



Mosquito (Diptera: Culicidae) surveillance for *Dirofilaria immitis* (Rhabditida: Onchocercidae) using a zoo as a focus for operational detection in central Utah

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Across the United States, *Dirofilaria immitis* (Leidy), commonly known as dog heartworm, poses a significant threat to common pets, including dogs, ferrets, and cats, but also to exotic and feral animals. This study focused on surveillance of *D. immitis* in mosquitoes collected in the Salt Lake Valley of Utah, with an emphasis on animals housed at a local zoo. Mosquitoes (Diptera: Culicidae) from the genera *Culex*, *Culiseta*, and *Aedes* were collected, identified, sorted, and subjected to DNA extraction. In the summer of 2023, extracted DNA from 320 pooled samples of 3,459 mosquitoes was tested by PCR with *D. immitis*-specific and panfilarial primers. Two samples collected at the zoo were positive for *D. immitis*, and 2 additional samples tested positive with panfilarial primers, though the precise filarial species could not be determined. In the summer of 2024, an additional 71 samples of 1,999 mosquitoes were collected from trapping at 12 locations across Salt Lake Valley, but none were positive for *D. immitis*. This study confirmed the presence of *D. immitis*-infected mosquitoes in the Salt Lake Valley, prompting the zoo to expand prophylaxis for at-risk animals and underscoring the importance of ongoing surveillance efforts. Further monitoring is recommended to assess and manage ongoing threats posed by mosquito-transmitted heartworm.

Keywords: heartworm, mosquitoes, parasite, roundworm, Culicidae

Introduction

Dirofilaria immitis (Leidy), commonly known as dog heartworm, is an enzootic parasitic nematode that was determined endemic within Utah following the detection of *Aedes sierrensis* (Ludlow) in 1965 (Nielsen et al. 1967). In 1987, *Ae. sierrensis* was first detected in Salt Lake County by the Salt Lake City Mosquito Abatement District (SLCMAD), and in the same year, the first autochthonous transmission of heartworm in Salt Lake Valley was identified in a dog (Erekson 1991, Hatch and Dickson 1991, Scoles et al. 1993). Detection in pet dogs continued to rise, reaching over 100 cases per year across Utah by 1998 (Rogers 2002). From 2014 to 2024, 309 veterinary cases of heartworm were reported to the Utah Department of Agriculture and Food (UDAF), with a peak of 59 cases in 2018 (Dr. Amanda Price, UDAF State Veterinarian, personal communication).

Though *Ae. sierrensis* may have driven the movement of *D. immitis* in Utah, it is unlikely that it is the only species now maintaining local transmission of the pathogen. Other

heartworm-competent vectors found in the Salt Lake Valley include *Culex pipiens* (L.), *Culex tarsalis* (Coquillett), *Aedes vexans* (Meigen), and *Culiseta inornata* (Williston) (Huang et al. 2013, McKay et al. 2013, Tran et al. 2022). However, current data on the prevalence and ecology of *D. immitis* in the Salt Lake Valley are lacking. In 2022, UDAF discontinued requesting reports of heartworm infections from veterinarians, as they categorize *D. immitis* as endemic. Additionally, there have not been contemporary surveys of heartworm in the Salt Lake Valley of Utah in recent decades. Operationally, this could be resolved by including molecular detection of *D. immitis* as part of routine surveillance trapping by SLCMAD (Rishniw et al. 2006, Holderman et al. 2021, Scavo et al. 2022, Tran et al. 2022).

