Dirofilariasis

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Learning Objectives

1. To ascertain that as a zoonotic filarial infection, the worms in humans do not produce larval microfilarial forms.
2. To understand the pathology of dirofilariasis in the infected lungs in humans.

Introduction

Dirofilariasis is an arbohelminthic disease of zoonotic importance, caused by *Dirofilaria* spp. *Dirofilaria immitis* and *Dirofilaria repens* are the most well-known species, with a diverse impact on both human health and veterinary health. While *D. immitis* is responsible for canine heartworm disease and human pulmonary dirofilariasis over wide geographic regions, *D. repens* typically causes subcutaneous dirofilariasis in both. *Dirofilaria* sp. is a also potential health concern for travellers also.

History

Amato Lusitano was probably the first to describe a girl with worms in her eyes in 1566, possibly *D. repens*. However, the first human dirofilariasis case was documented by De Magelhaes in 1887 from the left ventricle of a Brazilian boy on post-mortem examination. Subsequently, several reports originated from Europe regarding human ocular and subcutaneous infections. Canine heartworms were first discovered in 1856 on the south-east coast of the USA. Ercolani pointed out the fact that in microfilaraemic dogs, worms may be found in subcutaneous tissues apart from the heart. Grassi showed experimental transmission of parasites to mosquitoes in 1900. In the next 10 years, Demiaszkiewicz (2014) first described the nematode and named it *Dirofilaria repens*. Years later, the larval development in vector mosquitoes was published. In 1921, infection in cats was recognized. It was not until 1952 that human infection by *D. immitis* was documented in the USA. *Wolbachia* sp., an endosymbiont bacteria residing in filarial worms, was first discovered as bacterium-like bodies in *D. immitis* by electron microscopy in the 1970s. Later, in 1995 16S rDNA-based phylogeny of *Wolbachia* sp. was published from Italy during ongoing studies on *D. immitis*.

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Taxonomy

The genus *Dirofilaria* belongs to subfamily Dirofilariinae; family Onchocercidae; superfamily Filarioidea; suborder Spirurina; order Rhabditida; class Chromadorea and phylum Nematoda.

The genus *Dirofilaria* is divided into two subgenera: *Dirofilaria* (including *D. immitis*) and *Nochtiella* (more than 20 species including *D. repens*, *Dirofilaria striata*, *Dirofilaria subdermata*, *Dirofilaria sudanensis*, *Dirofilaria tawila*, *Dirofilaria tenuis* and *Dirofilaria ursi*).

Phylogenetic analysis of cytochrome c oxidase 1 gene is utilized for the identification of *Dirofilaria* sp. Studies revealed low genetic variability among *D. immitis* isolates over several countries compared to *D. repens,* which shows high intra-species variability. Recently, a *D. repens*-like filarial worm has been described as *Candidatus Dirofilaria hongkongensis* based on ITS-1 sequence difference. Yilmaz E et al. studied complete mitochondrial genomes of *D. repens* and this proposed new member and found their sequences clustered together as a common sister group to *D. immitis*. Their study strengthens the hypothesis that *C. D. hongkongensis* might be an independent species. That study also described microfilaria from Thailand, which could be another cryptic species *Candidatus Dirofilaria* sp. 'Thailand II' or a divergent population of *C. D. hongkongensis*.

Genomics and Proteomics

High-throughput Illumina technology has delineated *D. immitis* genome with assembly size of 78.16 MB with GC content of 28.3%. Interestingly, sequence analysis of *D. immitis* isolated from different countries showed very little (0.04%) genetic variation. Also, it harbours neither DNA transposons nor any active retrotransposon. Differential presence of home and nucleotide synthesis pathways possibly points out the metabolic mutualism between *D. immitis* and *Wolbachia* sp., endosymbiont bacteria. Very recently, *D. repens* genome was analysed revealing 17% larger size (99.59 MB) with fewer overlapping consensus regions of DNA or contigs (916 versus 11,654) and 0.7% lower GC content. Fewer proteins could be predicted compared to *D. immitis* (11,262 versus 12,344) as the protein-coding sequence was shorter (15.5% versus 18%). The *D. repens* genome contains a larger number of exons per gene (7 versus 5) than *D. immitis* does, exons being slightly shorter (136 bp versus 142 bp). This difference could be meaningful to explain their biological difference. Of identified proteins, 1.8% were dissimilar to *D. immitis,* but a few of them were biologically similar to *Loa loa*.

The genomic similarity of *D. repens* to *Loa loa* was also reflected in proteomic studies. Significant enrichment of *D. repens* proteins is in stark contrast to those of *D. immitis*. *D. immitis* proteins get clustered with complete nematodal proteome and included 3199 proteins (31% of total proteome) unique to *D. immitis*, a proportion similar to *B. malayi* (27%). Interestingly, 850 proteins are uniquely shared by both of them. Myosin-like antigen OVT1 in *O. volvulus* has two homologues in *D. immitis*, particularly in third- and fourth-stage larvae. Mass spectrometry data group the protein moieties in major four categories. Of these, two groups contain enzymes for anaerobic glycolysis and for the redox reactions and detoxification. Another group consists of actin-1, actin-2 and other molecules involved in motility. Heat shock proteins (HSP70, p27, etc.) belong to the fourth category. Overall, a significant proportion of *D. immitis* proteins are collagenase-susceptible acidic polypeptides ranging from 82 kDa to >200 kDa. A 35 kDa polypeptide has been identified as an immunodominant surface antigen in third-stage larvae (L3). Although abundant glycosylated molecules are found in the extract, these are not exposed on the surface of the intact worm.

The Parasite Morphology

Adult Worm

The adult worm is long, thin, cylindrical and whitish in colour.

Dirofilaria immitis: Adult female worms measure 230–310 mm in length and 1.0–1.3 mm in thickness. The terminal oral aperture lacks lips but is surrounded by 6 small median papillae and 2 lateral papillae. An anal opening is located subterminally at the obtuse caudal end. The vulva opens posterior to the oesophago-intestinal junction. These are ovoviviparous. Adult males are smaller and thinner, measuring 120–200 mm in length and 0.7–0.9 mm in thickness. A spirally coiled tail-end harbouring two lateral alae characterizes the male adult *D. immitis*. The cloacal opening is located near the caudal end (0.13 mm proximally). Three groups of papillae are located on the ventral side around it. The cuticle is smooth with a striated ventral surface of the last coil of the caudal end (Fig. 1).

