Multiple drug resistance in hookworms infecting greyhound dogs in the USA

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Background

- Ancylostoma caninum, the canine hookworm, is the most common and one of the most pathogenic nematode parasite of dogs.
- A recent study evaluating over 39 million fecal samples from 2012-2018, showed evidence of a steady yearly increase in prevalence from 2015 onwards, with an overall increase of 47%.
- In a study assessing intestinal parasites from 3006 dogs, fecal samples collected in 288 off-leash dog parks across the USA in 2019, the prevalence of A. caninum was 7.1%.
- Hookworm infections are generally regarded as being easy to treat.
- Multiple drug resistance (MDR) to benzimidazoles (BZs) and thiabendazole concentration and the control wells, respectively. (B) LDA 96 well plate with controls and increasing concentrations of kermidazole aglycone, and third-stage larvae.

Methods

- Investigate the prevalence of infection
- Investigate the range of in vitro and in vivo drug-susceptible/resistant phenotypes
- Investigate the frequency of benzimidazole-resistant β-tubulin genotypes in greyhound dogs infected with A. caninum

Hypothesis

A. caninum infecting greyhound dogs have high resistance levels and high SNP frequencies associated with BZ resistance.

Objectives

- Investigate the prevalence of infection
- Investigate the range of in vitro and in vivo drug-susceptible/resistant phenotypes
- Investigate the frequency of benzimidazole-resistant β-tubulin genotypes in greyhound dogs infected with A. caninum

Results

Table 1. Prevalence of hookworm infection from 219 samples collected from greyhound dogs originating from 16 different tracks or farms in 8 different states

<table>
<thead>
<tr>
<th>Track/Farm</th>
<th>Prevalence (%)</th>
<th>Total T. samples</th>
</tr>
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<tbody>
<tr>
<td>Texas</td>
<td>0.2%</td>
<td>75</td>
</tr>
<tr>
<td>Florida</td>
<td>0.3%</td>
<td>60</td>
</tr>
<tr>
<td>Arizona</td>
<td>0.4%</td>
<td>50</td>
</tr>
<tr>
<td>California</td>
<td>0.5%</td>
<td>40</td>
</tr>
</tbody>
</table>

Conclusions

- Clinical, in vitro, and genetic data provide strong evidence that racing and recently retired greyhound dogs in the USA are infected with MDR A. caninum.
- Very high IC50 and IC90 values were measured for both the benzimidazoles and macrocyclic lactones.
- The F167Y benzimidazole resistance polymorphism was detected in almost every sample.
- Clinical and genetic evidence strongly suggests that these MDR A. caninum evolved on greyhound breeding farms and kennels.

REFERENCES

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Figure 1. Deep amplicon sequencing assay (ADAS) showing how the amplicon sequence variants (ASVs) were generated.

Figure 2. (A) EHA showing no hatching and L1 larvae at the highest benzimidazole concentration and the control wells, respectively. (B) LDA 96 well plate with controls and increasing concentrations of benzimidazole aglycone, and third-stage larvae.

Figure 3. Allele frequency of the F167Y single nucleotide polymorphism (SNP) at codon 167 from 10 samples of hookworm infections of greyhound dogs originating from 16 different tracks or farms in 8 different states.

Figure 4. (A) EHA showing no hatching and L1 larvae at the highest benzimidazole concentration and the control wells, respectively. (B) LDA 96 well plate with controls and increasing concentrations of benzimidazole aglycone, and third-stage larvae.

Figure 5. Deep amplicon sequencing assay (ADAS) showing how the amplicon sequence variants (ASVs) were generated.