Native canine reservoirs for *D. immitis* in Utah include dogs, wolves, foxes, and coyotes (Ledesma and Harrington 2011, Huang et al. 2013), but many other non-canine species are also susceptible to infections. Non-native animals housed by Utah's

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Hogle Zoo at risk of *D. immitis* infection include Felidae (African lions, snow leopards, Amur leopards, Amur tigers, Pallas's cats), Otariidae (sea lions), Phocidae (harbor seals), Mustelidae (American river otters), Ailuridae (red pandas), Ursidae (grizzly bears, polar bears), and Herpestidae (meerkats) (Kimber and Kollias 2000, Neiffer et al. 2002, Matsuda et al. 2003, McCall et al. 2008, Estrada et al. 2009, Lan et al. 2012, Dantas-Torres and Otranto 2013, Delaski et al. 2015, Krucik et al. 2016, Otranto and Deplazes 2019, Roug et al. 2023). Native animals housed at the zoo include Canidae (red foxes, gray fox), Felidae (bobcat, cougar), Mustelidae (American badger), and Mephitidae (striped skunk) (Otranto and Deplazes 2019, Roug et al. 2023, Ramos et al. 2024). There is a risk of local transmission and spillover to these zoo animals, not only from domestic dogs, but also from coyote and fox populations residing in the nearby Wasatch Mountains. If local transmission occurs, intervention would be necessary; however, as with many parasitic diseases, proactive disease prevention is substantially less expensive and more efficacious than reactive treatments (Ledesma et al. 2019). As the zoo is within the SLCMAD, both the zoo and the surrounding residential neighborhoods receive integrated vector management, incorporating surveillance and regular larvicide treatments in tree holes, catch basins and other larval habitats. Our investigations report on the surveillance of *D. immitis* in the Salt Lake Valley, with a particular focus at Utah's Hogle Zoo.

Methods

Mosquito Collections

In 2023, from 18 May to 9 August, mosquitoes were collected at Utah's Hogle Zoo on the eastern side of Salt Lake City, UT, using SLC traps, which are based on the miniature CDC light

trap (Bibbs et al. 2024). The traps were baited with light-emitting diodes and CO₂. The traps were dispersed throughout the zoo, with 1 trap in each of 5 major areas, including near exhibits of primates, pinnipeds, elephants, crocodiles, and zebras and were deployed weekly for 24 h. The mosquitoes were counted and sorted by species from each trap site using morphological keys (Nielsen et al. 2002, Darsie and Ward 2005). Mosquitoes in the genera *Culex*, *Culiseta*, and *Aedes* were pooled as single species into 2 ml tubes. Samples ranged from 1 to 20 mosquitoes collected in the same trap and night.

During the 26 June to 31 July 2024 investigations, mosquitoes were collected at the zoo and 11 additional sites weekly for 24 h (Fig. 1). The zoo retained 2 CO₂-baited SLC traps at the primates and the newly opened Wild Utah Exhibit. Additionally, a gravid trap was located by the pinnipeds. These sites were selected as the primate and pinniped sites had collected the most mosquitoes in 2023 and had produced the filaria-positive results. SLC traps were used at all sites except Downington and Tracy Aviary, which had Salt Lake gravid traps deployed (Dewsnup et al. 2023). Gravid traps use fetid water to target ovipositing female *Culex* mosquitoes, whereas CO₂-baited traps attract host-seeking females. One sample per week of up to 30 *Cx. tarsalis* or *Cx. pipiens* were tested for *D. immitis* per trap, focusing efforts on the most likely species from 2023. No *Ae. sierrensis* were collected in 2024, or these would have been tested. To supplement the special samples for filaria testing, SLCMAD mosquito samples for routine arbovirus surveillance were occasionally recruited for this study, which contained 5 to 100 mosquitoes per sample. These samples were collected as part of the core mission of the mosquito abatement district for surveillance of West Nile, Western equine encephalitis, and St. Louis encephalitis viruses. Mosquitoes were stored at -80°C prior to testing.

