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Keywords: *Dirofilaria immitis* excretory/secretory antigens, *Dirofilaria immitis* somatic antigens, angiogenesis, HUVECs.

The relationship of *Dirofilaria immitis* with the angiogenic process as a possible survival mechanism has been previously described in investigations in which it has been demonstrated that the excretory/secretory antigens (DiES) promoted the expression of proangiogenic factors in addition to stimulating related cellular processes, while the somatic antigens (DiSA) only promoted the expression of vascular endothelial growth factor (VEGF). Thus, this study aims to deepen our understanding of the relationship of *D. immitis* with this process by evaluating the effect of DiES and DiSA on the expression of angiogenesis-related proteins.

For this purpose, we used an in vitro model of human umbilical vein endothelial cells (HUVEC) that were treated for 24 hours with the following stimuli: DiES, DiSA, VEGF-A, DiES+VEGF-A, DiSA+VEGF-A and unstimulated cells that were used as a control group. Subsequently, the supernatant was collected and a mass spectrometry study was performed, identifying between 360-450 proteins in all groups.

Gene ontology analysis was performed on the identified proteins to evaluate the effect of the different stimuli on the protein expression of the cells. The treatment of cells with DiES+VEGF-A produced significant differences in the expression of 143, 121 and 84 proteins compared to the control group, DiES-treated cells and VEGF-A-treated cells respectively, of which 26, 15 and 10 are related to the angiogenic process. Regarding DiES+VEGF-treated cells, significant differences were observed in the expression of 91,71 and 31 proteins compared to the control group, DiES-treated cells and VEGF-treated cells, respectively, of which 14, 8 and 3 are related to angiogenesis.

Stimulation with DiES+VEGF-A and DiSA+VEGF-A interacts with the angiogenic pathway by producing changes in the expression of proteins related to this process. Thus, and based on previous studies, angiogenesis is a potential survival mechanism of *D. immitis* in the host.

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O-38. REEVALUATING DOXYCYCLINE DOSAGE IN CANINE HEARTWORM DISEASE: IS LESS MORE?

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Keywords: *Dirofilaria immitis*, *Wolbachia*, C-reactive protein, anti-rWSP antibodies, doxycycline

Several aspects of heartworm disease adulticide treatment require further investigation, particularly regarding Wolbachia pipientis elimination. This study evaluated the acute phase response, specifically C-reactive protein (CRP) concentrations and anti-Wolbachia surface protein (r-WSP) antibody response, before (D0) and after (D30) treatment with oral ivermectin (6-12 µg/kg, monthly) and doxycycline for 30 days at three dosages: Group A (n=10, 10 mg/kg BID), Group B (n=16, 10 mg/kg SID), and Group C (n=13, 5 mg/kg BID). CRP concentrations were generally elevated at D0 (Group A: 15.3±22.7 mg/L; Group B: 27.6±45.3 mg/L; Group C: 13.9±14.2 mg/L) and slightly decreased at

D30 except in Group A (Group A: 25.9±63.3 mg/L; Group B: 17.7±15.7 mg/L; Group C: 12±9.4 mg/L). Anti-rWSP antibody response remained stable in Group A but significantly increased in Groups B and C at D30 ($p<0.05$). The increase in anti-WSP antibody optical densities may result from Wolbachia release during microfilarial death following ivermectin administration at D0 in microfilaremic dogs. Additionally, lower doxycycline doses in Groups B and C may have resulted in a minor Wolbachia reduction over 30 days. Also, it should be noted that the immune response is not well known and the serological persistence of these antibodies is unknown. Elevated CRP values at D0 confirm the strong inflammatory component of heartworm disease. By D30, slight CRP reductions in Groups B and C suggest decreased vascular inflammation due to Wolbachia elimination and microfilariae reduction. However, Group A showed increased CRP levels at D30, which may be due to other inflammatory factors or spontaneous adult worm death induced by antibiotic and macrocyclic lactone treatment. These findings support previous studies suggesting lower doxycycline doses may be sufficient for Wolbachia elimination in Dirofilaria immitis-infected dogs, and encourage further studies in this line of research.

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O-39. USE OF CORTICOSTEROIDS DURING ADULTICIDE TREATMENT IN DOGS WITH HEARTWORM: IS IT A RISK OR A BENEFIT?

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Keywords: *Dirofilaria immitis*, dogs, corticosteroids, pulmonary thromboembolism, D-dimer

D-dimer, a biomarker resulting from fibrin degradation, is a key protein used in the diagnosis of pulmonary thromboembolism (PTE). PTE is a life-threatening complication in dogs infected with *Dirofilaria immitis*. However, there are conflicting results regarding the effect of glucocorticoid therapy on these patients. This study aims to investigate the effects of low dose of corticosteroids on D-dimer levels in dogs with heartworm disease before, during, and after adulticide therapy.

Sera of 48 dogs with *D. immitis* undergoing adulticide therapy were evaluated on days 0, 30, 60, and 90. Additionally, parasite burden, presence of pulmonary hypertension, and microfilariae were assessed on day 0. Of them, 19 dogs (group B) received prednisone at decreasing doses (starting 0.5 mg/kg/BID) from day 0 during all study.

Dogs not receiving corticosteroids (group A) showed higher D-dimer concentrations on day 0 (0.13±0.15 µg/mL), which decreased on day 30 (0.10±0.04 µg/mL) and remained similar thereafter. Dogs receiving corticosteroids started with slightly lower D-dimer concentrations (0.09±0.00 µg/mL), which remained at similar levels to group A for the rest of the study.

D-dimer levels were found to be higher in older dogs, in dogs with high parasite burden, presence of microfilariae or symptoms, but not with pulmonary hypertension. There were no statistically significant fluctuations of D-dimer values between groups, suggesting that there was no increased risk of PTE due to the use of corticosteroids during adulticide treatment.

The results showed that the use of anti-inflammatory doses of corticosteroids did not produce significant variations in D-dimer concentrations during the adulticide treatment, contradicting previous results. Furthermore, anti-inflammatory doses of prednisone improved general symptoms, reduced local inflammation at the melarsomine inoculation site, and contributed to a faster recovery. Therefore, the benefits of corticosteroid administration may outweigh the potential risks, although further studies with larger sample sizes are necessary to confirm these findings.

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