circulatory system or connective tissues of their vertebrate hosts. Mature *D. immitis* worms normally reside in cardiopulmonary arteries of carnivores, but may be found in the right ventricle, right atrium and caudal vena cava of the heart (hence the common name of heartworm), and occasionally in femoral or hepatic arteries. Other species (e.g. *D. repens* and *D. tenuis*) are commonly found in subcutaneous connective tissues of their carnivorous hosts. Microfilariae accumulate in the peripheral blood and are transmitted to blood-feeding dipteran insects where larval development occurs mainly in the Malpighian tubules, and sometimes the fat bodies.

Pathogenesis: Many *Dirofilaria* infections are asymptomatic or subclinical and go unnoticed by their hosts. They are often sporadic and hosts remain amicrofilaraemic. However, infections by some *Dirofilaria* spp. may cause severe disease particularly in carnivores (notably canids, and sometimes felids, otariids and mustelids). Disease severity has been associated with high parasite burdens, long-term chronic infections, and aggressive host immune responses. Foci of clinical infections (hotspots) may arise due to a combination of host, parasite, and environmental factors (especially abundant hosts and competent vectors in favourable climatic conditions). In dogs, heartworm disease primarily involves the cardio-pulmonary system with mild-severe symptoms reflecting pulmonary hypertension and congestive heart failure. Clinical signs include inappetence, loss of condition, weight loss, cough (sporadic progressing to persistent), haemoptysis (coughing blood), dyspnoea (breathing difficulty), tiredness, exercise intolerance, jugular venous distention, cardiac arrhythmias, right-side murmurs and crackles upon auscultation, syncope (collapse), hepatomegaly and ascites. Pathological changes are not due to worm blockages but to a combination of host inflammatory and immune responses. While adult worms have been found to release toxic excretory/secretory metabolites, including vasoactive substances that cause blood vessel constriction, blood flow becomes restricted due mainly to thickening of the arterial walls due to endothelial (intima) proliferation, inflammation (arteritis) and fibrosis, often exacerbated by infiltrates of host immune cells (leucocytes, esp. eosinophils releasing destructive enzymes). Platelets may also be activated leading to disseminated intravascular coagulation. Dead and dying worms may cause granulomatous and villous inflammation and thrombosis. Thickened arteries become constricted, narrow, and occluded with reduced elasticity and turbulent blood flow. This results in pulmonary hypertension which eventually causes compensatory right heart enlargement (right ventricular dilatation and hypertrophy), cardiac insufficiency and possibly congestive heart failure. Thromboembolisms further contribute to the pulmonary hypertension. The liver may become enlarged due to chronic venous congestion (consistent with right-sided heart failure). In some instances, the presence of worms in the right atrium and venae cavae may cause caval syndrome characterised by inflow obstruction to the right heart, tricuspid insufficiency, hypertension, ascites, and haemolytic anaemia (the latter due to the lysis of fragile erythrocytes when passing through the mesh of tangled worms and fibrin deposits). Dogs present with dyspnoea, tachypnoea, cardiac thrill with heart murmur, jaundice, and haemoglobinuria. Heartworm infections may also lead to renal disease with glomerulonephritis and proteinuria occurring due to immune complex (antigen-antibody) formation. Occasionally, broncho-arterial fistulas may develop when weakened arteries rupture into the airways leading to marked haemoptysis especially after exercise. Rare ectopic infections may also result from aberrant migration of worms into the eye, brain, liver, peritoneal cavity, or skin. Infections are more prevalent and severe in dogs > 2 years of age, in larger breeds of dogs, and in dogs housed outdoors. While cats are atypical hosts and more resistant to *D. immitis* infections than dogs, they have comparatively smaller pulmonary trees so even a small number of worms can cause pulmonary manifestations. Clinical signs include peracute respiratory distress, tachypnoea, coughing, haemoptysis, vomiting unrelated to eating, seizures, collapse, and sudden death. Pathological changes are generally insufficient to cause persistent pulmonary hypertension or congestive heart failure. Instead, immature worms may produce an acute inflammatory reaction known as heartworm-associated respiratory disease, often misdiagnosed as allergic bronchitis or asthma. Degenerating worms have also been associated with symptoms similar to acute respiratory distress syndrome, involving pulmonary inflammation, oedema and thromboembolism contributing to respiratory failure. Infections in cats rarely cause caval syndrome, but even a few worms may cause tricuspid valve regurgitation with cardiac thrill and heart murmur. Ectopic infections have been detected in cats in systemic arteries in the central nervous system or body cavity. Several *Dirofilaria* spp. have been detected in humans: mostly *D. immitis* and sometimes *D. tenuis*, *D. ursi*, *D. striata* and *D. spectans* in the Americas; *D. repens* in Eurasia; and *D. hongkongensis* and *D. magnilarvatum* in Asia. Infections in humans appear to be accidental as worms do not mature or produce microfilariae. However, migrating and/or degenerating worms may cause pulmonary or subcutaneous lesions. *Dirofilaria immitis* and *D. repens* may cause nodular pulmonary granulomas resulting in chest pain, fever, cough, haemoptysis, and dyspnoea. *Dirofilara repens, D. tenuis* and other zoonotic species may cause wandering lesions and pruritic dermatitis in subcutaneous tissues, and occasionally in submucosal breast tissues, which eventually encapsulate into granulomatous nodules. Ectopic infections have been detected infrequently in humans in the conjunctiva, meninges, liver, testicles and mesenteric adipose tissue.