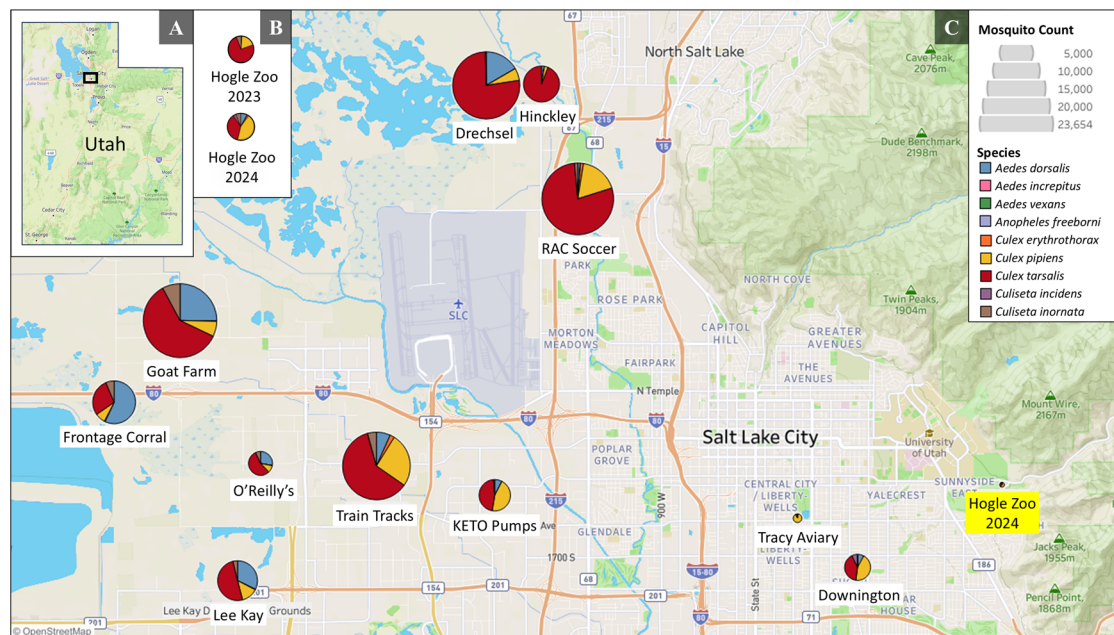


Fig. 1. Trap sites and collection results for study. A) Map of Utah with the study area indicated by black box. B) 2023 and 2024 Hogle Zoo species composition pie charts. These pie graphs represent all trap sites at the zoo across the collection periods of 7 May to 9 August 2023 and 26 June to 31 July 2024. The 2023 graph is scaled to the 2024 data in panel C (3,459 total mosquitoes); the 2024 data are enlarged for visibility. C) Map of Salt Lake City mosquito trap sites from which samples were taken for testing in summer 2024. Pie charts indicate species composition and abundance across the duration of this study. Hogle Zoo, emphasized in yellow, bottom right corner, is on the east side of Salt Lake Valley. Only one sample per week per trap of mosquitoes collected was tested for *D. immitis*.

DNA Extraction

DNA was extracted from mosquito samples using the Quick-DNA Miniprep Plus Kit per manufacturer's protocol (Zymo Research D4068). Samples were resuspended in water or phosphate-buffered saline and were triturated in their microcentrifuge tubes using a disposable plastic pestle. In 2023, mosquito samples with less than 10 specimens used 95 µl of nuclease-free water, 95 µl of blue tissue buffer, and 10 µl of proteinase K. All 2024 mosquito samples, and 2023 samples of 10 or more mosquitoes had double the amount of blue tissue buffer (190 µl), water (190 µl), and proteinase K (20 µl) to ensure all the mosquitoes were digested. Samples were incubated at 55 °C for 1 to 3 h in a water bath. After incubation, pre-wash, wash, and elution (50 µl) buffers were added to the spin columns per manufacturer's instructions and DNA was collected.

Quantitative PCR

Quantitative PCR (qPCR) was performed on extracted DNA using the SYBR Green Universal Master Mix (Applied Biosystems 4309155) using specific primers for the *D. immitis* cytochrome c oxidase subunit I gene (COI) (Table 1) (Rishniw et al. 2006). Each qPCR well contained 10 µl of SYBR Green Master Mix, 0.8 µl of DL_COI_F1 5'-AGTGTAGAGGGTCAGCCTGAGTTA-3' (10 µM); 0.8 µl of DL_COI_R1 5'-ACAGGCACTGACAATACCAAT-3' (10 µM), 4.4 µl of nuclease-free water, and 4 µl of template DNA for a total reaction volume of 20 µl. In 2023, nonspecific panfilarial parasite testing was performed as above in a separate reaction using the primers DIDR_F1 (5'-AGTGCGAATTGCAGACGCATTGAG-3') and DIDR_R1 (5'-AGCGGGTAATCAGACTGAGTTGA-3') (Rishniw et al. 2006). The panfilarial DIDR primers detect *Acanthocheilonema dracunculoides*, *A. reconditum*, *Brugia malayi*, *B. pahangi*, *B. timori*, *D. immitis*, *D. repens*, *Mansonella ozzardi*, and *Onchocerca volvulus*, which are all in the order Rhabditida and family Onchocercidae (Rishniw et al. 2006).