Dirofilaria repens: *D. repens* adults are smaller and stubbier than *D. immitis*. The cuticle contains the characteristic striations. Adult females measure 100–170 mm in length and 4.6–6.3 mm in thickness. Females are ovo-viviparous. The vulval opening is encircled by slightly projecting labia and is situated 1.84–1.92 mm from the cephalic end. The tail tip is obtuse and curves slightly to the ventral side. Adult males are 50–70 mm long and 3.7–4.5 mm thick. The ventrally curved caudal end bears 2 lateral alae and oblong pedunculate papillae.

Microfilariae

Dirofilaria microfilariae are devoid of a sheath unlike other microfilariae including *Acanthocheilonema dracunculoides* and *Cercopithifilaria grassii* found in infected dogs and cats (Fig. 2). Microfilariae of *D. immitis* are slightly shorter and thinner than those of *D. repens*. Microfilariae of *D. immitis* measure 290–330 μm in length and 5–7 μm in width, whereas those belonging to *D. repens* are 300–360 μm long and 6–8 μm thick. These can be differentiated by looking at the cephalic end, which is pointed in the former and obtuse in the latter. Also, the former has a pointed straight tail compared to a filiform/umbrella handle-like tail in *D. repens*. Sometimes non-sheathed microfilariae of *Acanthocheilonema reconditum* may be confused with these but the cephalic hook-like structure distinguishes it. Histochemical staining reveals two acid phosphatase activity spots in *D. immitis* microfilariae corresponding to the anal and excretory pores. Only one such spot is visible in *D. repens* microfilariae (anal pore), while *Acanthocheilonema* sp. shows spots over the whole microfilaria body. *D. striata* microfilariae measure 299 μm × 5–6.5 μm and are characterized by two prominent nuclei separated from the main body of the nuclear column within the cephalic space. *D. tenuis* microfilariae are longest, measuring 361–379 μm in length with a thickness of 7 μm.

Cultivation of Parasites

In vitro cultivation of filarial worms is difficult due to poor survival and developmental arrest. Attempts have been made to maintain *D. immitis* adult worms in a variety of media to extrude microfilariae. Sawyer and Weinstein (1963) first described successful development of microfilariae of *D. immitis* to the sausage-shaped late first-stage larva after inoculating host erythrocytes in a serum-supplemented chemically defined media NCTC 109. Insect mediums MM/MK and MM/VP_{12} improved survival up to 7 days without any development.

Laboratory Animals

Ferrets (*Mustela putorius*) have been utilized as the hosts for heartworm research. Upon a

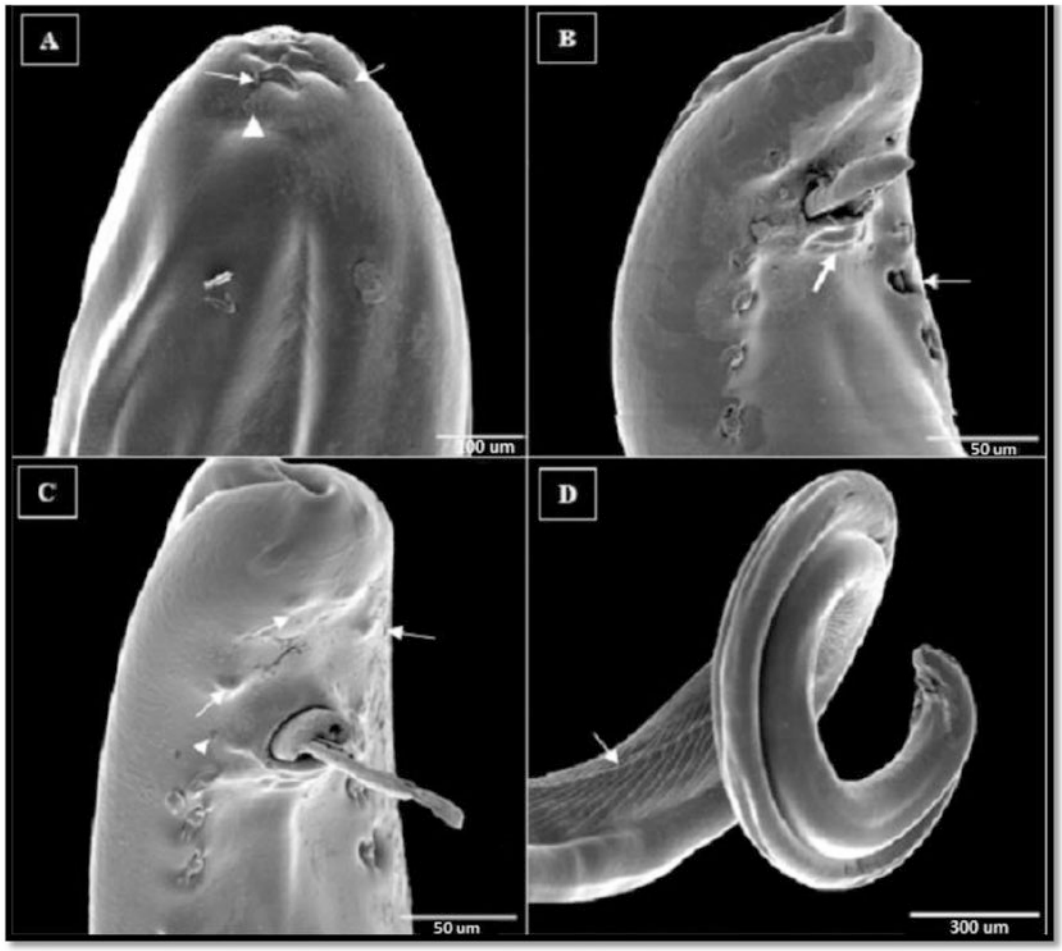

Fig. 1 Scanning electron micrographs of male *Dirofilaria immitis* showing (**a**) cephalic end with oral aperture details; (**b**), (**c**) and (**d**) showing posterior part ventral view showing papillae, anus, cloacal bumps, spicules and longitudinal striations. Image reproduced with permission from AR Meamar. Citation: Iranian Journal of Parasitology. 2020;15(1):57–66

successful infection, the adult worms are found mainly in the heart chambers and the associated veins and pulmonary arteries. Vena cava syndrome occurs commonly. Disease manifestation is similar to dogs but with a faster progression. The low parasitic load may cause death by pulmonary embolism. BALB/c mice have been utilized as animal models for immunological studies involving *Dirofilaria immitis*.