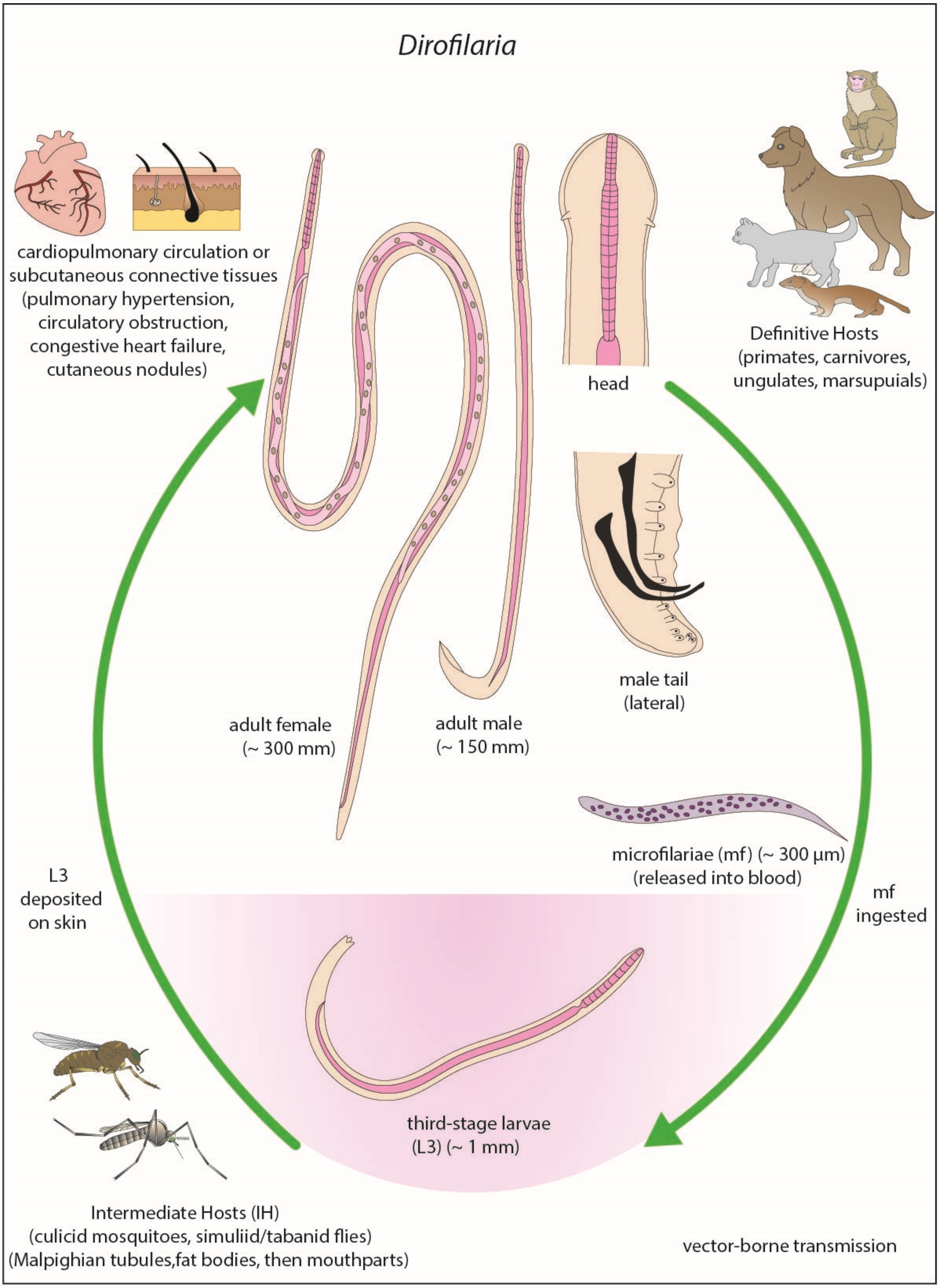
The recent detection of the rickettsial α-proteobacteria *Wolbachia pipientis* as intracellular endosymbionts in *D. immitis* provides somewhat of a conundrum between disease causation and treatment. The bacteria are present in all parasite stages and are considered to be essential for their normal development and survival (mutualistic symbiosis). Heartworm parasites cause significant inflammation in their vertebrate hosts in the form of proliferative pulmonary endarteritis, particularly when worms are dying and degenerating. Surface proteins from exposed (extracellular) bacteria have been shown to up-regulate host inflammatory responses, thus contributing to disease. Killing worms with anthelmintics and killing bacteria with antibiotics may have the unintended consequences of exposing the host to a flood of pro-inflammatory disease-causing substances, albeit transiently.