Mosquito-grown larval *D. immitis* preserved in ethanol were provided by Dr. Michael Reiskind (North Carolina State University, Raleigh, North Carolina) and used as a positive control. The qPCR cycling conditions on a QuantStudio 5 system (Applied Biosystems) were as follows: denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min. A melt curve (from 60 °C to 95 °C at 0.075 °C/s) and 2% agarose gels were used to determine if there were primer-dimers, nonspecific amplification products or multiple amplicons in the samples. Amplicons were Sanger sequenced with the same primers used to generate them (Epoch Life Science). The raw sequences were

evaluated in FinchTV 1.4.0 (Geospiza, Inc.; Seattle, Washington, United States) and aligned in MEGA11 (Tamura et al. 2021) by Clustal omega. Sequences were searched using the NCBI Basic Local Alignment Search Tool (Camacho et al. 2009). The minimum infection rate (MIR) was calculated as the number of positive samples divided by the number of mosquitoes tested, multiplied by 1,000.

Results

In 2023, trapping at Utah's Hogle Zoo yielded 320 samples of pooled mosquitoes (Fig. 1B). Of the 3,459 mosquitoes captured and tested, the predominant species was *Cx. tarsalis* ($n=2,657$; 76.8%), followed by *Cx. pipiens* ($n=538$; 15.6%), and *Cs. inornata* ($n=217$; 6.3%). No other species exceeded 1% of the collection, but *Ae. dorsalis* (Meigen), *Ae. sierrensis*, *Ae. increpitus* (Dyar), *Cs. incidens* (Thomson), and *Anopheles freeborni* (Aitken) were also collected (Supplementary Table S1).

Two samples of *Cx. tarsalis* tested positive by *D. immitis*-specific primers (Table 1; amplicon $T_m = 77.2$ °C). These were confirmed by Sanger sequencing. The trimmed sequences were 158 base pairs with 100% identity with the sequences for cultured *D. immitis* worms and published sequences of *D. immitis* COI (Hu et al. 2003). Both positive samples were from the pinniped enclosure and were collected on 5 July 2023. Panfilarial (DIDR) primers revealed 2 more positives for other non-specific filarial worms, which did not produce sequences with sufficient homology for further identification. These results produced a 0.7% *D. immitis* positivity rate by sample and an MIR of 0.58 for all mosquitoes and 0.75 for *Cx. tarsalis*. The panfilarial primers had 1.4% of samples test positive. Only 4 *Ae. sierrensis* mosquitoes were collected throughout the summer, and none tested positive for *D. immitis*.

For the summer of 2024, testing was expanded to 11 additional sites across Salt Lake City (Fig. 1C). *Culex* mosquitoes comprising 71 samples were tested with the *D. immitis*-specific primers (Rishniw et al. 2006). *Culex tarsalis* was the most abundant species tested ($n=1,704$; 85.2%) and *Cx. pipiens* comprised the rest ($n=295$; 14.8%). No *D. immitis* was detected in 2024 from any samples tested.

