fective as 10 mg/kg BID in reducing *W. pipientis*. On day 30, cortisol levels decreased in both groups, likely due to the significant reduction of *W. pipientis*. However, a high percentage of dogs still exhibited elevated cortisol levels, which may be attributed to the persistence of vascular damage caused by adult parasites. Additionally, external factors such as handling stress and shelter conditions should also be considered. In conclusion, the studied biomarkers decreased in both groups, with no significant differences between doxycycline doses, suggesting that both regimens may have similar efficacy in reducing the *W. pipientis* population.

Effect of doxycycline on *Wolbachia* levels in *Dirofilaria immitis* worms from five canine heartworm cases: Immunohistochemical staining analysis

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Thursday | 10:45–11:00 AM

Doxycycline is an essential component of the adulticidal treatment for canine heartworm, as it targets the elimination of *Wolbachia* pipientis, an endosymbiotic bacterium whose release triggers a proinflammatory innate immune response in the host. Therefore, the effective eradication of *Wolbachia* is crucial before proceeding with adulticidal therapy. This study aimed to evaluate the effects of doxycycline administered at two different doses (10 mg/kg BID and 5 mg/kg BID) on *Wolbachia* levels in *Dirofilaria immitis* worms harvested from dogs undergoing surgical parasite removal. The study involved five infected dogs, all of which had a high parasite load or vena cava syndrome. Two dogs did not receive any antibiotic treatment prior to surgery, two received doxycycline at 5 mg/kg BID, and one dog was treated with doxycycline at 10 mg/kg BID for 30 days. Parasite removal was performed under fluoroscopic guidance using Flexible Alligator Forceps through a right jugular incision, with 10–20 worms (males and females) extracted per animal. These worms were preserved in formalin and sent to a laboratory for processing, followed by immunohistochemical staining using anti-WSP (*Wolbachia* surface protein) antibodies to detect *Wolbachia*. Staining results demonstrated a significant reduction in *Wolbachia* levels in worms treated with both doxycycline doses compared to those in the control group, although there were still some sections with staining compatible with the presence of the bacterium in some points of the lateral cords at both doses. Additionally, treated female worms exhibited destruction of larvae and damage to morulae. These findings suggest that both doxycycline doses are equally effective in reducing *Wolbachia* levels, which may have important implications for improving the management and treatment of canine heartworm.

Effects of doxycycline dose rate and pre-adulticide wait period on heartworm associated pathology, adult worm mass, liver enzymes, and *Wolbachia* levels

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Thursday | 11:00–11:30 AM

The American Heartworm Society Canine Guidelines recommend treatment with doxycycline prior to adulticide administration to reduce levels of *Wolbachia* and its associated metabolites. Studies have determined that doxycycline administered at 10 mg/kg BID for 28 days is an effective dose for eliminating *Wolbachia*. The current guidelines also