Developmental cycle and mode of transmission: *Dirofilaria* spp. exhibit indirect heteroxenous life-cycles with adult worms forming in vertebrates (carnivores and primates acting as definitive hosts) and larval stages developing in invertebrates (insects acting as intermediate hosts). Cyclic transmission between hosts is vector-borne and associated with haematophagous feeding behaviours. Mature female worms release microfilariae which accumulate in the peripheral circulation and are ingested by blood-feeding dipteran insects. Most *Dirofilaria* spp. are transmitted by mosquitoes, notably female mosquitoes which require protein-rich blood meals for reproductive purposes. *Dirofilaria immitis* infections may utilize more than 70 species of culicid mosquitoes as intermediate hosts (including *Aedes, Anopheles, Armigeres, Coquilletidia, Culex, Culiseta, Mansonia, Psorophora, Wyeomyia* spp.), but most infections are transmitted by a few major vectors which vary according to their geographic distribution (including *Ae. albopictus, Ae. canadensis, Ae. caspius, Ae. notoscriprus, Ae. trivittatus, Ae. vexans, An. annulipes, An. maculipennis, An. punctipennis, An. quadrimaculatus, An. walkeri, Cx. annulirostris, Cx. pipiens, Cx. quinquefasciatus, Cx. theileri* and *Cq. richiardii*). A few *Dirofilaria* spp. do not use mosquito vectors, instead *D. ursi* is transmitted by simuliid flies and *D. roemeri* by tabanid flies. In all cases, ingested microfilariae migrate from the insect midgut through the haemocoel to the Malpighian tubules, except *D. corynodes* and *D. roemeri* which invade the fat body (in contrast, *Brugia* and *Wuchereria* larvae develop in the thoracic flight muscles of mosquitoes). The microfilariae form sausage-shaped first-stage larvae (L1) in 3-5 days, which then moult to form second-stage larvae (L2) in 7-10 days and then third-stage larvae (L3) in 11-16 days which migrate through the haemocoel to the mosquito's proboscis. Larval development takes longer when ambient temperatures are cooler, with the prepatent period in mosquitoes (time from infection to generation of infective L3) being extended up to 30 days at temperatures around 20°C, and even being arrested at temperatures lower than 15°C. Transmission occurs during blood-feeding when infective L3 exit the insect's labial sheath and are deposited on the skin of the mammalian host where they invade the puncture wound (contaminative transmission rather than inoculative salivary transmission). The larvae move into adjacent tissues and moult into fourth-stage larvae (L4) within a week. The L4 moult into young adults in submuscular membranes or subcutaneous tissues over 6-8 weeks and then migrate to their final predilection sites (arteries and/or connective tissues depending on species). Young *D. immitis* adults penetrate the vasculature and migrate to pulmonary arteries and the right heart 10-16 weeks after infection and reach maturity in another 8 weeks. Young *D. repens* and *D. tenuis* worms migrate primarily to subcutaneous connective tissues where they mature over several months. Following mating, female worms produce and release microfilariae which accumulate in the bloodstream. The prepatent period (time from infection to first release of microfilariae) ranges from 6-9 months and adult worms may live for up to 10 years while microfilariae may remain in the circulation for up to 2 years.