Conclusion

Due to the substantial time, effort, and money zoos invest to ensure the health of their diverse exotic species, zoos are a potential hotspot for arthropod-borne disease detection (Ludwig et al. 2002, McGregor et al. 2023). As such, monitoring these locations for pathogens spread by arthropods is of particular importance (Briggs et al. 2023, McGregor et al. 2023). The heartworm-infected mosquitoes at Utah's

Table 1. Positive filarial worm PCR results from summer 2023

Sample #	Date	Location in zoo	Species	# of mosquitoes	Primers generating amplicons (C _p)	Sanger Seq.	Result
D116	5 July 23	Pinnipeds	<i>Cx. tarsalis</i>	20	DIDR (36.5) & DICOI (33.7)	<i>D. immitis</i>	<i>D. immitis</i> confirmed
D118	5 July 23	Pinnipeds	<i>Cx. tarsalis</i>	20	DIDR (28.8) & DICOI (21.2)	<i>D. immitis</i>	<i>D. immitis</i> confirmed
D132	5 July 23	Primates	<i>Cs. inornata</i>	8	DIDR (35.2)	inconclusive	Filarial worm
D143	13 July 23	Primates	<i>Cx. tarsalis</i>	7	DIDR (35.1)	inconclusive	Filarial worm

Hogle Zoo in Salt Lake City were *Cx. tarsalis*, a known vector of several arboviruses and heartworm (Huang et al. 2013). The *D. immitis* positive samples were both collected on 5 July 2023 from the pinniped trap, indicating multiple infected mosquitoes circulating in that area simultaneously. Pinnipeds, such as seals and sea lions, are susceptible to *D. immitis* infections and can develop potentially fatal symptoms (Alho et al. 2017). The areas surrounding the zoo may also harbor infected mosquitoes, placing domestic dogs and cats at risk. The prevalence of *D. immitis* in mosquitoes reported here in Utah is substantially less than reports from Connecticut, Arkansas, and the San Joaquin Valley of California (Huang et al. 2013, McKay et al. 2013, Bagger et al. 2025), but higher than reported from Lake County, California (Tran et al. 2022). Models suggest that Utah may have lower rates of *D. immitis* infections compared to the majority of the continental United States (Wang et al. 2014). In Utah, heartworm cases in dogs have declined, from 125 cases in 2008 (Frampton 2010) down to 10 in 2021, the final year veterinary reporting was required (Price, personal communication). *Cx. tarsalis* were unusually abundant in the urban area of Salt Lake City in June and July 2023, which may have contributed to changes in heartworm ecology.

As a response to the 2 *D. immitis* detections within the zoo in 2023, surveillance efforts were expanded outside of the zoo in 2024. The goal was to investigate the range of potentially infected *Cx. tarsalis*, with special emphasis on areas expected to yield higher transmission. Two trap locations (Tracy Aviary and Lee Kay) were near dog parks and areas of outdoor recreation, while the other sites were dispersed across the Salt Lake Valley. However, despite the careful surveillance design, no *D. immitis* was detected in these mosquito samples, and the current risk in the greater Salt Lake Valley remains difficult to assess. Additional surveillance of dogs and mountain/peri-urban coyotes and foxes on the eastern side of Salt Lake Valley would provide insight into the dynamics of transmission, a further estimation of risk, and a better understanding of why so few positives were detected. Additionally, analyzing the bloodmeals taken by mosquitoes in the area would inform the zoo of the risk to specific animals and how their local feeding patterns factor into heartworm transmission dynamics.

Aedes sierrensis was historically considered the primary driver for *D. immitis* in Salt Lake City, due to its high vector competence and prevalence within urban and suburban habitats (Scoles et al. 1993). However, very few were collected in SLCMAD traps, and none tested positive for *D. immitis*. To further elucidate the current role of this species in local transmission, additional surveillance with *Ae. sierrensis*-targeted traps would be needed (Hougaard and Dickson, 1999; Chaves et al. 2020). Our targeted surveillance, focused around Utah's Hogle Zoo in 2023 and 2024, revealed a low but meaningful hazard for heartworm susceptible species. The zoo has modified their heartworm prevention program to include additional species which had not previously received prophylaxis, and has extended the preventative treatment to year-round instead of seasonally. Because *D. immitis* is endemic in Utah, but the exact risk of transmission is unknown, we recommend continued investigations into the ecology and specific involvement of local mosquito vectors of this pathogen.

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Supplementary Material

Supplementary material is available at Journal of Medical Entomology online.

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Conflicts of Interest

None declared.

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