The Emerging Role of *Wolbachia* Species in Heartworm Disease

Kristen Frank, DVM  
R. Dennis Heald, DVM, DACVIM  
Gulf Coast Veterinary Specialists  
Houston, Texas

**Abstract:** Heartworm disease was first recognized in dogs more than 100 years ago and is still prevalent among dogs and found in cats worldwide. The complications of heartworm disease can be devastating, and treatment carries risks. Wolbachia spp are gram-negative bacteria that infect filarial nematodes, including *Dirofilaria immitis*, and elicit an inflammatory response in cats and dogs. Antimicrobial therapy directed against these bacteria has resulted in decreased microfilarial loads, inhibition of the development of larval worms, female worm infertility, and reduced numbers of Wolbachia organisms. Antimicrobial therapy against *Wolbachia* spp may be useful in treating heartworm disease in cats and dogs, but further research is needed.

**Clinical Signs of Heartworm Disease**

Susceptible hosts are infected when feeding mosquitoes transmit 13-stage *D. immitis* larvae. Under ideal conditions,1 the larvae mature into adults in 6 to 7 months in dogs and 7 to 8 months in cats.4 While some dogs with heartworm infection are asymptomatic, most affected dogs develop clinical signs due to their immune response to *D. immitis*, resulting diseases include glomerulonephritis and allergic pneumonitis. Dogs with a high worm burden can also develop clinical disease secondary to the physical presence of the worms, such as congestive heart failure, caval syndrome, pulmonary hypertension, and pulmonary thromboembolism. Cats, too, can be asymptomatic when infected with heartworms. However, they can develop serious illness when infected with even a few heartworms. When clinical signs do occur in cats, they develop when the juvenile heartworms arrive in the pulmonary vasculature and when the adult heartworms die.5 Clinical signs in cats with heartworm infection are respiratory in origin and include dyspnea and cough due to an intense inflammatory response by the pulmonary arterial system to adult and juvenile heartworms.4,6 The clinical signs in these cats can be mistakenly attributed to asthma or allergic bronchitis, but they are part of a syndrome now known as heartworm-associated respiratory disease (HARD).5

**Wolbachia Species and *Dirofilaria immitis***

In the 1970s, intracellular bacteria were observed in various filarial nematodes, including *Onchocerca volvulus, D. immitis, Litomosoides sigmodontis,* and *Brugia malayi*, by electron microscopy.7 DNA sequence data have since identified these bacteria as belonging to the genus *Wolbachia*, gram-negative bacterial endosymbionts belonging to the Rickettsiaceae family. In filarial nematodes that harbor *Wolbachia* spp, the prevalence of infection appears to be 100%, suggesting an obligatory endosymbiosis.7 *Symbiosis* refers to a constant and intimate relationship between two
Dissimilar species, and an endosymbiont is any organism that lives within the body or cells of another organism.  Many instances of endosymbiosis are obligate; that is, neither the endosymbiont nor the host can survive without the other. Immunohistochemical, immunogold, and electron microscope studies have shown that Wolbachia spp usually occur as homogenous clusters of bacteria, principally in the lateral chords of the adult worms as well as in the microfilariae and all larval stages of D. immitis. In female worms, they are also found in the reproductive tract, including the oocytes, morulae, and microfilariae. The recognition of intracellular bacteria in D. immitis has raised concerns that these bacteria may contribute to the pathogenesis of heartworm disease and other filarial infections. Hosts are continuously exposed to Wolbachia spp released from the uterus of adult female worms; they can also be exposed when adult worms or larvae die due to natural causes, host immune defenses, or chemotherapy.

How Wolbachia Organisms May Contribute to Heartworm Disease

Data exist to support the potential role of Wolbachia spp in the development of the inflammatory reaction associated with heartworm disease in dogs and cats. In one study, cats developed an IgG immune response against Wolbachia surface protein (WSP) after infection with D. immitis. Additional studies have confirmed the IgG response to Wolbachia organisms in cats and that antibodies against Wolbachia spp increase after larvicidal therapy. In dogs, it has been shown that WSP activates canine polymorphonuclear neutrophils by inducing chemokinetic activity and IL-8 production. Human studies have shown that in river blindness (O. volvulus infection), Wolbachia organisms are direct and indirect sources of signals accounting for neutrophil accumulation around adult filariae. In one veterinary study, all dogs infected with D. immitis had circulating antibodies against WSP; these antibodies were detected independent of clinical signs of heartworm disease. In the same study, immunohistochemical staining for WSP in tissue from patients that had died from heartworm disease showed positive staining in lung, liver, and kidney samples. The Wolbachia organisms were localized within renal tubules and glomeruli and hepatic monocytes; they were found as extracellular bacteria in the lungs.