Differential diagnosis: Heartworm infections may be suspected on the basis of symptomatology involving signs of pulmonary or subcutaneous disease, particularly coughing and exercise intolerance in dogs infected with *D. immitis*, or pruritic dermatitis in humans infected with *D. repens*. Nonetheless, clinicians should pay careful attention to relevant patient history (geographic location, potential exposure, prophylaxis) as many other disease conditions may cause similar signs. Most clinical parameters (haematological and biochemical) are non-specific, so infections are generally confirmed by the microscopic detection of microfilariae in peripheral blood samples, either in wet mounts, fixed thick or thin smears, or concentrates obtained by membrane filtration (5 µm pore size), haematocrit tube centrifugation (microfilariae band immediately above buffy coat) or sedimentation (following haemolytic treatment with formalin (Knott's method), saponin or hypotonic saline). Permanent mounts may be stained with Giemsa, Delafield's haematoxylin or Field's rapid stain, while live organisms may be stained supravitally with cresyl blue or methylene blue. Some laboratories also stain microfilariae for alkaline phosphatase activity (*D. immitis* shows activity at the anal and excretory pores, *D. repens* only shows activity at the anal pore, while *Acanthocheilonema reconditum* shows diffusely distributed activity). *D. immitis* microfilariae are distinguished from those of other filarial worms by their large straight bodies with tapered heads and pointed tails and they exhibit nonprogressive (stationary) movement in wet preparations (in contrast, those of *Dipetalonema/Acanthocheilonema* have medium curved bodies with blunt heads and hooked tails and by their progressive movement in wet preparations). Caution is advised when interpreting the results of microfilarial examinations as quantitative studies have shown that the degree of microfilaraemia (number of circulating microfilariae) bears little relationship to the intensity of infection (number of adult worms). Indeed, many hosts have occult infections (adults present but no circulating microfilariae) for a variety of reasons: such as only one worm being present; all worms being the same sex; young worms not yet producing microfilariae; old worms no longer producing microfilariae; host immune responses rapidly clearing microfilariae; previous drug treatment(s) eliminating microfilariae or sterilizing adult worms; or worms infecting ectopic sites. On occasion, adult worms or fragments thereof may be examined after their collection by biopsy of subcutaneous or pulmonary lesions or during necropsy.

