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Canine *Dirofilariasis* by *Dirofilaria immitis* "An emerging Zoonotic Infection" [A review article]

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Abstract

Dirofilariasis commonly referred to as heartworm disease (HW), is a vector-borne zoonotic disease caused by Dirofilaria spp. It is a chronic and life-threatening cardiopulmonary disease predominantly affecting the stray dog population. The causative agent, *Dirofilaria immitis*, is transmitted by mosquitoes of the *Aedes*, *Anopheles*, and *Culex* genera and causes significant morbidity and mortality in infected animals. In Pakistan, the large population of stray dogs, particularly in rural and underdeveloped regions, remained neglected and subjected to mistreatment, leads to a high burden of parasitic infestation, including *Dirofilariasis*. Among the 27 species of Genus *Dirofilaria* species, *Dirofilaria (D.)immitis* is the most important due to its severe pathological effect and higher prevalence in dogs. The prevalence in India, Iran, China and Bangladesh - neighboring countries of Pakistan - is 22.08%, 5.4%, 7.4% and 50.0%, respectively. Other than dog, *D.immitis* can infect 30 species of animals includes wild cats, ferrets and wild canids. HW produces intense pulmonary vascular and parenchymal diseases which can lead to congestive heart failure, which may ultimately result in the victim's death. *Dirofilariasis* is believed to be emerging due to global warming, climate change and persistent movement of heartworm. Major diagnostic approaches includes Modified Knott's technique, PCR and ELISA. Treatment for this infection includes two approaches, either medical or surgical or both depending upon severity. The main objective of this review is to assess the prevalence and current distribution of canine *Dirofilariasis* in neglected areas in comparison with the globe. In addition, the current review aimed to assess possible diagnostic procedure and therapeutic protocols of *Dirofilariasis*.

Keywords: *Dirofilaria immitis*; Mosquitoes; Cardiopulmonary disease; Diagnostic methods; Treatment.

Introduction

Dirofilariasis, caused by *Dirofilaria* spp., is a significant parasitic disease with zoonotic implications, mainly affecting dogs as definitive hosts and humans as incidental hosts [1]. *Dirofilariasis* is a chronic and potentially fatal cardiopulmonary disease which mainly affects dog population [2]. The disease is also called heartworm (HW) disease and has a huge economic importance in dogs around the world [3]. It is one of the major health problem in tropical, sub-tropical and temperate regions [4].

By nature, It is a vector-borne parasitic disease caused by a small thread-like filarial nematode, *Dirofilaria immitis*. The transmission occurs entirely through the bite of infected mosquitoes belonging to the *Aedes*, *Anopheles*, and *Culex* genera, making direct host-to-host transmission impossible [5]. Adult heartworms typically reside within the right ventricle and pulmonary arteries [6], but in some cases, aberrant migration can result in their presence within the epidural space [7], brain [5], anterior chamber of the eye [8], lung parenchyma [9], or systemic arteries [10]. Although, Dogs serve as the definitive host of *D. immitis*, it can infect more than 30 species of animals includes foxes, wolves, wild canids,

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domestic and wild cats, ferrets and human as well [1]. Heartworm disease is responsible for a life-threatening systemic infection in dogs [11].

The genus *Dirofilaria* comprises 27 valid species, each with distinct geographic distributions and varying impacts on host species [12]. Among these, *Dirofilaria immitis* is the most clinically significant, primarily affecting dogs, where it causes severe cardiopulmonary disease [13-14]. Even light infection can lead to intense pulmonary vascular and parenchymal disease, posing a significant health risk [13]. In contrast, *Dirofilaria repens* is more commonly associated with subcutaneous infections in dogs and humans, with a wide distribution worldwide [6].

The cases of *Dirofilariasis* is found throughout the world wherever species of *Dirofilaria* are common [2]. The prevalence of HW disease is influenced by climate, socio-economic factors, topography and the presence of vectors [15]. *D. immitis* infection seems to be more prevalent in adult compared to growing dogs and in male than female dogs [16]. *Dirofilaria immitis* spp. is widely distributed in Africa, Asia, Australia, Latin America and Mediterranean countries [11].

The primary diagnosis is based upon clinical signs defining parasitism by a HW [17]. Antigen detection assays, such as ELISA, are the diagnostic method of choice for *D. immitis* due to their superior sensitivity and specificity, particularly in detecting circulating adult female heartworms [11]. It is recommended to use combine different diagnostic approaches [18] to reduce the chances of false-negative results and develop a more accurate diagnostic test with higher sensitivity and specificity to better detect the disease. There are two treatment approaches based on severity of the infection that might be considered. The primary; medical approach includes melarsomine dihydrochloride as the first choice for treating HW disease [2-3], several macrocyclic lactones to control heartworm disease as preventive approach [19] and doxycycline [20]. The secondary; is the surgical approach that includes surgical removal of worms in dog from right ventricle and pulmonary artery through a forcep [21].