Life Cycle of *Dirofilaria* spp.

Hosts

Definitive Hosts

A variety of mammals including carnivores, primates and dogs are definitive hosts. Humans are the incidental hosts (Fig. 3).

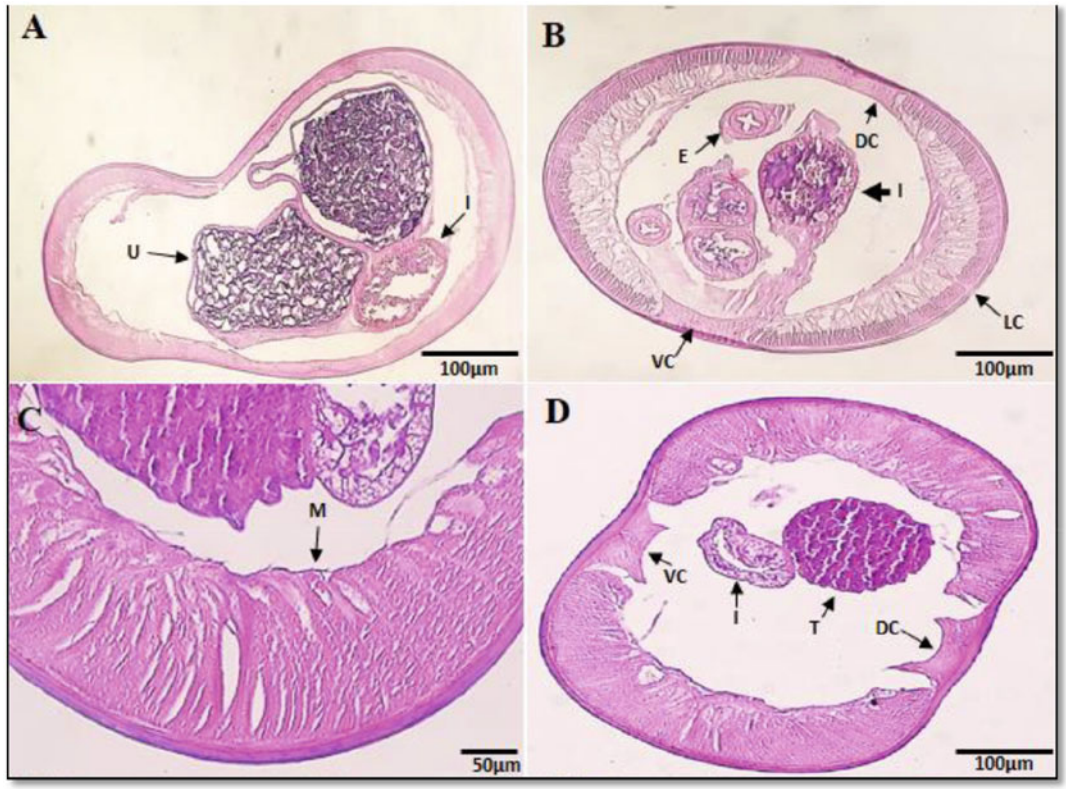

Fig. 2 Adult *Dirofilaria immitis* cross-sectional anatomy (**a**–**d**, **h** & **e**): (**a**) female (**b**) male. *DC* dorsal cord, *E* oesophagus, *I* intestine, *LC* lateral cord, *M* tail end muscle layer, *T* testis, *VC* ventral cord, *U* uterus. Image reproduced with permission from AR Meamar. Citation: Iranian Journal of Parasitology. 2020;15(1):57–66

Intermediate Hosts/Vectors

Aedes, *Anopheles*, *Culex*, *Culiseta* and *Mansonia* sp. are the arthropod mosquito vectors. The most important intermediate hosts are those species without the buccopharyngeal armature that damages the microfilarial cuticle.

Infective Stage

Third-stage larvae (L3) are the infective stage.

Transmission of Infection

A typical sylvatic life cycle involving carnivorous mammals is common. All the species depend on an arthropod vector (mosquito) to be infected by infective third-stage larvae (L3) during a blood meal from mammals. These L3 larvae invade on their own into the soft tissues of skin. Several reach the muscle sheaths too. These are the locations for moulting and maturing, which takes around 4 months. Thereafter, migration to the heart begins. Studies suggest that in six months, they fully mature into sexually competent adults and mate inside the pulmonary arteries. After mating, the female becomes gravid and begins to release microfilariae into the bloodstream. The mosquito takes the blood meal and gets infected. Up to several thousand microfilariae (first-stage larvae or L1) are shed daily. An infected dog may circulate several hundred microfilariae per ml of blood. Ingested L1 larvae reach malpighian tubules and undergo two subsequent temperature-dependent moultings to develop into third-stage larvae (L3). L3 larvae migrate to the labial sheath lumen in the vector mouthpart to initiate the cycle again.

