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A molecular survey of hemoplasmas in domestic dogs from Turkey

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ABSTRACT

Mycoplasma haemocanis (*Mhc*) and *Candidatus* Mycoplasma haematoparvum (*CMhp*) are cell-wall-deficient bacterial pathogens that localize on the vertebrate erythrocyte surface. Prevalence of infection with these hemoplasmas and associated risks factors were ascertained from archived DNA samples collected from 621 domestic dogs (201 strays, 262 from shelters, 158 domestic pets) by species-specific PCR assay. The overall prevalence of infection was 15.3% (CI 12.5–18.4), with *Mhc* and *CMhp* single infection prevalence of 4.5% (CI 3.0–6.4) and 4.3% (CI 2.9–6.3), respectively. Dual infection with *Mhc* and *CMhp* was found in 6.4% (I 4.6–8.715). Infection rates for male (15.8%, CI 12.5–18.4) and female (14.5%, CI 10.5–19.4) dogs were not significantly different ($P > 0.05$), and the frequency of hemoplasmas was higher in adult dogs (18.9%, CI 15.1–23.2) than in young (9.5%, CI 6.1–14.0) ($P < 0.05$). Shelter and free-roaming stray dogs had higher prevalence of infection compared to domestic pet dogs ($P < 0.05$), but there was no significant difference in prevalence between free-roaming and shelter dogs ($P > 0.05$). A significant association between hemoplasma infection and the presence of *Rhipicephalus sanguineus sensu lato* was observed ($P < 0.05$). Presence of tick-borne pathogens (*Babesia*, *Hepatozoon*, *Anaplasma, Ehrlichia*) was also significantly associated with canine hemoplasmas ($P < 0.05$).

1. Introduction

Hemoplasmas are cell-wall-deficient bacterial pathogens that localize on vertebrate erythrocyte surface (Messick, 2004). *Mycoplasma haemocanis* (*Mhc*) and *Candidatus* Mycoplasma haematoparvum (*CMhp*) are known canine-specific hemotropic mycoplasmas that cause acute and chronic disease (Messick, 2004). Clinical symptoms are related to stress, concomitant infections, splenectomy, and immunosuppression (Wengi et al., 2008). Risk factors for canine mycoplasmosis are associated with breed, living conditions, gender (Barker et al., 2010; Novacco et al., 2010), arthropod infestation (Valle et al., 2014), and multiple infections with arthropod-borne parasites (Roura et al., 2010).

Mycoplasma species are probably cosmopolitan, occurring in dogs in Europe (Kenny et al., 2004; Novacco et al., 2010; Roura et al., 2010; Tennant et al., 2011), Asia (Sasaki et al., 2008), Australia (Barker et al., 2012), Africa (Aquino et al., 2016), and the Americas (Compton et al., 2012; Soares et al., 2016). Canine hemotropic mycoplasmas have been identified in dogs from Turkey (Aktas and Ozubek, 2017; Guo et al., 2017), but their frequency and distribution are not fully documented. Hence, we aimed to evaluate their frequency in healthy dogs from nine provinces of Turkey, as well as identify possible risk factors.

2. Materials and methods

2.1. Samples

A total of 621 archived genomic DNA (gDNA) samples extracted from apparently healthy dogs in nine provinces of Turkey (Elazig, Erzurum, Ankara, Nevşehir, Adapazarı, İzmit, Mersin, Giresun, İzmir) during 2010–2012 were included in this study. Samples were classified according to source: 201 from free-roaming dogs (recently owned and abandoned), 262 shelter dogs, and 158 domestic pets. Free-roaming dogs were obtained from the Firat University Veterinary Faculty and private veterinary clinics where the dogs were taken for neutering. Shelter dogs came from municipal shelters, where they were kept outdoors in a confined space. Samples from domestic pets were obtained at several veterinary clinics during routine health control. The dogs had not been outside of Turkey, and no dog was splenectomised.