The Life Cycle of Dirofilaria immitis

Dirofilaria immitis has an indirect life cycle as it involves mosquito as its intermediate host [22]. Adult *D. immitis* primarily resides in the pulmonary arteries, but as a of severe infection, they maybe colonized in the right ventricle of the heart [23]. Males can grow up-to 100-150mm while Female can grow up-to 250-300 mm in length [24]. In dogs, the adults can live up-to 5 years or possibly longer [2]. In this period, adult female worm produces microfilaria, which represent the first larval stage (L1) which will circulate in the bloodstream.

The intermediate host, mosquito engulfs the microfilaria. The mosquito serves as an obligatory intermediate host in the lifecycle of *D. immitis*. Following ingestion of microfilariae during a blood meal, the larvae undergo two developmental molts within the mosquito over approximately 14 days, maturing into second-stage larvae (L2) before progressing to the infective third-stage larvae (L3). Afterwards the L2 molts to become an infectious stage (L3), which is settled in the head of mosquito. When the mosquito landed upon a host it takes a blood meal [22]. At this point the larvae burst out of the proboscis. The larvae gather around the mosquito's stylet (the sharp part of its mouth) in a pool of hemolymph. When the stylet is withdrawn, the larvae enter the host through the hole it left. Once inside the host, the larvae (L3) follow a complex migration path. They stay near the entry site for about 1-12 days, during which they molt into fourth-stage larvae (L4). This process usually finishes by day 4 but can sometimes take up to 12 days after infection [2].

The molt to the juvenile adult stage typically happens by day 58. Over the next 99 to 152 days, the first worm reaches its final destination, the pulmonary artery. By 184 to 210 days post-infection, the worms become sexually mature and start producing microfilariae, completing their life cycle [2].

Historical Milestones in Heartworm Disease Research

The earliest documented observation of canine heartworm disease dates back to 1626, when Francesco Birago, a Lombard nobleman, recorded the presence of adult *Dirofilaria immitis* in the hearts of his hunting dogs. Although Birago misidentified the parasites as larvae of another species, his findings laid the foundation for the eventual recognition of *D. immitis* as a distinct species and the etiological agent of heartworm disease in canines [25].

In 1856, significant progress was made by Joseph Mellick Leidy, an American paleontologist and parasitologist. Leidy was the first to relate the species name 'immitis' and provided a detailed description of the worm's morphology. His work positioned the groundwork for the modern thoughtful of this parasite and helped distinguish it from other similar organisms [26].

Despite its recognition in dogs, the zoonotic budding of *Dirofilaria immitis* was not understood until much later. The first recognized human case of *Dirofilariasis* was reported in 1952 in the United States. This discovery knowingly expanded the understanding of the disease, highlighting its ability to infect not only animals but also humans [26]. Following the first human case in the United States, additional cases were reported worldwide, especially in regions with high mosquito activity. These cases

have led to a broader consciousness of the zoonotic potential of heartworm and the importance of monitoring and controlling the disease in both animal and human inhabitants [27].

Presently, *Dirofilariasis* is caused by parasites from the genus *Dirofilaria*. It mainly affects dogs but can also poison humans [14].

Global and Regional Prevalence of Canine Dirofilariasis

Dirofilariasis is found throughout the world wherever species of *Dirofilaria* are common [2]. The pervasiveness of *D. immitis* infection in dogs worldwide is 10.91% [28]. Prevalence of *Dirofilaria immitis* in different countries has been accessible in Table 1.

This prevalence is influenced by multiple factors, including climate, socio-economic factors, topography and the presence of vectors [15]. [16] investigated that *D. immitis* infection was more prevalent in adult (55.56%) compared to growing (0.0%) dogs and in male (72.73%) than female (36.84%) dogs.

The epidemiological landscape of *Dirofilariasis* is rapidly evolving, with an increasing prevalence in endemic regions and its spread to previously unaffected areas [55], Contributing factors include global warming and climate change, which are expanding the range of *Dirofilaria* species to new territories [56]. Since its first discovery in Iran in 1969 by Sadighian, *Dirofilariasis* has been reported across all continents where mosquitoes, the primary vectors, are found [56-57].

Prevalence varies widely across countries due to factors like mosquito distribution, mosquito fertility and density, animal activities, environmental temperature, living conditions, and the average age of the host [28]. The disease is predominantly prevalent in regions with temperate, semitropical, or tropical climates, such as the United States, Canada, Australia, Latin America, and southern Europe, where environmental situations favor the life cycle of *Dirofilaria immitis* [2,4]. In the U.S., the highest rates are found from Texas to New Jersey and along the Mississippi River and its tributaries [56]. Wild canids together with stray dogs, domestic dogs and coyotes represents a potential reservoir host for *Dirofilaria immitis* [58].

Etiology of Heartworm Disease: The Role of Dirofilaria spp.

Dirofilariasis, caused by zoonotic filarial nematodes of the genus fitting to family Onchocercidae, is a significant parasitic disease affecting both animals and, less commonly, humans [1,59]. Among the 27 valid species within this genus, *Dirofilaria immitis* is documented as the most clinically significant [25] due to its severe pathological effects, high prevalence, and incidence, especially in canine hosts [14]. Unlike other species, such as *Dirofilaria* repens, which primarily affects subcutaneous tissues, *D. immitis* inhabits the pulmonary artery and right ventricle, leading to cardiopulmonary complications in diseased dogs [60].