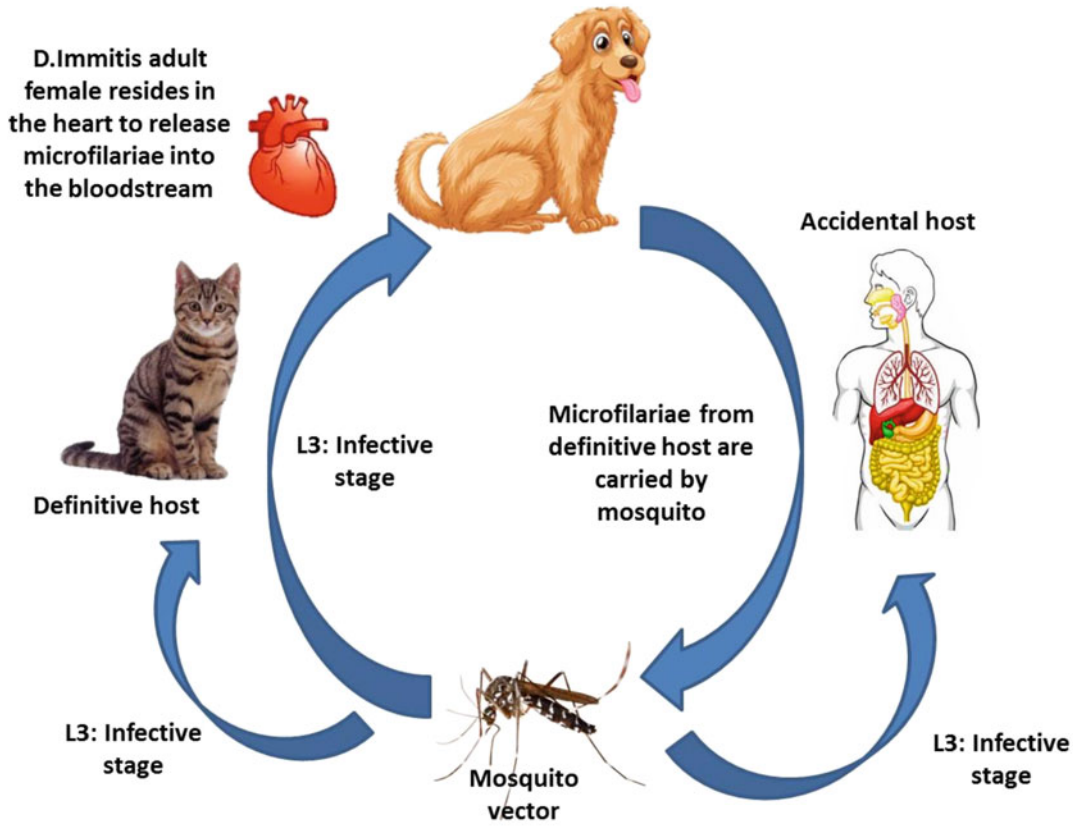

Fig. 3 The life cycle of *Dirofilaria immitis*

Humans act as an incidental host. The larvae embark on their journey but fail to mature. Their premature death results in granuloma formation in different internal organs and subcutaneous tissue.

Pathogenesis and Pathology

Lesions in animals predominantly occur in the pulmonary vessels and lung by *D. immitis*. They cause parasite load-dependent pulmonary hypertension ultimately leading to congestive heart failure. Generally, the larger right caudal lobar artery accumulates more worms than the left. The earliest lesions are characterized by endothelial cell junction disruption/dislodgement and denudation of the intimal surface. Intimal thickening narrows down the vascular lumen to cause pulmonary hypertension. The condition is associated with physical trauma, and metabolic and immune-mediated cytotoxicity by the parasite. In the cross-section, these ridges have a villous appearance, which is considered pathognomonic. Pulmonary blood flow is impeded primarily by a reduction in the cross-sectional area of the arterial vascular bed due to obliterative endarteritis of small peripheral branches. Eventually, with an increase in infection, the pulmonary vascular resistance becomes fixed and congestive cardiac failure gets manifested. Microfilariae play a minor pathogenic role in constituting pneumonia and glomerulonephritis.

Pathologically, the presence of a nodule is the characteristic. The nodule represents a pre-adult worm trapped in the defensive immune reactions causing ultimate death and disintegration of the

parasite. Histopathologically, four types of patterns are seen: (a) abscess type, the majority, characterized by necrotic matter containing neutrophils and eosinophils surrounding the nematode; (b) central zone containing nematode surrounded by epithelioid cells, histiocytes and foreign body giant cells; (c) decomposed nematode surrounded by occasional inflammatory cells inside fibrous tissue, mostly seen in lung; and (d) mixed pattern where nematode is surrounded by necrotic leucocytic infiltration with demarcating fibroblastic elements, seen in breast, epididymis, spermatic cord and mesentery.

Wolbachia spp. plays a key role in the pathogenesis of dirofilariasis. *Wolbachia* is the endosymbiont alpha-2 proteobacteria belonging to the *Rickettsiales* order inhabiting filarial worms, hexapods, crustaceans, etc. Studies suggest their role in moulting and embryogenesis of filariae. They are also found in hypodermal cords of adults of both genders and the female genital organs, suggesting their role in the long-term survival of adult worms. Experimental data suggest that *Wolbachia* provides the haem group to filariae, which is essential for cytochrome P450. Tetracycline antibiotics can block the intrauterine development of *D. immitis* microfilariae by depleting *Wolbachia*. *Wolbachia* surface proteins take part in the immunopathogenesis of dirofilariasis.

Immunology

The host–parasite relationship in dirofilariasis is immunologically complex due to (a) the wide range of hosts involved; (b) the presence of endosymbiont *Wolbachia* sp. contributing additional sets of antigens; and (c) immune evasion mechanisms.

Cell-mediated immunity plays a minor role as it gets obtunded by *D. immitis* proteins with detoxification and antioxidant properties.

Different antibodies of class IgM, IgG and IgE have been observed against every developmental stage with the highest levels corresponding to microfilaremia. While antibody-mediated complement activation and antibody-dependent cellular cytotoxicity provide a defence against microfilaria, they are ineffective against adult worms. Dead microfilaria and adult worms upon disintegration release *Wolbachia* spp. into the bloodstream. *Wolbachia* surface protein (WSP) is a potent immunogen and has been implicated in granuloma formation. Polyclonal antibodies against WSP have been detected in multiple tissues and immune cells of heartworm-infected dogs. Compared to dogs, a stronger immune response is seen in cats and possibly in humans, making them relatively unfavourable hosts.

