

A dirofilariasis mouse model for heartworm preclinical drug testing

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The filarial nematode, *Dirofilaria immitis*, is the cause of veterinary heartworm disease; a potentially life-threatening infectious disease of companion animals, and the cause of zoonotic pathologies in humans. Heartworm preventative and curative drug R&D has been reinvigorated by the identification of macrocyclic lactone drug-resistant isolates and incidence of chemoprophylactic treatment failures in pets. With no small animal model available, cats and dogs are utilised in primary *in vivo* drug screening against *D. immitis*. We therefore assessed lymphopenic immunodeficient mice with ablation of the interleukin-2/7 common gamma chain (γ_c) as susceptible *D. immitis* hosts with utility to test heartworm preventative drug efficacy. Non-obese diabetic (NOD) Severe Combined ImmunoDeficient (SCID) $\gamma_c^{-/-}$ (NSG) mice produced consistent yields of viable *D. immitis* larvae at two weeks post-infection across multiple experiments and different batches of infectious larvae inoculates. Developing larvae were found in subcutaneous tissue, the natural site of this stage of the heartworm life cycle in dogs. Larvae retrieved from NSG mice were morphologically mid-L4 stage of development. Compared with age-matched *in vitro* propagated larvae, *in vivo* derived L4 were significantly larger and contained expanded intracellular *Wolbachia* titres, determined by QPCR of the single copy *Wolbachia* surface protein gene and Fluorescent *in situ* Hybridisation (FISH) of *Wolbachia* 16S ribosomal RNA. We validated the NSG mouse model and an *ex vivo* L4 screening system against the reference macrocyclic lactone, moxidectin and the anti-*Wolbachia* reference drug, doxycycline. We have subsequently adopted the mouse model screen to assess efficacy of novel, fast-acting azaquinazoline anti-*Wolbachia* compounds. We contend that future adoption of the mouse model of heartworm will benefit end-user laboratories conducting R&D of novel heartworm preventatives and provide reduction and refinement in long-term procedures requiring cats or dogs.