Wolbachia spp have been shown to contain lipopolysaccharides (LPS) that can induce monocyte activation in vitro. LPS, which are released by gram-negative bacteria, are the major mediators of the inflammatory response in heartworm disease. It was initially believed that the death of adult filarial nematodes was the primary cause of generation of an anti-WSP response because this event increases the levels of Wolbachia DNA in the bloodstream of people. However, exposure to the L3 larval stage of D. immitis initiated the strongest immune response in human and mouse studies, indicating that the role of Wolbachia spp in the inflammatory response in heartworm disease may be most significant in the early stages of infection.

It has also been shown that Wolbachia spp induce a helper T1 (T h1) lymphocyte response during filarial infection, whereas nematode antigens may be responsible for the helper T2 (T h2) lymphocyte response seen in heartworm infections. T h1 lymphocytes secrete cytokines that support cell-mediated immune responses to viral and bacterial infections, characterized by enhanced cytotoxicity, recruitment of inflammatory cells to the infected site, and production of selected classes of antibodies. T h2 lymphocytes, which produce cytokines that generally stimulate antibody production, are activated during parasitic infections and allergic reactions; these responses are characterized by the production of all classes of antibodies and the stimulation of eosinophils and mast cells.

The presence of Wolbachia bacteria results in an inflammatory response in humans, dogs, and cats. In one study, 65.6% of heartworm-positive dogs were found to have Wolbachia DNA in their blood. Because of the high prevalence of Wolbachia spp in heartworm-positive dogs in this report and the evidence supporting the role of Wolbachia spp in inflammatory responses seen in heartworm disease, the potential for including chemotherapy to eliminate Wolbachia spp in the heartworm treatment protocol is being studied.

Current and Potential Therapies

The current therapy for canine heartworm disease is melarsomine dihydrochloride, an organoarsenic with an unknown mechanism of action that is effective in killing mature and immature adult D. immitis worms. Although the safety of melarsomine is better than that of thiacytarsamide (the organoarsenic previously used to treat heartworm disease in dogs), adverse effects of therapy still occur. Adverse effects associated with melarsomine administration include irritation and pain at the injection site, swelling, tenderness, and reluctance to move. Sterile granulomas or abscesses may also form at the injection site. Additionally, neurologic complications following melarsomine administration have been reported, consisting of marked paraparesis or paralysis of the hindlimbs. Pulmonary thromboembolism is a potential adverse effect of treating heartworm disease in dogs.

Melarsomine therapy is currently not recommended in cats due to previous studies that indicated melarsomine was toxic to cats even at low doses. Symptomatic therapy for heartworm disease continues to be recommended for affected cats; therapies including corticosteroids, balanced electrolyte solutions, bronchodilators, and oxygen have been used.
Because *Wolbachia* spp are members of the Rickettsiaceae family, research has investigated the effect of antibiotics commonly used to treat rickettsial infections on these bacteria.26–29 *Wolbachia* spp are susceptible to tetracycline, doxycycline, rifampin, and azithromycin but are not affected by chloramphenicol, erythromycin, or ciprofloxacin.26–29 Use of effective antibiotics for *Wolbachia* spp in animals and humans with filarial nematode infections has resulted in decreased microfilarial loads, inhibition of the development of larval worms, and female worm infertility.28,30,31 Direct adulticidal effects on the filarial nematodes with antibiotic chemotherapy were not demonstrated, and there are conflicting results on whether antibiotic therapy completely eliminates *Wolbachia* DNA.31,52 However, this may be a result of the various antibiotic protocols used in these studies. A protocol has not been established for the treatment of *Wolbachia* infection, but effects on the fertility of filarial nematodes and a reduction in the number of *Wolbachia* bacteria present have been reported in dogs and humans treated with doxycycline and tetracycline at doses recommended for rickettsial infections.27,31

**Conclusion**

Despite efforts by the veterinary community to increase pet owners’ awareness of heartworm disease in dogs and cats and to emphasize the importance of heartworm preventives, heartworm disease continues to be a major problem in veterinary patients. The complications of heartworm disease, such as congestive heart failure, glomerulonephritis, allergic pneumonitis, eosinophilic granulomatosis, pulmonary thromboembolization, and HARD (in cats), can be life threatening. The treatment available for heartworm disease is not without risk in canine patients, and only supportive therapy is recommended in cats. Research has proven that the *Wolbachia* bacteria present in *D. immitis* cause an inflammatory response in both cats and dogs. Additionally, these bacteria have been found in the lungs and kidneys of heartworm-infected dogs. The emerging role of *Wolbachia* spp in the pathogenesis of heartworm disease offers the potential for novel therapies for this disease that may reduce the complications of heartworm infections as well as the adverse effects associated with treatment. In cats, antibiotic therapy has the potential to reduce clinical signs and the risk of acute death associated with heartworm disease. Furthermore, the ability to detect *Wolbachia* antigens may lead to the development of new testing methods that may enhance the diagnostic sensitivity of heartworm testing in cats and dogs with a low worm burden.