Different subclasses of antibodies are noted in human pulmonary dirofilariasis cases with IgE, IgM and IgG antibodies formed against the excretory/secretory (E/S) antigens. Antibodies of IgG in nature against WSP are seen significantly associated with pulmonary dirofilariasis but not in *D. repens*-associated subcutaneous dirofilariasis.

The pulmonary nodule in human dirofilariasis cases is characterized by anIgG1-based pro-inflammatory response to WSP in comparison with IgE-based Th2 response against the parasitic proteins (aldolase and galectin) in those without pulmonary affection. Experimental data suggest that WSP contributes to the extension of the inflammation by the promotion of neutrophil chemotaxis and inhibition of apoptosis. Also, *Wolbachia* spp. possibly interacts with macrophages via lipopolysaccharide receptors.

Important immune evasion mechanisms employed by *Dirofilaria* spp. include (a) a short-term mechanism in L3 larvae by releasing large amounts of 6 kDa and 35 kDa surface antigens and (b) a long-term mechanism by pre-adult and adult worms by masking their body surface with glycolipids and heat shock proteins. E/S antigens also stimulate prostaglandin E2 and plasmin activation and retard monocyte transmigration.

Infection in Humans

Humans are considered to be accidental and dead-end host in dirofilariasis, although mature female *D. repens* carrying microfilariae have been reported in the literature. Pulmonary nodules are

commonly seen with *D. immitis* infection. These are frequently misdiagnosed as malignancy owing to their typical asymptomatic nature and incidental radiological discovery. A single peripherally located pulmonary nodule of diameter 1–3 cm is commonly seen; even five nodules have been described in a single person mimicking metastasis, histoplasmosis and Wegener's granulomatosis. Right lung and subpleural regions are frequent sites. Non-specific symptoms like chest pain, cough and haemoptysis are usually seen. Rarely, pleural effusion may be seen.

Subcutaneous dirofilariasis cases including ocular/periorbital cases have been reported extensively. These present as insidiously growing subcutaneous firm nodules. Mostly female individuals above the age of 40 years are affected, except in Sri Lanka where children also acquire the disease. Mostly the nodule is located at subcutaneous tissue, deep dermis, or submucosa, and rarely in muscle, lymph node or deep viscera. The upper half of the body (including periorbital) and upper limbs are more frequent sites.

D. repens has been the commonest pathogen in this scenario along with *D. tenuis*, *D. ursi*, *D. subdermata*, *D. striata* and *D. immitis* causing a minority of cases. A parasite nodule is always present except localization in subconjunctiva where it could be migratory and not trapped by the host's reaction. Data suggest the speed of migration in subcutaneous tissue could be 30 cm in 2 days, which is facilitated further by hot compress and ultrasound therapy. This could very well present as delusional parasitosis cases. Orbit, eyelid, subconjunctiva and intravitreous tissues are commonly affected, causing mild visual dimness and floaters to grave complications like cataract and retinal detachment. Subcutaneous *D. immitis* has been reported to involve liver, mesentery, conjunctiva, ocular chambers and even testicular arteries. The male external genitalia along with the spermatic cord and female breast have been affected by *D. repens*. Rarely, *D. repens* has been isolated from the lungs.

Infection in Animals

Canine cardiopulmonary dirofilariasis is a potentially lethal disease in dogs caused by adult *D. immitis*. The disease has a chronic course starting from the pulmonary arteries to lung parenchyma and right side of the heart. Worms cause proliferative endarteritis of the pulmonary arteries. Tunica intimal hypertrophy coupled with mechanical trauma leads to perivascular seepage of plasma proteins and blood cells into the lung parenchyma. Severity is proportional to the duration of infection, parasite load and host immune response. Affected arterial walls become rough and velvety and get ruptured resulting in haemoptysis and severe lung haemorrhage. Thromboembolism develops following death and disintegration of the worm resulting in severe inflammation. Inflammation along with arterial narrowing gives rise to pulmonary hypertension causing circulatory overload and tricuspid valve dysfunction. All these lead to congestive cardiac failure.

Immune-mediated glomerulonephritis is seen. The presence of IgG antibodies against *Wolbachia* in urine corresponded to microfilariae in renal capillaries. Heartworm-associated respiratory disease with primary pulmonary involvement is the characteristic clinical presentation in infected acts. Subcutaneous/ocular dirofilariasis is most commonly seen in dogs and is caused by *D. repens*, and very rarely by *D. immitis*.

Epidemiology and Public Health

Human dirofilariasis has been reported sporadically from different countries. Compared to *D. repens*, which is exclusive to the Old World, *D. immitis* has wider distribution globally (Table 1, Fig. 4). Cases have been reported sporadically from Costa Rica, Argentina, Venezuela and Colombia. Subcutaneous/ocular dirofilariasis due to rare species like *D. tenuis* and *D. ursi*-like species has been reported from North America. In the last decade, a greater rise of subcutaneous/ocular dirofilariasis cases has been attributed to

Table 1 Distribution of some *Dirofilaria* species of importance in humans

Species	Major distribution	Recognized vectors	Usual definitive host
Dirofilaria immitis	Temperate and tropical areas	Mosquito (*Aedes, Anopheles, Culex, Culiseta*)	Dogs, carnivores, cats
Dirofilaria repens	Old World	Mosquito (*Aedes, Anopheles, Culex, Mansonia*)	Dogs, carnivores, cats
Dirofilaria tenuis	North America	Mosquito (*Aedes taeniorhynchus*, *Anopheles quadrimaculatus*, *Psorophora* sp.)	Racoons
Dirofilaria striata	USA (Florida)	Mosquito (*Aedes taeniorhynchus*, *Anopheles quadrimaculatus*, *Culex quinquefasciatus*)	Panthers, bobcats
Dirofilaria ursi	North America	Black fly (*Simulium* sp.)	Bears
Dirofilaria subdermata	North America	Black fly (*Simulium* sp.)	Porcupines

the expansion from Southern Europe to the central and northern parts. Human dirofilariasis cases in India have been reported from coastal Karnataka, Kerala and Maharashtra. A few *D. tenuis* cases have also been reported from India.