In human hosts, *Dirofilaria species* are unable to complete their lifecycle, as the human immune system prevents the larvae from maturing into adult worms. However, microfilariae can migrate to various tissues, where they may trigger localized inflammatory reactions. Microfilariae are stereotypically absent in the bloodstream of infected humans [61-62], preventing the nematodes from accomplishment maturity within the heart or skin, as seen in *D. immitis* infections in dogs [42].

Transmission Dynamics of Dirofilaria immitis

Mosquitoes from the Aedes, Anopheles, and Culex genera are primary vectors in the transmission of *Dirofilaria immitis*, the causative agent of heartworm disease [63-64]. Female mosquitoes, vaguely, play a crucial role in this transmission cycle as they are the ones that feed on blood, which is obligatory for the maturation of their eggs [65-66].

The transmission of D. immitis initiates when a mosquito ingests microfilariae during a blood meal from an infected host [66]. These microfilariae undergo several developmental stages within the mosquito, culminating in the infectious third-stage larvae (L3) [22,67]. Upon biting a susceptible canine host, the mosquito deposits these larvae into the skin, from where they migrate into the bloodstream, ultimately reaching the pulmonary arteries and right ventricle to mature into adult heartworms [66].

Once in the heart and pulmonary arteries, the *Dirofilaria immitis* worms mature into adults. Adult worms can rise up to 31 cm long and can live for several years within the host. Their existence in the cardiovascular system causes signify cant physical damage to the heart and lungs. The worms can obstruct blood flow, leading to a range of severe circumstances, including pulmonary embolism, which is the blockage of arteries in the lungs by clots [68]. This blockage can cause lung tissue to die due to lack of oxygen (pulmonary infarction) and may lead to the materialization of cavities within the lung tissue [69]. Additionally, the presence of heartworms can lead to spontaneous pneumothorax, a condition where air leakages into the space between the lung and chest wall [70].

Pathogenesis of Dirofilaria immitis: Pulmonary and Cardiovascular Impact

The pathogenesis of heartworm disease is influenced by the worm burden, the host's immune response, duration of infection, and the level of physical activity [33].

Heart disease has many clinical manifestations an d is caused by adult and primary parasites (microfilar iae). Adult heartworms predominantly inhabit the pulmonary arteries, where they cause the most prominant pathological changes, and may extend into the right ventricle in cases of heavy worm burdens [6]. The initial lesion occurs in the pulmonary arteries and lung parenchyma and is usually caused by adult intravascular parasites. If untreated, they can cause pulmomary hypertension [71], Pulmonary hypertension places extra pressure on the right side of the heart, which can cause right ventricular dysfunction, arrhythmias, and ultimately, right-sided heart failure [72]. Other symptoms are related to the blockage of blood flow due to location of *D. immitis* at the level of heart's tricuspid valve in the right atrium [71]. In rare cases, aberrant migration of adult worms occurs, leading to their presence in a typical locations such as epidural space [7], brain [5], anterior chamber of the eye [8], lung parenchyma [9], or systemic arteries [10]. These unusual locations are often associated with severe or advanced infestation and can result in localized inflammation, obstruction, or other organ-specific complications.

The day after *Dirofilaria immitis* enters the arterial system, it starts damaging the walls of the pulmonary arteries. The endothelium develops smooth muscle structures, and the arteries become hardened, which blocks blood flow. This process is driven by Platelet Derived Growth Factor. The endothelial damage and inflammation lead to surrounding tissue swelling. In active dogs, the increased oxygen demand causes heightened cardiovascular activity. This frequent exertion for meeting required demands of oxygen increases pulmonary arterial pathology and may leads to congestive heart failure [73].

Studies have demonstrated that the intensity of *Dirofilaria immitis* larval invasion in dogs leads to significant changes in blood parameters, which worsen as the larval density increases. These changes are primarily driven by the inflammatory response and vascular damage caused by presence of larvae and adult worms. In dogs with an invasion intensity of 20–40 larvae/cm³, there is a noticeable decrease in red blood cells by 11.92%, hemoglobin by 16.33%, and hematocrit by 12.70%, likely due to the mechanical damage caused by the worms and their metabolites which can lead to anemia, thrombocytopenia, and alterations in red blood cell indices. White blood cells increase by 15.17%, reflecting the immune system's activation in response to combat the parasitic infection. With a higher invasion intensity of 40–60 larvae/cm³, these effects are more pronounced: erythrocytes decrease by 25.54%, platelets by 34.03%, hemoglobin by 30.86%, and hematocrit by 20.56%, with an increase in leukocytes by 32.75% and ESR by 1.84 times. At the highest invasion intensity, over 60 larvae/cm³, the reductions are even more severe: erythrocytes drop by 47.21%, platelets by 42.84%, hemoglobin by 42.27%, and hematocrit by 41.57%, with leukocytes increasing by 44.98% and ESR by 2.51 times [74].

