Prevalence of *Dirofilaria immitis* in dogs from Hatay province, Turkey

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Abstract

The present study was conducted to determine the prevalence of canine dirofilariosis in Hatay province, south of Turkey. A total of 269 blood samples were collected from owned dogs in this multi-centre survey between March and July 2006. Blood samples were examined by modified Knott and enzyme-linked immunosorbent assay (ELISA) techniques to detect circulating microfilariae and antigens of *Dirofilaria immitis*, respectively. Seventy out of 269 dogs (26.0%) were positive for *D. immitis*. However, 61.4% of positive dogs had occult infection. The prevalence of canine dirofilariosis was heterogeneous in Hatay province, with higher values in shoreline (33.0%) and riverside (30.9%) areas followed by the lowland (25.5%) or mountainous (15.2%) areas. No statistically significant differences were observed in relation to sex (females, 33.3%; males, 24.4%, *P* > 0.05). When evaluating the prevalence of *D. immitis* by age, the highest prevalence was observed in dogs older than 4 years of age. Large breeds (29.6%) and the dogs living outdoors (30.2%) showed a higher prevalence in comparison to small breeds (21.4%) and the dogs living indoors (10.5%), respectively. In conclusion, according to the results of this study canine dirofilariosis had a high prevalence in Hatay province. Therefore, prophylaxis against heartworm is advisable to decrease the incidence of canine dirofilariosis.

Introduction

Canine heartworm disease (dirofilariosis) caused by the *Dirofilaria immitis* is a zoonotic parasitic disease inhabiting the right ventricle and pulmonary artery of infected dogs. Dirofilariosis is a disease with worldwide distribution, endemically seen in temperate, tropical and subtropical countries (Marquardt *et al.*, 2000). In recent years, many epidemiological studies have been performed in many countries, including Tanzania (*Matola*, 1991), Japan (*Hatsushika et al.*, 1992), USA (*Theis et al.*, 1999; *Nelson et al.*, 2005), Spain (*Aranda et al.*, 1998), Canada (*Klotins et al.*, 2000), Italy (*Cringoli et al.*, 2001), Argentina (*Rosa et al.*, 2002; *Vezzani et al.*, 2006), South Korea (*Song et al.*, 2003), Brazil (*Reifur et al.*, 2004), Europe (*Genchi et al.*, 2005) and South America (*Labarthe & Guerrero*, 2005).

It is also known that Turkey is an endemic region for dirofilariosis. Several studies have been reported regarding the prevalence of dirofilariosis in dogs from different regions of Turkey (table 1). It is of interest to determine the geographical distribution of the disease, which may be associated with ecological factors, such as existence of water, rich vegetation and mosquitoes.
Materials and methods

Study areas and sampling

Hatay province is located in the south of Turkey on the east Mediterranean coast. The climate of this province is typical of the Mediterranean region, with warm, wet winters and hot summers with high humidity (higher than 69%). A total of 269 owned dogs (older than 6 months) were randomly selected from six districts of Hatay province in this multi-centre survey. Iskenderun (shoreline area), Antakya (riverside area), Kirikhan and Hassa (lowland area), and Yayladagi and Altinozu (mountainous area) were selected for sampling between March and July 2006 (fig. 1). A 4 ml blood sample was withdrawn from the cephalic vein of each dog, and transferred to an EDTA-containing tube during daytime. Whole blood samples were centrifuged at 3000 rpm at room temperature, and the plasma samples obtained were stored at −18°C until analysis. A questionnaire was recorded regarding the dog’s age, sex, breed (large breed or small breed), and housing (indoor or outdoor), and prophylactic measures, if used for heartworm disease.

Questionnaire

A total of 269 owned dogs with ages ranging from 6 months to 14 years were examined in the present study: 221 dogs (82.2%) were male and 48 dogs (17.8%) female; 196 dogs (72.9%) were of larger breeds heavier than 20 kg (Kangal dog, pointer, collie, German Shepherd, boxer, setter, dalmatian, husky, golden retriever, and mixed breed) and 73 dogs (27.1%) were of smaller breeds (terrier, poodle, cocker, Yorkshire terrier, pincher, and mixed breed). One hundred and five dogs (39.0%) were aged 0.5–2 years, 103 (38.3%) were 2–4 years old, 26 (9.7%) were 4–6 years old and 35 (13.0%) were older than 6 years; 212 dogs (78.8%) were living outdoors and 57 dogs (21.2%) were living indoors. None of the dogs had received specific heartworm prophylaxis.

Detection of microfilariae and D. immitis adult antigen

One millilitre of each blood sample was processed using a modified Knott method (Georgi et al., 1990). The identification of filarial species was based on well-established morphological criteria (Watson et al., 1973). Circulating D. immitis antigen was detected by use of a commercial antigen enzyme-linked immunosorbent assay (ELISA) kit (DiroCHEK, Symbiotics Corp., San Diego, California, USA) with spectrophotometry, according to the manufacturer’s instructions. DiroCHEK canine heartworm antigen test kit is an enzyme immunoassay designed to detect the presence of circulating antigen from adult females of D. immitis.