Both *D. immitis* and *D. repens* are endemically widespread in European countries. Northern and Central European countries including France reported a higher prevalence of *D. repens*. The relative preponderance of *D. repens* over *D. immitis* has also been reported from Iran and Sri Lanka. Southern European countries and Italy are highly endemic for *D. immitis*. Central Asia has a high prevalence of *D. immitis*. Elevated prevalence rates have been reported from Malaysia, South Korea, Taiwan and Australia. *D. immitis* infection in canines has been well documented in the north-eastern states of India. Both *D. immitis* and *D. repens* infections have

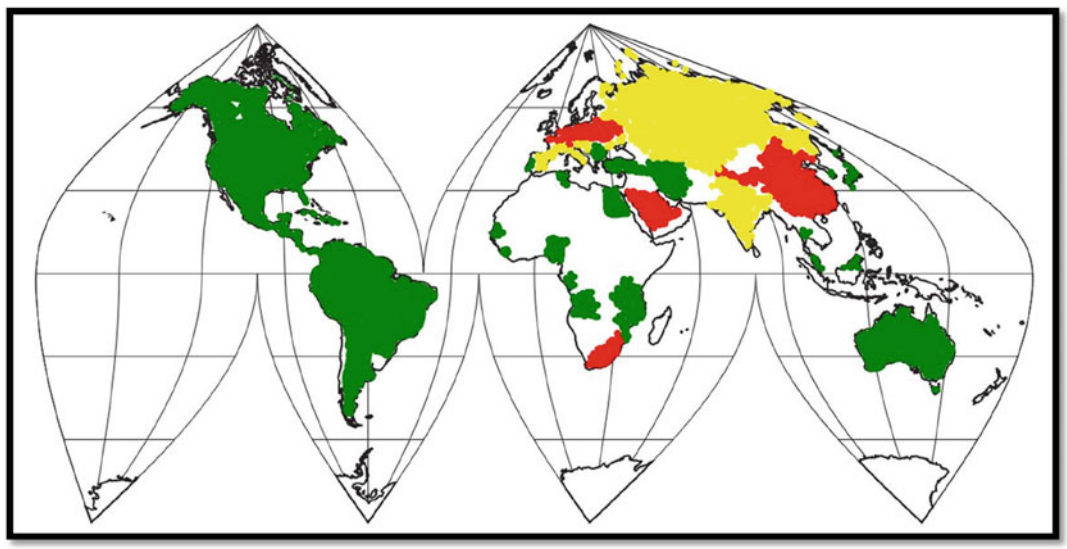

Fig. 4 Global endemicity of dirofilariasis. Green: *Dirofilaria immitis*; *red*: *Dirofilaria repens*; *yellow*: both *Dirofilaria immitis* and *Dirofilaria repens*

been reported from India. Feline dirofilariasis cases correspond to the highest level of endemicity in dogs and have been reported from Canada, Brazil, Venezuela, northern Italy, Japan and Australia. Based upon epidemiological studies, focal regions have been identified for coyotes, red wolves and foxes in Texas, California, Sierra Nevada and San Francisco, with *D. immitis* being the most prevalent species.

Culicid mosquitoes are efficient vectors due to their adaptability extending from the coastal areas to the mountain ranges. Multiple species of *Aedes*, *Culex* and *Anopheles* are involved. *Culex pipiens* is considered to be the potential primary vector.

Apart from the common species, human subcutaneous nodular granuloma has been reported with *D. tenuis*, *D. ursi*-like species, *D. subdermata* and *D. striata* from the USA. Black flies (*Simulium* spp.) have been implicated as a vector for some of these. Human infections caused by *D. ursi* have been reported along the US–Canadian border. *Simulium* sp. acts as the vector.

The actual public health importance of the non-overt human pulmonary dirofilariasis lies in the seriousness of the differential diagnoses including malignancy, tuberculosis and fungal infections.

Diagnosis

Diagnosis in Humans

Laboratory diagnosis of pulmonary dirofilariasis in humans (Table 2) is based on the following tests.

Microscopy

Fine-needle aspiration is done without any risk of parasitic embolization since the parasite has died already.

In the majority of subcutaneous/ocular dirofilariasis cases, the diagnosis is based on histological examination of the nodule (Figs. 5 and 6). The presence of external longitudinal cuticular ridges is characteristic for *Nochtiella* sp./*D. repens*, distinguishing it from *D. immitis*. Specific differentiating features include 95 to 105 longitudinal ridges placed at intervals measuring slightly more than the width of an individual ridge. Periodic acid–Schiff, Masson's trichrome and haematoxylin/eosin are the commonly employed stains. Nodules with dead and disintegrated parasite pose a challenge for identification. Careful histological examination may reveal decomposed parasite surrounded by occasional inflammatory cells. Special attention needs to be exercised for searching trilamellar cuticle, thick somatic muscle bundle and reproductive tubules. Eosinophilia in peripheral blood can be seen in only 20% of patients.

Serodiagnosis

ELISA and indirect haemagglutination assay for demonstration of specific antibodies in the serum are of limited value in the diagnosis of Dirofilaria infections in humans, due to their poor sensitivity and specificity.

Molecular Diagnosis

Polymerase chain reaction (PCR)-based techniques are helpful in diagnosis. However, the common practice of sending tissue samples in 10% formalin instead of methyl alcohol diminishes PCR positivity.

Other Tests

Chest radiogram shows homogenous spherical/ovoid opacity with well-defined borders. Calcified nodules might present as angiocentric lesions and may disappear.