Clinical Manifestations of Canine Heartworm Disease

Although many dogs infected with *Dirofilaria immitis* may exhibit no clinical signs initially [79], if left untreated, these dogs are at risk of developing nonspecific and varied cardiorespiratory complications [80]. These complications, including exercise intolerance, coughing, pale mucous membranes, dyspnea, anorexia, and weight loss [79], can be subtle and are often misdiagnosed, being confused with other cardiorespiratory diseases [81]. As the infection advances, more severe signs such as hemoptysis, tachypnea, and syncope may emerge, indicating advanced cardiopulmonary involvement [72].Others,[82] emphasized that the severity of clinical symptoms is directly correlated with the worm burden in the heart and pulmonary arteries. A high worm burden obstructs blood flow, increasing resistance within the pulmonary arteries and leading to an increase in pulmonary arterial pressure. This persistent pressure overload puts strain on the right ventricle, eventually resulting in right-sided heart failure [72,83]. Additionally, the physical presence of worms irritates the arterial walls, leading to an inflammatory response characterized by endothelial cell damage, leukocyte infiltration, and cytokine release. This inflammation contributes to swelling of the artery walls, which further narrows the lumen and aggravates pulmonary hypertension. The increased vascular resistance can also cause fluid to accumulate in the pleural cavity as a result of elevated hydrostatic pressure, while the inflammatory mediators induce lung parenchymal and bronchial inflammation, as well as alveolar damage. Laboratory findings in affected dogs often expose microfilaremia, leukocytosis, eosinophilia, thrombocytopenia, elevated myoglobin levels [83], and increased cardiac troponin sensitivity, which are indicative of significant heart damage [84].

In severe cases, heartworm infection can tip to life-threatening conditions such as pulmonary thromboembolism, heart failure, and caval syndrome. Without timely intervention, these conditions may result in the death of the affected animal. Therefore, early diagnosis and prompt treatment are critical to handling heartworm disease and improving the prognosis for infected dogs [72].

Advances in Diagnostic Methods for Canine Dirofilariasis

A diagnosis is often based on a dog's exposure to mosquitoes in areas where *Dirofilaria immitis* is common and on clinical signs of heartworm infection. However, some clinical signs can mimic heartworm disease. Therefore, diagnosis should

include antigen detection in serum, plasma, or whole blood. Traditional methods, like blood smears, Knott,s or filtration test or histochemical staining of microfilaria which identify microfilariae by their appearance, are also used for all species with blood-circulating microfilariae [86]. These clinical tests to diagnose *D.immitis* are based on parasitological, serological and molecular methods and may vary in sensitivity and specificity [87].

Modified Knott's Technique: A Conventional Method for Microfilariae Detection

The modified Knott's test is the most widely used method among concentration tests in parasitology [88], and is currently considered as the gold standard among conventional tests for detection of circulating microfilariae in the blood. The sensitivity and specificity of modified Knott's method is 85.71% and 92.85% respectively [78]. [89] observed that, modified Knott's test was more sensitive comapared to direct smear test, because it concentrates the microfilaria.

It involves use of whole blood mixed with 2% formalin which is then centrifuged at 2,300 relative centrifugal force (RCF) for 5 min. The supernatant is removed and the remaining pellet is mixed with 1% methylene blue stain, the number of microfilariae are then counted and examined under a microscope using 10 × objective [90].

The most common canine filarial species with microfilariae in the blood are *Dirofilaria immitis*, *D. repens*, *Acanthocheilonema dracunculoides*, and *A. reconditum* [27]. These species release microfilariae into the blood of their final hosts, making the detection of circulating mf the primary method for diagnosing canine filariosis [24]. Thus, the number of circulating microfilariae does not relate with the number of adult *Dirofilaria immitis* and thus does not demonstrate illness seriousness [91]. Specific identification of those tiers is important for an accurate analysis and for choosing the ideal treatment [24]. The MK test is highly sensitive for detecting microfilariae because it concentrates the sample, allowing for microscopic examination of mf morphology, which is essential for distinguishing *D. immitis* from *D. repens*, *Acanthocheilonema dracunculoides*, and *A. reconditum* [92].

Molecular Diagnostics: The Role of PCR in Detecting Dirofilaria immitis

Polymerase chain reaction (PCR) is currently suggested as a species-specific method for the diagnosis of *D. immitis*. PCR is considered as gold standard and can be used to estimate the tests [87]. It is used to detect DNA and enable sequencing for species differentiation and confirmation [93]. Combining a qPCR assay with the *D. immitis* antigen test offers an effective alternative for heartworm screening. The qPCR method is more precise than the MK test, specifically detecting *D. immitis* DNA without cross-reacting with A. reconditum [92].

The "cytochrome oxidase subunit I (COI)" gene is nominated as the target for detecting *Dirofilaria immitis* [94]. The sensitivity and specificity of multiplex qPCR system is 100% and 99.3% respectively with almost perfect agreement with the true positive rate, as indicated by a kappa value (k) of 0.98 for Wolbachia endosymbiont of *D. immitis* [95].

