Ph.D. THESIS

Vector-borne pathogens of dogs in Galapagos in the context of conservation medicine

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ABSTRACT

The archipelago's colonization began with the arrival of pirates and whalers in the 1500s and 1700s, leading to the near-extinction of several species, including some species of giant tortoises—a consequence of intensive hunting (Jackson, 1993; Townsend, 1925; Conrad and Gibbs, 2021; Jimenez et al., 2024). The introduction of domestic animals and other invasive species, including black rats and house mice, has further compounded the anthropogenic pressures on local fauna (MacFarland et al., 1974; Smith, 1979; Phillips et al., 2012; Jimenez et al., 2024). While the direct impacts of invasive species on native fauna have been well documented, the effects of parasites linked to these invasive hosts are still not thoroughly explored. This represents a critical area for further investigation and poses an ongoing threat to the unique biodiversity of the Galapagos Islands.

The endemic Galapagos sea lion (*Zalophus wollebaeki*), listd as endangered by the IUCN. Galapagos sea lion has exhibited significant population declines, particularly in colonies near human settlements like Puerto Baquerizo Moreno, where the largest population resides (Páez-Rosas and Guevara, 2017; Páez-Rosas et al., 2020; Ruiz-Saenz et al., 2023). The prevalence of domestic and free-roaming dogs across the four inhabited islands increases the risk of pathogen transmission. Although investigations into canine parasites in the Galapagos have been limited, studies suggest a high diversity of pathogens, with ectoparasites such as ticks posing pronounced threats to domestic animals, wildlife, and humans. The free-roaming dog population is especially susceptible to tick infestations, serving as potential reservoirs for a variety of parasites and pathogens.

This thesis explores the epidemiology of canine vector-borne pathogens within the Galapagos Islands, grounded in the shared evolutionary lineage of domestic dogs (*Canis lupus familiaris*) and Galapagos sea lions (*Zalophus wollebaeki*). The study's general objective was to determine the distribution and prevalence of these pathogens across inhabited islands, emphasizing the potential for pathogen spillover to endemic wildlife. The specific objectives were to evaluate the epidemiology of vector-borne pathogens in domestic dogs across San Cristobal, Isabela, Santa Cruz, and Floreana, understanding the transmission cycle of *Dirofilaria immitis* by identifying mosquito population, assessing the host range of mosquitoes in urban and coastal habitats, and exploring the role of Galapagos sea lions as potential reservoir hosts.

Chapter II.1 of the current thesis, demonstrates the presence of circulating *Dirofilaria immitis* microfilariae in dogs on San Cristóbal Island. Previous studies in other islands from Galapagos focused on antibody or antigen detection (Barnett, 1985; Levy et al., 2008; Gingrich et al., 2010; Adams et al., 2016; Diaz et al., 2016; Jimenez et al., 2020) or DNA (Jimenez et al., 2020), but this work completes that picture by directly demonstrating the presence of circulating microfilariae. The prevalence (1.7%) was lower than in Santa Cruz (6.9%) (Jimenez et al., 2020) and Isabela (34%) (Levy et al., 2008), but higher than in some other oceanic islands (Little et al., 2011; Montoya-Alonso et al., 2011). The study highlighted the importance of climatic factors (Montarsi et al., 2015; Younes et al., 2021) and mosquito vector presence (Meriem-Hind and Mohamed, 2009; Ledesma and Harrington, 2015; Torres and Mena, 2018; Šebesta et al., 2011; Montero-Serra et al., 2014) in the endemic biological cycle. Positive dogs were found

near the Galapagos sea lion (GSL) rookery, raising concerns about potential transmission to this endangered species. The study's key finding is the presence of *D. immitis* microfilariae in domestic dogs, posing a potential infection source for GSLs due to their proximity.

Chapter II.2 of the thesis expands existing data on vector-borne pathogens in dogs Galapagos archipelago. The prevalence of *D. immitis*, *Babesia vogeli*, and *Hepatozoon canis* was determined using molecular methods. The presence of *B. vogeli* and *H. canis* in dogs is reported for the first time in Galapagos islands. The higher prevalence of *D. immitis* in older dogs is consistent with previous studies (Montoya-Alonso et al., 2006; Yaman et al., 2009; Montoya-Alonso et al., 2011). The study also highlighted the potential role of cats as a neglected reservoir (Gingrich et al., 2010; Torres and Mena, 2018; Ash, 1962; Traversa et al., 2010; Simón et al., 2012; Pennisi et al., 2020). The study highlighted the significance of *D. immitis* for conservation medicine due to its potential transmission to GSLs. The study also provided the first report of *B. vogeli* and *H. canis* in inhabited islands of the arhipelago.

Chapter II.3 evidenced key ecological interactions between mosquitoes and their bloodmeal sources. Mosquito life cycles are strongly affected by climate, habitat, and host availability (Thiemann et al., 2011; Simpson et al., 2012; Asigau et al., 2019). *Aedes aegypti* showed a preference for humans (Causton et al., 2006; Asigau et al., 2017; Asigau et al., 2019), while *Aedes taeniorhynchus* preferred coastal habitats (Barnett, 1985; Asigau and Parker, 2018; Asigau et al., 2019; Becker et al., 2020). *Culex quinquefasciatus* exhibited opportunistic feeding behaviour, adapting to urban and mangrove environments (Zinser et al., 2004; Takken and Verhulst, 2013; Asigau et al., 2019). The observed feeding patterns highlight the crucial role of urbanization in shaping mosquito host selection and availability. This research demonstrates complex relationships between mosquito species and hosts. *Culex quinquefasciatus*' adaptability enhances its role in pathogen transmission.

The Chapter II.4 provides new epidemiological data on *D. immitis* in pinnipeds. The study found a positive case of *D. immitis* microfilariae in a GSL, suggesting that GSL could act as a suitable definitive host. Monitoring and effective conservation strategies are essential.

Chapter II.5 presents the first molecular confirmation of *A. phagocytophilum* in the Galapagos. A high prevalence (20.3%) in dogs was observed, along with *A. platys*. The study suggests that *Rhipicephalus linnaei* could act as a vector (Šlapeta et al., 2022; Almazán et al., 2024; Teo et al., 2024; Nieto-Cabrales et al., 2024) for *A. phagocytophilum* and *A. platys*, highlighting the global spread of *Anaplasma* spp. and public health concerns.

The research identified several key findings: the presence of *D. immitis* in domestic dogs indicates a risk for endangered Galapagos sea lions, particularly near canine populations; the adaptability of *C. quinquefasciatus* as a vector increases pathogen transmission risk; and the detection of *D. immitis* microfilariae in GSL suggests their potential role as definitive hosts. The identification of *R. linnaei* as a possible vector for *Anaplasma* spp., *Babesia vogeli*, and *Hepatozoon canis* enhances understanding of tick-borne pathogen dynamics in the archipelago.

In considering these findings, future research should focus on developing comprehensive conservation strategies to reduce the risks of pathogen transmission to endemic species. This necessitates continuous surveillance and specific interventions.

Collaboration among local authorities, conservation organizations, and public health entities is crucial for the development of integrated vector control strategies and wildlife health management in this unique and vulnerable ecosystem. This research enhances our comprehension of zoonotic disease dynamics in the Galapagos and highlights the necessity for proactive conservation strategies.

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