Statistical analysis

The chi-square test was performed to compare the prevalence of D. immitis according to sex, age, district, housing and breed categories (large breed or small breed).

Results

Prevalence of infection

Seventy out of 269 tested dogs (26.0%) were positive for D. immitis infection with microfilariae and/or antigen detection tests. The individual results of the DiroCHEK heartworm antigen and microfilariae tests are shown in table 2. Sixty-three (23.4%) out of 269 samples tested positive with the antigen-detecting ELISA. In addition, 43 (61.4%) of the positive dogs were determined to have infection with microfilariae and/or antigen; however, seven dogs (10.0%) were antigen negative but microfilariae positive. The regional distribution of canine heartworm disease in
Hatay province is also shown in Table 2. Canine heartworm prevalence showed a heterogeneous pattern in different regions of Hatay province. The highest rates were detected in the shoreline area (33.0%) and riverside (30.9%), followed by lowland (25.5%) and mountainous (15.2%) areas.

The results of the association analysis of different factors with heartworm disease are presented in Table 3. No differences in prevalence were observed between the sexes (females, 33.3%; males, 24.4%, $P > 0.05$). The mean overall positivity rates of *D. immitis* infection were 15.2% in the 0.5–2-year-old group, 29.1% in the 2–4-year-old group, 38.5% in the 4–6-year-old group, and 40.0% in the group older than 6 years. In the present study, the prevalence of dirofilariosis in heavier dogs (>$20$ kg) (29.6%) was found to be statistically higher compared to that of lighter dogs (16.4%, $P < 0.05$). The prevalence rates of dogs kept outdoors (30.2%) and indoor dogs (10.5%) were statistically different ($P < 0.05$).

**Discussion**

Dirofilariosis is a serious disease and has long been known to spread from tropical and subtropical provinces to temperate-zone countries such as Turkey. The geographical distribution of the disease is important for the biological cycle of *D. immitis* (Rosa et al., 2002). The weather is a critical factor affecting the prevalence of this disease. Hot weather and suitable temperatures are necessary for the development of mosquitoes to produce third-stage larval development in the intermediate host; the worm needs temperatures higher than $18^\circ C$ for nearly 1 month (Montoya et al., 1998). Hatay is located in a temperate zone with a hot and humid environment from April to November ($17.2-32.4^\circ C$) and the mean humidity is higher than 69% throughout the year (Turkish State Meteorological Service, 2008). This is conducive for growth and reproduction of mosquitoes. In Turkey, the prevalence of *D. immitis* ranges from 1.5 to 46.2% (Table 1).
These different prevalence rates may reflect different testing methodologies (post-mortem inspection, detection of microfilariae and serological testing) or regional differences. In the present study, it was found that *D. immitis* had a high prevalence (26.0%) in the dogs from Hatay province. This result showed that Hatay province was the second highest prevalence following the Van province, located in Van Lake (46.2%), and the value is close to that of shoreline areas like Kocaeli province (18.3%). However, the lowland areas of Turkey (Kayseri, Elazig and Ankara) presented a lower prevalence of heartworm infection compared to our result (nearly 10%).

The prevalence of canine heartworm disease varied regionally, with higher values in shoreline areas and riversides and lower values in the lowland and mountainous areas. The geographic distribution of *D. immitis* is mainly on riverside and shoreline areas with an extended habit over a large tropical part of the world (Theis et al., 1999). A previous study of dirofilariosis demonstrated that prevalence was significantly higher in coastal areas than in urban and mountainous areas (Song et al., 2003). Rosa et al. (2002) reported that an increase in prevalence may occur in dogs that live in riverside areas because of the higher probability of being exposed to additional risk factors (types of confinement and presence of intermediary hosts). The present data revealed higher values in shoreline (33.0%) and riverside areas (30.9%), followed by the lowland (25.5%) or mountainous (15.2%) area.

Diagnosis of dirofilariosis is usually based on concentration techniques for detecting microfilariae in the blood. Immunodiagnostic techniques have recently been developed, enabling the diagnosis of occult infections. Occult dirofilariosis is defined as the presence of parasite antigens, indicating the presence of adult worms, and the absence of microfilariae. Occult infections may occur in up to 30% of infected dogs (Rawlings et al., 1982). In the present study, sixty-three (23.4%) out of 269 samples, tested positive with the antigen-detecting ELISA. In addition, 61.4% of positive dogs had occult *D. immitis* infection. This result is close to the previous report by Alves et al. (1999) (57.1%). The high percentage of occult infection is not uncommon and has also been reported by several other researchers (Labarthe et al., 1997; Reifur et al., 2004). Occult infections may be due to different causes, including treatment with macrocyclic lactones or immune-mediated reactions that are able to eliminate the microfilariae or to decrease the fecundity of adult worms. In the present study, none of the dogs had received specific heartworm prophylaxis; however, some of the dog may have received ivermectine injections as antiparasitic treatment against ectoparasites. Labarthe et al. (1997) suggested that the abusive use of