Serological Approaches: ELISA for Heartworm Antigen Detection

ELISA is used to identify antigen of *Dirofilaria immitis* using IDEXX Snap™ 4Dx® or DiroCHEK®. It is more actual in serological diagnosis of heartworm [93]. It's sensitivity and specificity, which range from 85.7% to 100%, depend on the number of mature female *D. immitis* present [96] which helps the detection of hidden infections. [93] detected that the PCR positivity rate (15.5%) was lower than the ELISA rate (29.1%). This could be due to hidden contaminations, issues with DNA extraction, or low levels of *D. immitis* microfilariae that PCR couldn't detect. [97] observed similar results that the positive rate by ELISA was 18.03% while the positive rate by PCR was only 13.93%.

ELISA can be a better investigative test to detect infection with a low number of worms than the haematological diagnostic tests. It can give more precise indication of the level of infection when used along with other techniques e.g. modified Knott's and PCR [78]. Diagnosing *D. immitis* infection in dogs depends on distinguishing antigens produced mainly by adult female worms, along with a microfilaria detection test [92].

Ultrasonography and Radiography: Imaging Tools for Disease Severity

A chest X-ray and a comprehensive echocardiogram are suggested for all dogs testing positive for *D. immitis*. Echocardiography should focus on the occurrence and severity of pulmonary hypertension and is important in analyzing pulmonary hypertension [98]. Echocardiography was used to confirm the diagnosis of vena caval syndrome [99]. Ultrasound diagnostics is a recent and convenient examination method for estimating the general condition of animals and humans and detecting various diseases in the body [100]. [100] mentioned that number of nematodes found depends on the microfilariasis infestation density index: no nematodes are found when the index is less than 40 larvae/cm3 by echocardiography. Beyond this measurement, [100] found 1-10 parasites in the right ventricular and right atrial cavities and in the tricuspid valve leaflets.

Treatment Protocols for Heartworm Disease in Dogs

Treating a heart disease in dogs is difficult and often has risky side effects like Pulmonary Thromboembolism due to of destruction of adult parasites and their embolization within the blood vessels. Therefore, it is necessary to choose the appropriate treatment. Before treatment, each animal's condition should be evaluated, including parasites, age and size [2]. Treatment of an established HW infection in dogs requires long-term medication, exercise limitation and sometimes surgery [102].

Pharmacological Management: Macrocyclic Lactones and Melarsomine Dihydrochloride

Arsenamide sodium, used for treating adult *D. immitis* since the 1940s, required hospitalization due to potential liver and kidney toxicity. In the 1990s, melarsomine dihydrochloride replaced arsenamide because it is safer and more effective. Melarsomine dihydrochloride remains the preferred treatment for heartworm disease. While it reduces the need for hospitalization, dogs still need exercise restriction to avoid thromboembolism [3]. Some authors [20] found that treating with doxycycline orally for 30 days and applying 10% imidacloprid plus 2.5% moxidectin topically for 9 or 10 months, starting at either 105 or 149 days post-infection, was 100% effective in controlling heartworm infection. Research by [103] showed that combining macrocyclic lactones with doxycycline has a synergistic effect against both microfilariae and adult heartworms. Currently, heartworm prevention primarily uses macrolide drugs such as ivermectin, milbemycin oxime, and moxidectin [19]. Selamectin administered topically at a minimum dose of 6 mg/kg body weight for 36 months was 100 % effective for *D. immitis* prevention in dogs [104].

Dogs with a high risk of complications should undergo stabilization prior to the administration of melarsomine. Treatment aimed at stabilization typically involves cage rest, oxygen therapy, corticosteroids, and a 1–2 month course of doxycycline. Administering doxycycline before starting the split-dose melarsomine (three doses) protocol helps reduce the risk of severe reactions associated with the death of heartworms. Strict cage rest is advised for several weeks after adulticide treatment to lower the risk of serious complications like secondary pneumonia and thromboembolism, which can occur as the dying adult worms move into the lungs [105,106]. [106] recommends a range of treatments for dogs that include using adulticide drugs like melarsomine, along with pre-treatment with a macrocyclic lactone such as ivermectin, helps reduce the number of circulating microfilariae.

Surgical Intervention: Managing Severe Heartworm Burdens

High pulmonary artery pressure, reduced cardiac output or low blood pressure, large heartworm burden, simultaneous or delayed maturation of worms, and prophylactic or adulticide therapy can result in migration of heartworm from the pulmonary arteries into the heart resulting in life threatening caval syndrome (CS), also known as *Dirofilaria* hemoglobinuria [108]. Caval syndrome occurs in 55% of heartworm-positive dogs [109]. Diagnosis of caval syndrome is based on sudden onset of severe lethargy, dyspnea, pale mucous membranes, and weakness frequently appear along with hemoglobinemia and hemoglobinuria and a loud systolic murmur of tricuspid regurgitation [80].

Surgical removal of worms in dog is accomplished using light sedation, local anesthesia, and either a minivascular snare catheter [107] or forceps is introduced through jugular vein and enters the right ventricle and finally into the pulmonary artery , guided with help of a fluoroscope. The heartworms are pulled out by the jaws of forcep and the process is repeated until all the parasites are removed [21].