All DNA samples were tested by PCR for *Babesia*, *Theileria*, *Hepatozoon*, *Anaplasma*, and *Ehrlichia* species to evaluate canine tick-borne infectious disease in healthy dog populations as previously reported (Aktas et al., 2015a,b). Ixodid ticks had also been collected from the dogs (unpublished data). These results were used to assess their association with hemoplasma infections. The dogs were categorised as: having ixodid ticks and having parasite co-infections, or not.

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2.2. PCR amplification

gDNA samples were screened by PCR for *Mhc* and *CMhp* infection using primer sets specific for each canine hemoplasma species (Torkan et al., 2014). The primers [forward (5′-GAAACTAAGGCCATAAATGA CGC-3′ and reverse 5′- ACCTGTCACCTCGATAACCTCTAC-3′)] were used to amplify a 328 bp fragment of the 16S ribosomal RNA gene of *Mhc*. For identification of *CMhp* infection, the primers [forward (5′-ACGAAAGTCTGATGGAGCAATAC-3′) and reverse (5′- TATCTACGCAT TCCACCGCTAC-3′)] were used to amplify a 309 bp fragment of the 16S rRNA gene of *CMhp*. PCR reaction and thermal cycling conditions were adapted from Torkan et al. (2014). DNA from *Mhc* and *CMhp* previously isolated from naturally infected dogs (GenBank accession nos. KY368749 and KY368750) were used as positive templates in the PCR. Sterile water and canine genomic DNA were used as negative controls. Amplicons were visualized by UV transillumination in a 1.5% agarose gel. To avoid cross-contamination, DNA extractions and PCR amplifications were performed in separate rooms.

2.3. Sequencing

To confirm species identity, representative PCR amplicons were purified using the QIAquick Gel Extraction Kit (Qiagen GmbH) according to the manufacturer's instructions and submitted for sequencing. The products were separated in an automated DNA genetic analyzer (ABI 310 Prism; Perkin-Elmer Corporation, Foster City, CA) and manually edited by Chromas-Lite® (www.technelysium.com.au). The sequences were registered in GenBank under the accession numbers: MF377458 for *Mhc* and MF666879 for *CMhp*.

2.4. Statistical analysis

The data were compared by the Pearson Chi Square (χ^2) test. Association of hemoplasma infection with the categorical variables gender, age, source (free-roaming, shelter, or pet), presence of ixodid ticks, and having co-infections (babesiosis, hepatozoonosis, ehrlichiosis and anaplasmosis) was tested. *P*-values ≤ 0.05 were considered significant.

2.5. Ethic statement

This work was approved by Firat University Experimental Animal Ethics Committee (Approved Protocol no. 16.02.2010-15).

3. Results

3.1. Prevalence of hemoplasmas in apparently healthy dogs

Of the 621 samples examined by PCR, 95 (15.3%, CI 12.5–18.4) were infected with hemoplasmas. The highest positivity was detected in dogs from İzmir Province with the ratio of 35% (CI 23.1–48.4), and lowest was in Nevşehir Province with 3.9% (CI 0.5–13.4) (Table 1).

The gender, age, source, presence or absence of ticks and tick-borne disease, and prevalence of total, single, and combined hemoplasma infections are presented in Table 2. Twenty-eight of the 621 samples tested (4.5%, CI 3.0–6.4) were positive for *Mhc*, and 27 (4.3%, CI 2.9–6.3) were positive for *CMhp* by species-specific PCR assays. No significant differences were seen in the frequency of *Mhc* and *CMhp* ($P > 0.05$). Both pathogens were found in 40 samples (6.4%, CI 4.6–8.7). Difference in infection rate of male (15.8%, CI 12.5–18.4) and female (14.5%, CI 10.5–19.4) dogs was not significant ($P > 0.05$); however, infection was more prevalent in adult dogs (18.9%, CI 15.1–23.2) than in youngs (9.5%, CI 6.1–14.0) ($P < 0.05$). There was a significant difference in pathogen prevalence among the dog populations. Domestic pets were less infected (7.6%, CI 4.0–12.9) compared to shelter (14.5%, CI 10.5–19.3) and free-roaming dogs (22.4%, CI 16.8–28.8) ($P < 0.05$). However, prevalence of hemoplasmas did not differ in free-roaming and shelter dogs ($P > 0.05$). A significant association of hemoplasma infection with the presence of *Rhipicephalus sanguineus sensu lato* was observed ($P < 0.05$). Among the 95 PCR-positive dogs, 61 (35.5%, CI 28.3–43.1) were infected with at least one other pathogen (*Babesia*, *Hepatozoon*, *Anaplasma, Ehrlichia*). Co-infections were also significantly associated with canine hemoplasmas ($P < 0.05$).

