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Laboratory assessment of treatment with artemisin against canine leishmaniosis: a case report

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Background

In canine leishmaniosis (CanL), there is a need for additional therapies other than antimonials and miltefosine, in cases of lack of efficacy or unacceptable side effects. *Artemisia annua* (ART) and its derivates have been studied *in vitro* and *in vivo* against various species of *Leishmania*, including *L. infantum* [1,2]. Multiple mechanisms of action against *Leishmania* have been described, namely an improvement of macrophages' activity and externalization of phosphatidylserine, which leads to the loss of mitochondrial membrane potential, cell-cycle arrest, and parasite's programmed cell death. *In vivo* studies have shown that the leaves and seeds of ART caused increased production of Th1 cytokines, and decreased Th2 cytokines [2]. The presence of iron is fundamental for the efficacy of the therapy: iron in excess binds to hemoglobin and activates ART, creating free radicals that destabilize *Leishmania* [2]. This case presentation describes a dog with leishmaniosis that did not respond to conventional anti-*Leishmania* treatment and was treated with a powdered source of ART and iron supplementation.

Case report

A medium-sized mongrel female, spayed, 16 months old, was rescued from a shelter, with a known seropositivity to *L. infantum* and under treatment for two months with oral allopurinol. At the admission, the dog had polyarthritis and laboratory alterations suggestive of visceral leishmaniosis. According to the LeishVet Clinical Staging [3], the dog was classified as stage III. As part of their practice [4], the authors assessed different acute phase proteins (APPs) including serum ferritin, which was markedly increased (1425 ng/ml, RR 38-272), and *Leishmania* real-time PCR from bone marrow aspirates, which showed a high protozoal burden (1.096.000.000 copies of kinetoplast/ml). Allopurinol (10 mg/kg twice daily) and meglumine antimoniate (50 mg/kg subcutaneously once daily) were attempted but stopped after a few days due to the development of acute kidney injury. The dog was hospitalized, treated with fluid therapy, and after partial recovery treated with miltefosine, for twenty-eight days, at the standard dose, always with allopurinol (stopped after 4 months because of xanthine urolithiasis). After one month from the last dose of miltefosine, the dog had clinically improved, but APPs were still elevated as well as *Leishmania* PCR from bone marrow (399.600.000 copies of kinetoplast/ml). Considering the normalization of creatinine, meglumine antimoniate was again prescribed (50 mg/kg subcutaneously twice daily) for 30 days. After one month, the bone marrow PCR for *Leishmania* showed 5.240.000 copies of kinetoplast/ml, relevant APPs were still increased; therefore, the injectable therapy with meglumine antimoniate was maintained for 30 more days. At the end of the treatment, *Leishmania* PCR from bone marrow showed 605.000 copies of kinetoplast/ml and all APPs were still abnormal. At this point, ART (Luparte 2.0, 1400 mg/m^2/day PO) and iron supplementation (LupoVet LuCefer Iron Powder, 0.9 gr/kg/day PO) were proposed, according to the producers (Lupovet GmbH, Müllheim/Schwarzwald, Germany) and a recent review [5]. After one month of therapy, and up to the most recent check-up (six months after the introduction of ART), the dog did not show any clinical signs, *Leishmania* PCR from bone marrow was negative, all APPs had normalized, and creatinine and urinary protein-to-creatinine ratio were normal. The only test that was not normalized was a quantitative ELISA test, which resulted in a high positive for anti-*Leishmania* antibodies.

Conclusions

In this severe case of CanL, the use of both commonly prescribed leishmanicidal drugs failed to resolve clinical signs, clear the bone marrow from protozoal DNA, and normalize APPs used by the authors to monitor treatment progress. Through oral administration of an herbal extract and iron supplementation, and without any side effects, we achieved recovery from all clinical signs and clinicopathological abnormalities, except for the persistent high seropositivity. We believe that further studies should explore the potential of ART or its derivates, which are already stablished as powerful drugs for the treatment of Malaria [2].

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References

1. Cortes, S., Albuquerque, A., Cabral, L.I.L. et al.. In Vitro Susceptibility of *Leishmania infantum* to Artemisinin Derivatives and Selected Trioxolanes. Antimicrob Agents and Chemoth. 2015;59(8):5032–5.
2. Loo, C.S.N., Lam, N.S.K., Yu, D. et al.. Artemisinin and its derivatives in treating protozoan infections beyond malaria. Pharmacological Research. 2017;117:192–217.
3. Solano-Gallego, L., Cardoso, L., Pennisi, M.G.et al. Diagnostic Challenges in the Era of Canine *Leishmania infantum* Vaccines. Trends in Parasitol. 2017;33(9):706–17.
4. Ceron, J.J., Pardo-Marin, L., Caldin, M. et al. Use of acute phase proteins for the clinical assessment and management of canine leishmaniosis: general recommendations. BMC Vet Res. 2018;14:196–201.
5. Schäfer, I., Müller, E., Naucke, T.J. Ein Update zur Leishmaniose des Hundes: Diagnostik, Therapie und Monitoring. Tierarztl Prax Ausg K Kleintiere Heimtiere. 2022;50(6):431–45.