Prevention and Control Strategies for Canine Dirofilariasis

Prevention of *Dirofilariasis* centers on reducing mosquito exposure and employing prophylactic treatments. Measures include minimizing outdoor activities during peak mosquito activity periods, using insect repellents, and utilizing insecticide-treated bed nets. The HW disease in dogs can be prevented by administering diethylcarbamazepine daily during and after heartworm season [110]. Routine and reliable screening tests are recommended to be performed in dogs for *D. immitis* infection in endemic areas [28].

The macrocyclic lactones are the most commonly used chemoprophylaxis agents, most MLs are administered either in oral, topical, or parenteral formulations at an interval of a month or 6 months or 12 months. Most commonly used MLs in case of HW infection are ivermectin, selamectin, eprinomectin, and abamectin and moxidectin [111]. When used correctly, chemoprophylaxis is almost 100% effective against heartworm disease [2,112]. Preventive treatment using macrocyclic lactones should be started in puppies as early as possible, but no later than at 8 months of age [113]. The above mentioned MLs are effective against *Dirofilaria immitis* third-stage larvae (L3) and fourth-stage larvae (L4), and the extreme sensitivity of early L4s to MLs can effectively prevent the larval stages of *D. immitis* from being developed to the pathogenic adults and thus prevent HW disease [112].

Resistance to macrolide anthelmintics has been observed in various nematode species. Studies indicate that resistance develops when a higher proportion of parasites survive a specific drug dose compared to a normal population [111].

Zoonotic Implications of Dirofilaria immitis

Heartworm disease is a zoonotic illness, meaning people in areas where it is common are more prone to the infestation [114]. In humans, *D. immitis* infections are typically aborted, with larvae failing to reach maturity. Upon reaching the pulmonary arteries, these immature larvae induce inflammatory responses, resulting in granuloma formation and occasional pulmonary nodules, which may mimic malignancy on radiographic examination [111]. Most of the patients infested with *Dirofilariasis* remains asymptomatic and those with symptoms have a cough, chest pain, fever and pleural effusion [110]. Prevalence of *Dirofilariasis* in human is 7.8% in Italy and Tunisia [115].

Vector-borne zoonotic diseases cause human and animal deaths and economic losses on a global scale, and greatly affect the economic development of developed and developing countries. The first case of human pulmonary *Dirofilariasis* due to *D. immitis* was announced from Mumbai, India [110]. Since the natural transmission of heartworm disease is through microfilariae and microfilariasis never occurs in humans, heartworm disease cannot be transmitted from one person to another [2,14].

Future Directions and Recommendations for Heartworm Management

There is a need for new therapeutic agents targeting adult worms, as resistance to current treatments like macrocyclic lactones is emerging [111,112,116]. Thus, researcher should focus on understanding the mechanisms behind this resistance and developing new therapeutic strategies to overcome resistance.

Raising public awareness about the importance of heartworm prevention, especially in endemic areas, can significantly reduce the incidence of the disease. The [80] emphasizes regular use of preventive medications and routine testing. Veterinarians should be more vigilant about this zoonotic disease and take appropriate steps to prevent it in dogs [28]. *Dirofilariasis* should be reduced by designing active surveillance programmes at national and regional level.

Vector control remains crucial in preventing heartworm transmission. Studies have highlighted the effectiveness of integrated mosquito management programs in reducing vector populations. [110] suggested exposing as little skin as possible, using of insect repellent, sleeping under an insecticide treated bed net, walking dogs earlier in evening before mosquitoes become active and using of mosquito larvicides to avoid or reduce mosquito bites.

There is a need for more rapid, accurate, and cost-effective point-of-care diagnostic tests that can detect *Dirofilaria immitis* infections early in both symptomatic and asymptomatic dogs. These tests should be easy to use in various settings, including remote areas with limited veterinary access. [18] and [117] recommended to combine different diagnostic approaches to reduce the chances of false-negative results.

Conclusion and Global Implications of Canine Dirofilariasis

Heartworm disease is a vector-borne parasitic zoonotic disease found throughout the world, which is caused by *Dirofilaria immitis*. This review underscores the considerable variation in the prevalence and geographic distribution of canine Dirofilariasis, with neglected regions bearing a disproportionate disease burden. Understanding the multifactorial drivers behind these variations, including climatic conditions, vector ecology, and socio-economic factors, is crucial for developing targeted prevention and control strategies that protect both animal and public health.

The review underscores the need for a global effort to address canine *Dirofilariasis*, with particular attention to neglected regions where the disease burden remains high. By doing so, it is possible to reduce the incidence of heartworm disease and protect both canine and human health in the affected regions. Therefore, lifelong chemoprophylaxis is needed to prevent canine *Dirofilariasis*.

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Conflict of interest

Authors have no conflict of interest.