Although the role of *Wolbachia* spp in inflammation has been proven and chemotherpy with tetracycline, doxycycline, rifampin, or azithromycin reduces worm viability, to our knowledge, no studies have yet been performed establishing a protocol for antibiotic use in dogs and cats to eliminate, reduce, or sterilize *Wolbachia* bacteria. One study of dogs that had been experimentally infected with *D. immitis* found that dogs treated with doxycycline in combination with melarsomine and ivermectin had less pulmonary pathology compared with dogs treated with melarsomine alone.25 Additionally, the combination of ivermectin and doxycycline has been shown to be adulticidal.33 Antimicrobial therapy for *Wolbachia* infection may be useful in treating heartworm disease in cats and dogs by affecting *D. immitis* development and reproduction and by decreasing the inflammatory responses associated with infection and treatment. At our practice, a 3-week course of doxycycline at 10 mg/kg/day is administered before adulticidal therapy is initiated. Prospective studies are needed to evaluate whether adding antibiotics to standard heartworm therapy in naturally infected dogs and cats improves clinical outcome and survival. The potential for adverse effects of therapy, including antibiotic resistance and gastrointestinal signs, must be considered when deciding whether to use antibiotics in dogs and cats with heartworm disease.

**Key Facts**

- Heartworm disease remains prevalent among dogs and is found in cats worldwide.
- Heartworm infection is caused by the filarial nematode *Dirofilaria immitis*.
- *Wolbachia* spp are endosymbiotic gram-negative bacteria found in filarial nematodes, including *D. immitis*.
- The presence of *Wolbachia* bacteria evokes an inflammatory response in cats and dogs.
- Antimicrobial therapy with tetracycline, doxycycline, rifampin, or azithromycin has resulted in decreased microfilarial loads, inhibition of the development of larval worms, and female worm infertility.

**References**

8. Daida JM, Grasso CS, Stanhope SA, Ross SJ. Symbiontism and complex adap-
Wolbachia Species in Heartworm Disease

1. *D. immitis*, the filarial nematode that causes heartworm disease, does not infect
   a. domestic cats.   d. domestic dogs.
   b. birds.   e. humans.
   c. ferrets.

2. The infective stage of *D. immitis* is the ______ stage.
   a. L1   d. L4
   b. L2   e. L5
   c. L3

3. ______ are clinical signs of heartworm disease in cats.
   a. Heart murmur and pleural effusion
   b. Vomiting and diarrhea
   c. Elevated liver enzyme activities
   d. Dyspnea and cough
   e. Dysphagia and regurgitation

4. *Wolbachia* spp are ______ belonging to the Rickettsiaceae family.
   a. gram-positive bacteria
   b. anaerobic bacteria
   c. viruses
   d. gram-negative bacteria
   e. fungi

5. In one study, cats infected with *D. immitis* developed an ______ immune response against WSP.
   a. IgA
   b. IgD
   c. IgE
   d. IgG
   e. IgM

6. *Wolbachia* bacteria were isolated in tissue samples taken from the ______ of dogs infected with *D. immitis*.
   a. heart
   b. eyes
   c. kidneys
   d. intestines
   e. lymph nodes

7. ______ is not a known adverse effect of treatment for heartworm disease.
   a. Injection site abscess
   b. Paraparesis
   c. Pulmonary thromboembolism
   d. Injection site pain
   e. Blindness

8. *Wolbachia* bacteria are not susceptible to
   a. ciprofloxacin.
   b. tetracycline.
   c. doxycycline.
   d. rifampin.
   e. azithromycin.

9. The use of antimicrobial therapy directed against *Wolbachia* spp in filarial nematode infections, including *D. immitis* infections, has resulted in
   a. decreased microfilarial loads.
   b. inhibition of the development of larval worms.
   c. female worm infertility.
   d. none of the above
   e. a, b, and c

10. The optimal antimicrobial against *Wolbachia* spp to add to the treatment protocol for cats and dogs with heartworm disease is
    a. doxycycline.
    b. azithromycin.
    c. tetracycline.
    d. rifampin.
    e. unknown.