Table 2 Diagnostic methods for dirofilariasis

Diagnostic approaches	Methods	Targets	Humans	Animal
Direct microscopy	Biopsy, larval extraction, necropsy	Larval/parasitic anatomical details (trilamellar cuticle, reproductive tubule, cuticular ridges)	Confirmatory Often difficult to identify the disintegrated worm anatomy particularly from the pulmonary cases	The adult worm can be visualized; morphological identification and speciation can be done
	Concentration of venous blood (modified Knott test, filter test)	Microfilaria	Not applicable	Good sensitivity and specificity; morphological identification and speciation can be done Poor performance in cats
Immunodiagnostics	Antigen detection (ELISA)	Adult female *Dirofilaria immitis* antigen (*Dirofilaria immitis* somatic antigen/DiSA, excretory antigen/DiE/S)	Variable sensitivity and poor specificity	Highly sensitive and nearly 100% specific Occult infection can be detected Poor performance in cats
	Antigen detection (immunochromatography)	Adult female *Dirofilaria immitis* antigen (*Dirofilaria immitis* somatic antigen/DiSA, excretory antigen/DiE/S)	Not available	Highly sensitive and specific Occult infection can be detected. Poor performance in cats
	Antibody (indirect haemagglutination assay)	Anti-*Dirofilaria immitis* antibody	Variable sensitivity and poor specificity	Useful in cats only
Molecular assays	PCR, sequencing, high-resolution melting analysis (HRMA)	Cytochrome oxidase subunit 1 (cox1), 18S-ITS1-5.8S, ITS1-5.8S-ITS2	High sensitivity and specificity; poor performance in formalin-preserved tissue samples	High sensitivity and specificity; HRMA can help in rapid diagnosis
		Wolbachia16S rRNA	Supportive diagnostic role	Supportive diagnostic role

Diagnosis in Animals

Laboratory diagnosis of pulmonary dirofilariasis in animals (Table 2) is based on the following tests.

Microscopy

Adult worms extracted from animal samples are examined microscopically for diagnosis. Poor sensitivity of fresh venous blood smear in demonstrating microfilaria mandates concentration methods like modified Knott test or filter test.

Modified Knott test is considered a sensitive and specific test for diagnosis of dirofilariasis in the canine population. In this method, venous

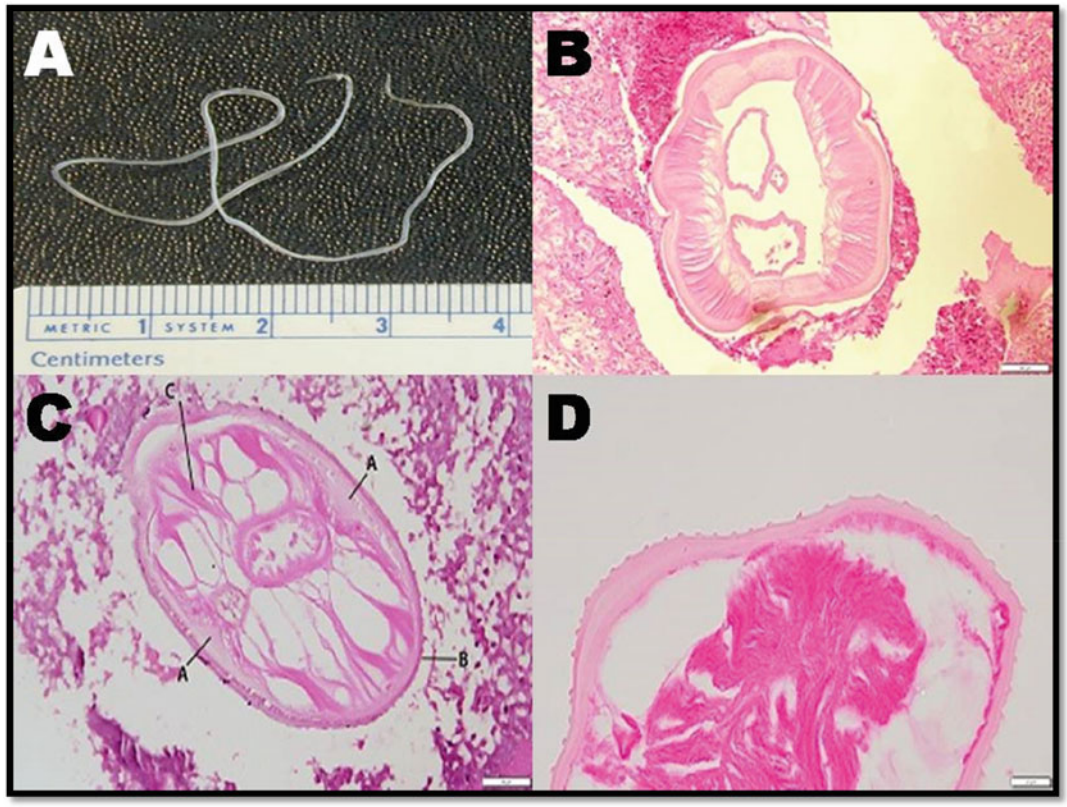

Fig. 5 Human dirofilariasis cases (**a**–**d**). (**a**) *Dirofilaria* sp. removed from the eye; (**b**) section of a *Dirofilaria immitis* worm showing the typical smooth cuticle (without ridges), musculature, paired uteri and small intestine; (**c**) section of *Dirofilaria tenuis*: '**a**' denotes the internal ridge, '**b**' denotes the cuticle (ridges) and '**c**' refers to the tall musculature; (**d**) biopsy specimen from breast nodule showing high-crested cuticular ridges. Note the distally spaced arrangement. These features distinguish *Dirofilaria subdermata* and *Dirofilaria ursi* distinctly among the subgenus *Nochtiella* (image courtesy: DPDx, CDC; https://www.cdc.gov/dpdx)

blood is mixed with 2% buffered formalin (1:10) followed by centrifugation (1500 rpm for 3–5 min). This is followed by staining of the sediment with methylene blue (1:1000) and microscopic examination. Filter test using millipore filter is another test used in the diagnosis. The test excludes the need for the centrifuge but is costly and shrinks microfilariae, thereby altering the measurements.

For differentiation of *Dirofilaria* species, morphological characteristics are compared.