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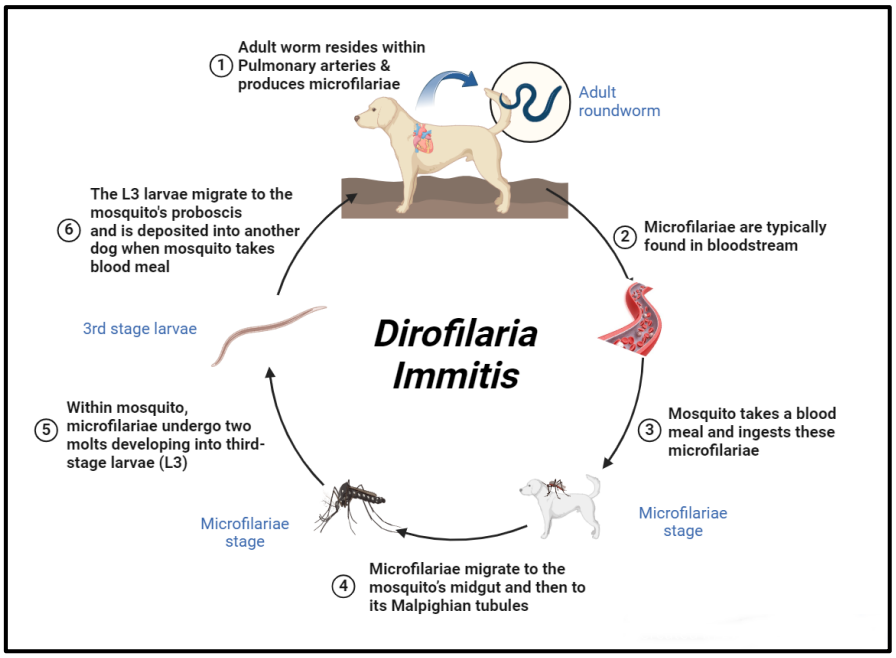

Fig. 1. Image showing the Life cycle of *Dirofilaria immitis*

TABLE 1. Prevalence rate of *Dirofilaria immitis* in different countries

Country	Diagnostic methods	Overall Prevalence	References
India	Wet blood film method & modified Knott's technique	22.08%	[29]
West indies	ELISA	17%	[30]
Bulgaria	Enzyme immune assay technique	34.33%	[31]
Cambodia	N/A	33.33%	[32]
China	16S rRNA gene PCR	7.4%	[118]
Portugal	PCR & modified Knott's technique	48.4%	[33]
Romania	Necropsy records	4.36%	[34]
Brazil	ELISA	7%	[35]
Slovakia	N/A	64.0%	[36]
Thailand	COI-PCR	57.89%	[37]
Iran	ELISA	5.4%	[38]
Central Italy	Serological assays	7.75%	[39]
Lithuania, Latvia & Poland	SNAP	Absent	[40]
Bangladesh	Antigen Test Kit	50.0%	[16]
Serbia	Antigen Test Kit	25.30%	[41]
Moscow, Russia	N/A	3.4%	[42]
Hungary	Necropsy records	2.7%	[43]
Nigeria	Wet mount & Buffy coat method	40.00%	[44]
Nepal	Modified Knott's technique	19.3%	[45]
Central Greece	ELISA	21.7%	[46]
Malaysia	N/A	3.85%	[47]

Country	Diagnostic methods	Overall Prevalence	References
Taiwan	Antigen Test Kit	22.8%	[48]
Sri Lanka	PCR & Direct smear test	Absent	[49]
Turkey	PCR & Antigen test kit	10.0%	[50]
USA	Quantitative PCR	6.3%	[11]
Australia	modified Knott's test[1] & Antigen test kit[2]	16.7%[1] 22%[2]	[51]
Philippines	PCR	13.6%	[52]
Indonesia	PCR & Antigen test kit	Absent	[52]
Singapore	PCR & Antigen test kit	2.6%	[52]
Myanmer	PCR	28.0%	[53]
Iraq	Wet mount technique & Modified Knott's test	27.8%	[54]

Table 2. Clinical hematology and serum biochemistry of dogs infected with *Dirofilaria immitis*

Parameters	Infected Dog	Normal Dog	References
WBC (×103cells/µL)	17.16 ± 5.65	11.10 ± 2.13*	[75]
RBC (x106/uL)	4.81 ± 0.13	7.20 ± 0.92*	[74]
Platelets	141.50 ± 21.60	160.00 ± 13.24	[76]
ALT (U/L)	94.92±26.31	48.39±16.52	[77]
BUN (mg/dL)	26.14±12.60	60.00±31.12	[77]
Creatinine (mg/dL)	2.09 ± 0.82	1.20 ± 0.42*	[75]