3.2. Sequence comparison

Sequence comparisons revealed that the *Mhc* sequences identified in this study (MG594501) exhibited 99.6% similarity to the *Mhc* isolate dd9 (KY368749) identified in dog from Turkey. The *CMhp* sequences (MG594499) was 99.7% identical to the corresponding published sequences for *CMhp* previously isolated from a dog in Turkey (KY368750).

4. Discussion

Previous studies surveying related hemoplasmas in Turkey showed 38.3% (n = 282) and 31.9% (n = 192) of shelter dogs to be infected in Diyarbakır (Aktas and Ozubek, 2017) and Konya (Guo et al., 2017) provinces, respectively. The lower prevalence in our study might be due to the geographical distribution of the pathogens or to the type of population sampled. Another possible explanation for the lower prevalence could be that the DNA samples used in the present study were archived samples and some could have degraded.

The overall prevalence of hemoplasmas in our study was similar to reports from the south of France with 15.4% (Kenny et al., 2004), Spain 14.9% (Roura et al., 2010), Romania 18% (Andersson et al., 2017) and Tanzania with 19% (Barker et al., 2010), but higher than reported in most other areas such as Nigeria 7.7% (Aquino et al., 2016), USA 1.3% (Compton et al., 2012), Trinidad 4.9% (Barker et al., 2010), Switzerland 1.2% (Wengi et al., 2008), Greece 10.6% (Tennant et al., 2011), Italy 4.5% (Ravagnan et al., 2017), and Brazil 3.4–6.4% (Valle et al., 2014; Soares et al., 2016). In contrast, the prevalence of the disease was 40% in Portugal (Novacco et al., 2010), 44% in Australia (Barker et al., 2012), 42.3% in Sudan (Inokuma et al., 2006) and 23% in Iran (Torkan et al., 2014). These worldwide variations in prevalence could be attributed to environmental variations, the sampled populations, and clinical status of the dogs, as well as to the manner in which the dogs are housed and cared for in homes and being kennelled (Inokuma et al., 2006; Novacco et al., 2010; Roura et al., 2010; Tennant et al., 2011; Valle et al., 2014). This study revealed prevalence in domestic pets to be lower than in shelter and free-roaming stray dogs. The findings are consistent with the possibility that holding in shelters, as well as ranging free, increases exposure to causal agents and the rate of direct transmission and may be a risk factor for hemoplasma infection (Kemming et al., 2004; Barker et al., 2012; Ravagnan et al., 2017). Group housing may also be a source of stress that increases susceptibility to infection (Brennan et al., 2008).

Studies from Nigeria (Aquino et al., 2016), Australia (Barker et al., 2012), Spain (Roura et al., 2010), Greece (Tennant et al., 2011), Iran (Torkan et al., 2014), and Brazil (Soares et al., 2016) found *Mhc* to be more common than *CMhp*. However, canine hemoplasma studies in France (Kenny et al., 2004), Sudan (Inokuma et al., 2006), and the USA (Compton et al., 2012) found *CMhp* to be more common. In agreement with other hemoplasma studies (Kenny et al., 2004; Barker et al., 2010; Novacco et al., 2010; Roura et al., 2010; Tennant et al., 2011; Alves et al., 2014; Valle et al., 2014), we found 6.4% (CI 4.6–8.7) of dogs to be positive for both *Mhc* and *CMhp*, and no significant difference between *Mhc* (4.5%, CI 3.0–6.4) and *CMhp* (4.3%, CI 2.9–6.3) infections ($P > 0.05$). *CMhp* was first detected in blood of a splenectomised dog in the USA in 2005 (Sykes et al., 2005).

Of greater concern is the report of *CMhp* in a human patient in 2013