Worms may be identified by their characteristic morphotypic features, especially with respect to size, cuticular features, lateral chords and somatic muscle development. Over the last several decades, recourse has been made to the immunological detection of either host antibodies or parasite antigens in blood samples. Several generations of antibody tests were developed, most based on enzyme immunoassays, but many demonstrated poor diagnostic sensitivity and specificity due to antigenic cross-reactivity, the absence of antibodies in the first 6-8 weeks of infection or the persistence of antibodies for years after infections have been eradicated. Several generations of antigen tests were then developed using polyclonal or monoclonal immunoreagents in enzyme immunoassays or immunochromatographic tests. These antigen tests demonstrated a good threshold of detection (able to detect one worm) and little cross-reactivity with other nematodes (especially tests using monoclonal reagents). However, false negative results may occur as many tests use glycoprotein antigens from the reproductive tracts of female worms and therefore will only detect mature infections (> 6 months). False positive results may also occur occasionally when antigens persist in the circulation although adult worms are dead. Modern medical imaging techniques, including X-ray (radiography), ultrasound (sonography, echocardiography), computed tomography (CT) scans and magnetic resonance imaging (MRI), have been used to detect characteristic pathological changes to pulmonary arteries (increased size, tortuosity, 'coin' lesions) or to the heart (right-sided cardiomegaly), and sometimes to reveal worms themselves in arteries (the worm cuticle is hyperechogenic and may be visualized as parallel lines). More recently, molecular biological techniques have been used to detect parasite DNA in fresh, frozen, or fixed blood/tissue samples following polymerase chain reaction (PCR) amplification of specific nuclear gene sequences (ribosomal RNA, internal transcribed spacer regions, cuticular antigens with multiple tandem repeats) and mitochondrial gene sequences (12S mtRNA, cytochrome c oxidase 1).

Treatment and control: Existing heartworm infections can be treated chemotherapeutically, but different anthelmintic drugs are used to kill adult worms and developing larvae. Animals should be tested before treatment to determine their infection status, so the correct treatment is used. Animals should also be hospitalized during and shortly after adulticide treatment as the risk of pulmonary embolisms due to fragments of dying worms is high and supportive therapy may be required to manage shock, including intravenous corticosteroids, balanced electrolyte solutions, bronchodilators, and oxygen therapy. The drug of choice for killing adult *D. immitis* worms > 4 months old is the organoarsenical melarsomine, but it does not act on microfilariae. Previously, another organoarsenical thiacetarsamide was used but it was not well tolerated and caused hepato-renal toxicity. Treatment should involve multiple graded doses, so worms are eliminated in stages, rather than single heroic doses which often causes complications. Side-effects to adulticide treatment include rapid onset of pulmonary oedema (managed with dimercaprol), hypersensitivity (managed with corticosteroids or non-steroidal anti-inflammatory drugs), but mostly pulmonary thromboembolisms usually occurring 5-21 days after treatment (minimised using anti-thrombotic and anti-inflammatory drugs). Some degree of pulmonary thromboembolism will occur as worms are destroyed so supportive therapy should be continued for several weeks after adulticide treatment. Animals should not be exercised during this recovery period and cage rest may be warranted for boisterous animals. In some instances, pre-treatment with drugs active against developing larvae may be used prior to adulticide treatment to ensure infections do not re-establish (the risk of delaying adulticide treatment should be considered on a case-by-case basis). The most commonly used pre-treatment drugs are macrocyclic lactones (e.g. milbemycin) which are active against L3, L4, sub-adult worms as well as microfilariae. A complication of such therapy in microfilaraemic dogs is an anaphylactic reaction to antigens released from microfilariae, especially in small dog breeds with heavy parasite loads. Dogs have also been pre-treated with tetracycline antibiotics (doxycycline) which act against the endosymbiotic *Wolbachia*-bacteria and cause worm sterility. However, bacterial surface proteins have been implicated in pro-inflammatory host responses and may contribute to heartworm disease pathogenesis. Nevertheless, heartworm-positive dogs pretreated with macrocyclic lactones and doxycycline prior to melarsomine treatment had less pulmonary pathology associated with worm death. An alternative soft-kill approach using monthly macrocyclic lactones (ivermectin) with antibiotics (doxycycline) to slowly kill worms over 9-12 months was trialled, but it was not as effective as melarsomine treatment and has mostly been discontinued. In dogs in which caval syndrome has developed, recourse is made to the surgical removal of worms occluding the vena cava and/or the right atrium. In humans, surgical removal of subcutaneous nodules and even lung granulomas is the preferred option. The prevention of infections is based around chemoprophylaxis, vector control and protective barriers. Anthelmintic drugs that are active against developing larvae include the macrocyclic lactones (ivermectin, milbemycin, moxidectin and selamectin) and the diethylenediamine (diethylcarbamazine, DEC). The former are available as topical, oral or injectable formulations, usually applied monthly (injectables yearly), while DEC is available orally but must be given daily or on alternate days (missing a dose temporarily voids protection). DEC is currently being phased out due to the strict treatment regimen and adverse side effects, notably hypovolaemic shock and death. Dogs should be tested for infections before beginning chemoprophylaxis and treated with adulticides if infected. In endemic areas, puppies should commence preventive medication no later than 8 weeks of age. Various strategies are available for controlling insect vector populations, including the use of insecticides to kill adult mosquitoes, the elimination of mosquito breeding sites, and using protective screens, clothing or insect repellents to avoid mosquito bites. Public education campaigns have done much to raise public awareness and protect pets at risk.