Serodiagnosis

Antigen-based ELISA and immunochromatographic tests are used for diagnosis. These tests detect adult female *D. immitis* antigens with good sensitivity and nearly 100% specificity when two or more worms are present in the infected animals. These tests are not helpful if male worms only or immature female worms are present. When a negative antigen test result does not correlate with the presence of microfilaria or suspected active disease, heat treatment of serum (104 °C for 10 min in a water bath) is recommended to release blocked antigens. Dogs develop detectable antigenaemia 5–6.5 months

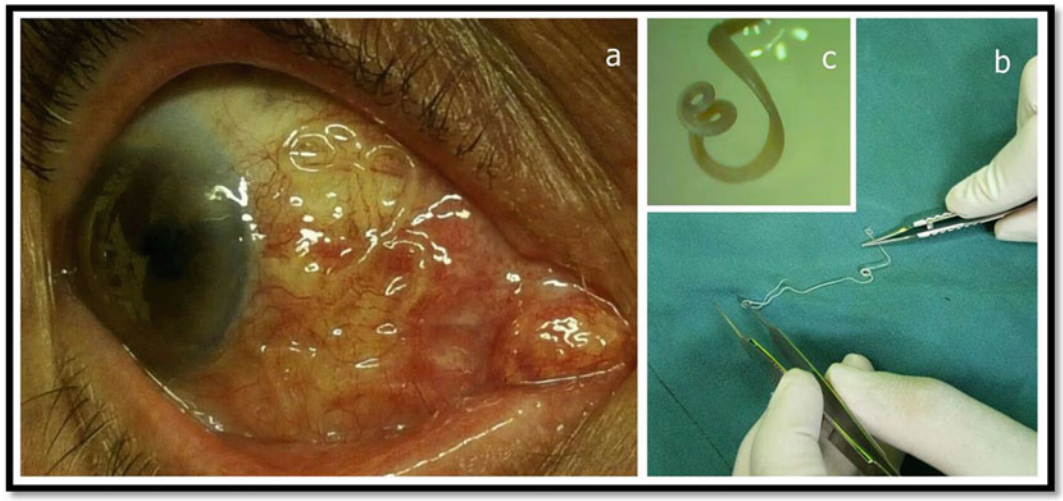

Fig. 6 Human dirofilariasis case (**a**) *Dirofilaria* visualized in subconjunctival space; (**b**) the extracted worm; inset (**c**) the coiled tail. Image reproduced from *BMC Infect Dis* **20,** 520 (2020) (http://creativecommons.org/licenses/by/4.0/)

after infection. Antigen tests are also useful to confirm the success of adulticide treatment when tested 5 and 9 months later. Both microfilariae and antigen tests are less useful in cats. Antibody-based tests hold promise in diagnosis of infection in cats.

Molecular Diagnosis

PCR-based methods can reliably differentiate *Dirofilaria* sp. from other filarial worms from animal samples. Molecular methods are best suited to diagnose in case of morphological abnormalities of microfilaria, particularly in dogs treated with medications or with co-infections. A recent method combining PCR and high-resolution melting analysis (HRMA) provides a rapid differentiation between *D. immitis* and *D. repens* from canine samples.

Treatment

In humans, the main modality is surgical extraction of the nodule or the worm for treatment of subcutaneous dirofilariasis. Pulmonary dirofilariasis needs no treatment as the parasite is already dead. Antinematodal medications like levamisole and thiabendazole have been tried in the past but are not used currently. The American Heartworm Society (AHS) in their latest guidelines (2020) advocated a 3-dose regimen of melarsomine (2.5 mg/kg; one injection followed at least 1 month later by two injections 24 h apart) for treatment in both symptomatic and asymptomatic dirofilariasis in dogs.

In cats, AHS recommends waiting for a spontaneous cure in the absence of overt clinical signs even if radiological evidence suggests dirofilariasis. Surgical extraction of the worm is always preferred.

Prevention and Control

Prevention of mosquito bite and breeding control are important steps in the prevention of human dirofilariasis. The use of mosquito nets and mosquito repellents is effective. Travel to endemic locations needs to be carefully planned with precautions.

For the canine population, regular *Dirofilaria* antigen along with microfilaria testing is

recommended over 7 months of age. Year-round administration of FDA-approved preventive drugs and EPA-registered mosquito repellents and ectoparasiticide application is endorsed by AHS. AHS recommends preventive medications for all cats in endemic areas during transmission season (warmer months) including kittens above 8 weeks of age. Monthly ivermectin (24 μg/kg) and milbemycinoxime (2 mg/kg) are oral formulations. Topical moxidectin (1 mg/kg) or selamectin (6 mg/kg) is also recommended.

Case Study

A 42-year-old male patient presented with dry cough and chest pain. The patient is a smoker (4 pack-days over the last 20 years) and has no contact with pets. In the last 1 year, he had a business meeting in Greece. Chest skiagram revealed a coin-shaped solitary pulmonary lesion in the right upper lobe. Clinical examination and laboratory parameters (leucocyte count, C-reactive protein, erythrocyte sedimentation rate, liver function test and serum electrolytes) were unremarkable. Tuberculin test and sputum testing were non-contributory. Follow-up after 2 months showed the absence of symptoms but no radiological change. CT scan of the chest showed a 1.6 cm non-calcified nodule in the right upper lobe abutting the parietal pleura without lymphadenopathy. Bronchoscopy and immunological/vasculitis profile were found to be negative. Surgically, a 1.6 cm greyish-yellow nodule was resected. Histopathological examination showed no malignancy but the presence of necrotic elements with fragments of a parasite characterized by a smooth surface and internal longitudinal ridges. The patient had an uneventful recovery without any further medical treatment.

Questions

1. How did the patient in the case study acquire the infection?
2. What could be the species involved? How would you proceed to differentiate?
3. What are the differences between *D. immitis* and *D. repens*?
4. What are the agents causing subcutaneous dirofilariasis?

Research Questions

1. What is the mechanism of high adaptability of *Dirofilaria* spp. in a vast animal population?
2. What may be the actual pathogenesis and pathology in dirofilariasis?
3. What is the effect of antibiotics on endosymbiont *Wolbachia* spp. in the killing of *Dirofilaria*?
4. Which anti-parasitic agent can be useful in eliminating the infection in animals?

Further Readings

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