### Table 2. Overall heartworm prevalence in Hatay province.

<table>
<thead>
<tr>
<th>Research area</th>
<th>No. of dogs examined</th>
<th>No. of infected dogs</th>
<th>Rate (%)</th>
<th>Microfilariae (+)</th>
<th>Microfilariae (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoreline area (Iskenderun)</td>
<td>88</td>
<td>29</td>
<td>33.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Riverside (Antakya)</td>
<td>55</td>
<td>17</td>
<td>30.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Lowland (Kirikhan and Hassa)</td>
<td>47</td>
<td>12</td>
<td>25.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6</td>
<td>−</td>
</tr>
<tr>
<td>Mountainous (Yayladagi and Altinozu)</td>
<td>79</td>
<td>12</td>
<td>15.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>269</td>
<td>70</td>
<td>(26.0%)</td>
<td>20 (28.6%)</td>
<td>7 (10.0%)</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> The different letters indicate a significant difference among groups by chi-square test (P ≤ 0.05).

<sup>x</sup> Prevalence rate of occult *Dirofilaria immitis* infection.

### Table 3. The prevalence of *D. immitis* correlated with sex, age, breed (large breed or small breed) and housing.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of dogs examined</th>
<th>No. of positive dogs</th>
<th>Mean (%)</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>48</td>
<td>16</td>
<td>33.3</td>
<td>1.193</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Male</td>
<td>221</td>
<td>54</td>
<td>24.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2</td>
<td>105</td>
<td>16</td>
<td>15.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.519</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2–4</td>
<td>103</td>
<td>30</td>
<td>29.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>26</td>
<td>10</td>
<td>38.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 6</td>
<td>35</td>
<td>14</td>
<td>40.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larger</td>
<td>196</td>
<td>58</td>
<td>29.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.122</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smaller</td>
<td>73</td>
<td>12</td>
<td>16.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outdoors</td>
<td>212</td>
<td>64</td>
<td>30.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.029</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Indoors</td>
<td>57</td>
<td>6</td>
<td>10.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>269</td>
<td>70</td>
<td>26.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b</sup> The different letters indicate a significant difference among groups by chi-square test.
microfilaricidal agents contributed substantially to the elevated rate of occult dirofilariosis. Therefore, veterinarians should be aware of the risk of misdiagnosing heartworm patients when testing them only for microfilariae, especially if microfilaricidal drugs are used for the control of ectoparasites (Alves et al., 1999). In the present study, seven dogs (10.0%) were antigen negative but microfilariae positive, as well. The status of being antigen negative but microfilariae positive may be explained by undetectably low levels of heartworm antigen. It has been reported that commercial ELISA kits may have low sensitivity when the parasite burdens are below five D. immitis adult parasites (Martini et al., 1996; Klotins et al., 2000; Atkins, 2003). Furthermore, immune clearance of antigen–antibody complexes (Menda, 1989) and the presence of microfilariae for 1–3 years after the death of adult females, would cause negativity against adult D. immitis while being microfilariae positive.

When evaluating the prevalence of D. immitis in terms of sex, there are contradictory results. According to some of the researchers, no significant differences have been detected between the sexes (Oge et al., 2003; Song et al., 2003; Simsek et al., 2008). Whereas, some researchers (Selby et al., 1980; Montoya et al., 1998) have reported that male dogs had higher infection rates. The generally higher infection rates in male dogs may be due to their stronger attraction to mosquitoes. However, in the present study, there was no statistically significant difference in prevalence between males (24.4%) and females (33.3%).

Regarding the prevalence of D. immitis in terms of age, the results of the present study were similar to those of Yildirim et al. (2007), that older dogs have more time and more opportunities to become infected with heartworm. The likelihood of heartworm infection has been found to increase with an increased period of exposure to mosquitoes (Lee, 1993; Rosa et al., 2002).

In the present study, the prevalence of D. immitis infection in the larger dogs was found to be higher compared to that of smaller dogs. This result was consistent with other research (Butts, 1979; Selby et al., 1980). Selby et al. (1980) showed an increased prevalence of heartworm infections in hunting, sporting dogs. They speculated that this was because of their use for field training or hunting purposes, which likely contributes to an increase of the exposure risk to the infected mosquitoes (Theis et al., 1999). Similarly, in the present study most of the infected dogs were sheepdogs, hunting dogs or guard dogs, which were living outdoors.

In conclusion, this survey demonstrated that canine dirofilariosis is prevalent in Hatay province (26.0%) of Turkey, especially in shoreline and riverside areas (more than 30% prevalence). Since dirofilariosis is a zoonosis and is frequently diagnosed in dogs in the Hatay province, preventive treatments for dogs should be considered, to decrease the incidence of canine dirofilariosis.

References


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