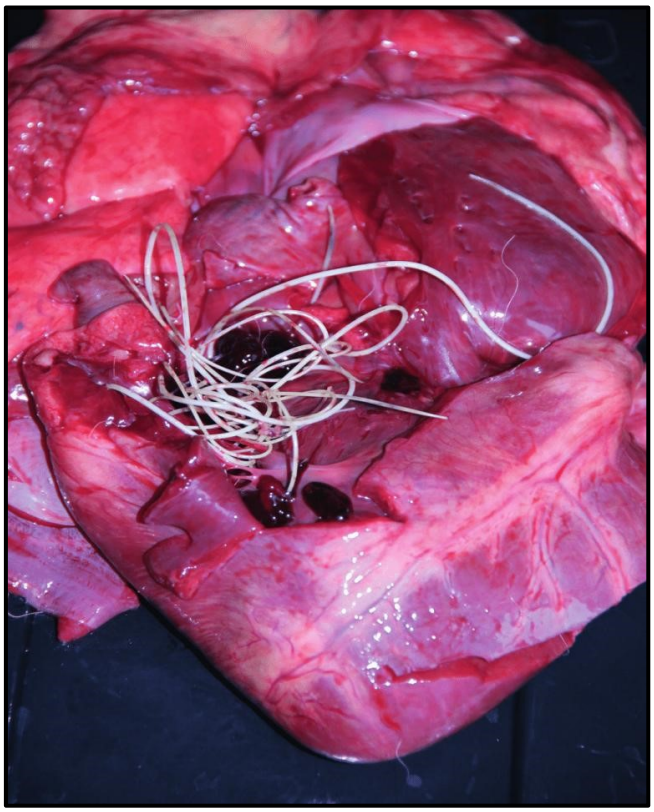

Fig. 2. Adult *Dirofilaria immitis* worms in the right heart ventricle of Tibetan Mastiff male dog [85]

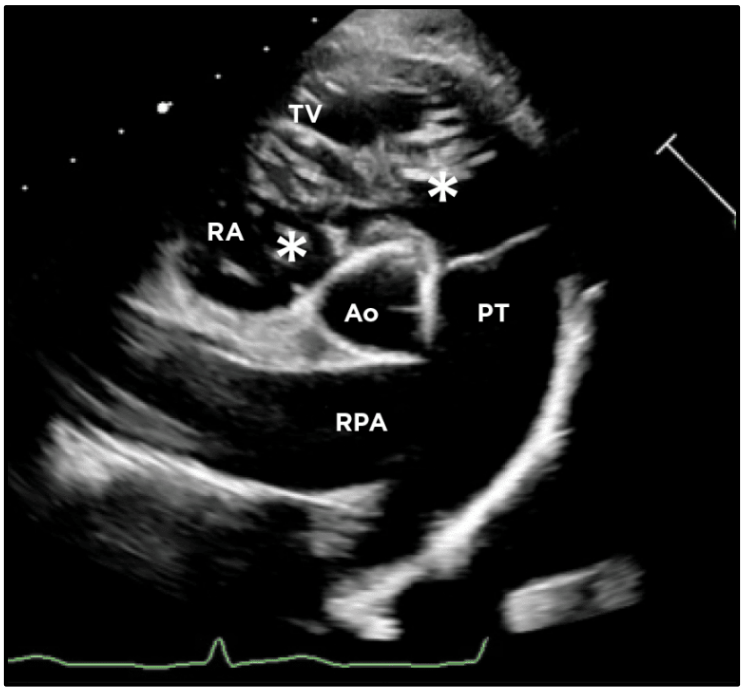

Fig. 3. Right parasternal short-axis echocardiographic view showing a mass of heartworms (long and short equal signs; white asterisks) traverses the tricuspid valve [101]

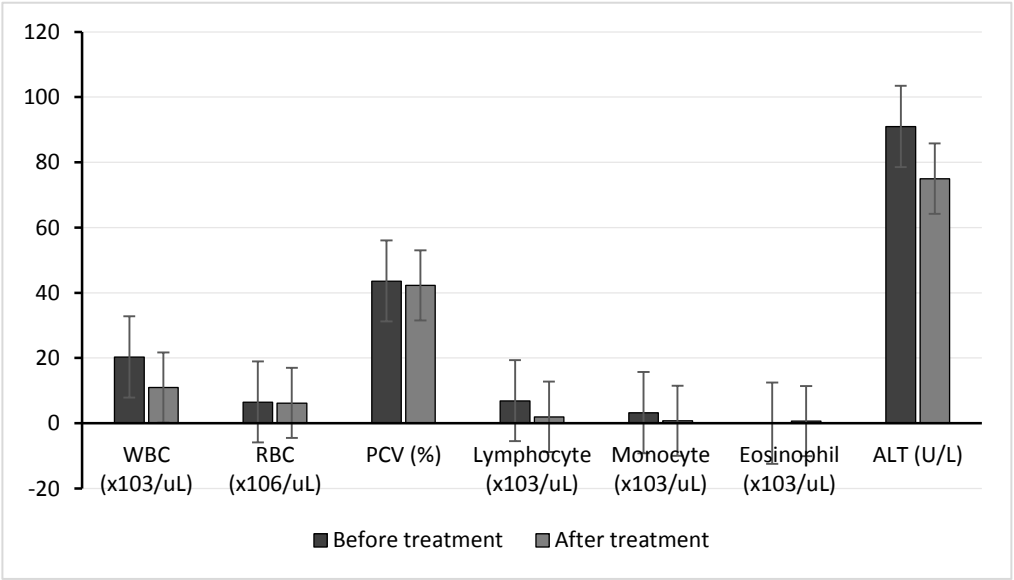

Fig. 3. Hematological and serum biochemical parameters before and 1 month after heartworm removal of cat and dog [Data compiled from 107]

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