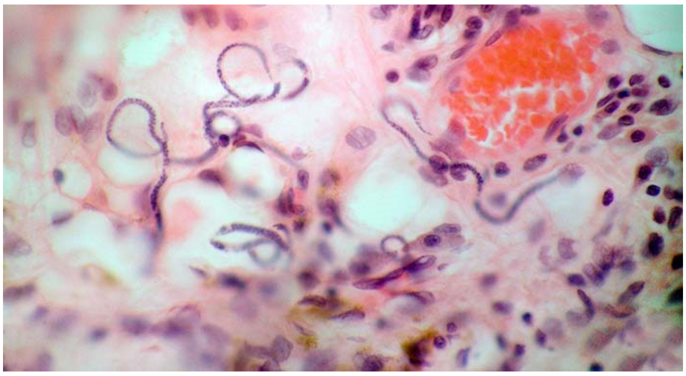
Dirofilaria
cardiopulmonary circulation or
subcutaneous connective tissues
(pulmonary hypertension,
circulatory obstruction,
congestive heart failure,
cutaneous nodules)
head
Definitive Hosts
(primates, carnivores,
ungulates, marsupuials)
male tail
(lateral)
adult female
(~ 300 mm)
adult male
(~ 150 mm)
microfilariae (mf) (~ 300 µm)
(released into blood)
L3
deposited
on skin
mf
ingested
third-stage larvae
(L3) (~ 1 mm)
Intermediate Hosts (IH)
(culicid mosquitoes, simuliid/tabanid flies)
(Malpighian tubules,fat bodies, then mouthparts)
vector-borne transmission

Dirofilaria adult worms

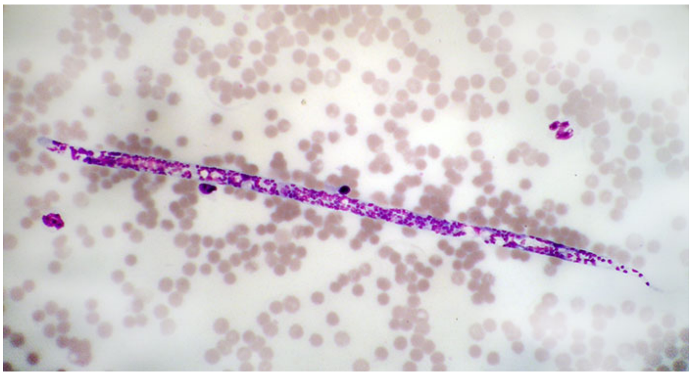
Dirofilaria adult worms

Dirofilaria adult worm, head

Dirofilaria adult worm, male tail



Dirofilaria microfilariae in skin



Dirofilaria microfilaria in blood