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Identification, occurrence and clinical findings of canine hemoplasmas in southern Brazil

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ABSTRACT

Hemoplasmas are ubiquitous pleomorphic and epicellular bacteria detected in erythrocytes in several species. In Brazil, studies on hemoplasmas have not included information on occurrence, clinical signs, and risk factors in dogs. This paper investigates the occurrence of hemoplasmas in dogs, focusing on risk factors and clinical status. Conventional PCR for the four types of canine hemoplasmas was performed in 331 blood samples collected from dogs clinically treated at a teaching veterinary hospital. Of all samples, 17/331 (5.1%) were positive for *Mycoplasma haemocanis* and 6/331 (1.8%) were positive for a '*Candidatus Mycoplasma haemominutum*-like' organism. Risk factors included the presence of vectors, old age, dog bite wounds, and neoplastic diseases. In the multivariate analysis, a 4.40 odds ratio in dogs with vector-borne diseases indicated risk for hemoplasmosis. There was correlation between hemoplasma infection and neoplastic disease, suggesting that neoplastic conditions are a risk factor for hemoplasma infection in dogs.

1. Introduction

Hemotropic mycoplasmas (hemoplasmas) are pleomorphic, epicellular bacteria that do not grow in conventional culture media and target erythrocytes of several animal species [1]. Hemoplasmas (formerly *Haemobartonella* and *Eperythrozoon*) are classified into the class Mollicutes and into the genus *Mycoplasma* based on 16S rRNA gene [2–4]. In dogs, five hemoplasma species have been described: *Mycoplasma haemocanis* [3], '*Candidatus Mycoplasma haematoparvum*' [5,6], '*Candidatus Mycoplasma haemominutum*' [7], '*Candidatus Mycoplasma turicencis*' [8] and *Mycoplasma ovis* [9].

All hemoplasmas detected in dogs are ubiquitous, with variable occurrence, and the study population, environmental conditions, and the diagnostic method used are of relevance to the different prevalence rates recorded by

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different studies worldwide. In Europe, prevalence rates range from 15.4% in France [10] (9.6%, 3.3% and 2.6% for '*Candidatus M. haematoparvum*', *M. haemocanis*, and both hemoplasmas, respectively) to 1.2% in Switzerland [11] (0.9% for *M. haemocanis* and 0.3% for '*Candidatus M. haematoparvum*'). In Tanzania and Trinidad [12], the prevalence rates were 19% and 4.9% for *M. haemocanis*, respectively, and 2.7% for '*Candidatus M. haematoparvum*' in Trinidad. The only Brazilian prevalence study conducted in the northeastern region of the country revealed that 0.48% (1/205) of the dogs was positive for *M. haemocanis* [13].

Transmission of *M. haemocanis* by the brown dog tick (*Rhipicephalus sanguineus*) has been demonstrated experimentally [14]. However, the role of this tick in natural transmission of *M. haemocanis* and other hemoplasmas that infect dogs is unknown. Blood transfusions [15–17], splenectomy [5,6,15,16,18], immunosuppression, concomitant clinical conditions [19–22], and kennel-raised dogs [19,23–25] have been recognized as risk factors for *M. haemocanis* infection. To the best of our knowledge, risk factors associated with other hemoplasma infections in dogs have not been investigated.

Severe anemia may occur after *M. haemocanis* infection, but more typically, a clinically asymptomatic chronic infection is established and the infected dog may develop overt disease if splenectomized or immunocompromised [15,16,18,26]. Chronic infections may also be exacerbated by concurrent diseases such as ehrlichiosis [27], babesiosis [23,28] and septicemia [19,21]. Dysregulation of the immune response might explain the development of opportunistic infections in some animals with latent hemoplasmosis. There is a lack of information about the clinical course of disease in dogs infected with '*Candidatus M. haematoparvum*'. The clinical course of '*Candidatus M. haemominutum*' and '*Candidatus M. turicencis*' infections in the dog is largely unknown; cases in which infection was previously described included asymptomatic animals [7,8].

Various molecular assays have been developed for detecting hemoplasmas in the cat and dog. Conventional polymerase chain reaction (PCR) [29] and a real-time PCR assay, which has a higher sensitivity and specificity, have been used to amplify *M. haemocanis* from the blood of infected dogs. The same primer sets applied for the amplification of *M. haemofelis* in cats have been successfully used to amplify the 16S rRNA gene of *M. haemocanis* in the dog [30,31]. While the 16S rRNA gene sequence is highly preserved between these two hemoplasmas, genes unique to *M. haemocanis* or that show greater sequence divergence could be targeted for the development of a species-specific assay [32]. A real time PCR assay was previously reported for amplification of '*Candidatus M. haematoparvum*' [12] in the dog. The specificity of this assay was not tested against '*Candidatus M. haemominutum*,' however, amplification of this hemoplasma, as shown in our laboratory, is also possible. A conventional PCR has been used for detection of '*Candidatus M. turicencis*' in cats and the same protocol has also been used to amplify this hemoplasma species from dogs [8].

The aim of the present study is to investigate the occurrence of hemoplasmas in dogs in southern Brazil using conventional PCR analysis of four hemoplasma species and to assess the risk factors for infection based on clinical data obtained from household dogs.

2. Materials and methods

2.1. Sample collection and medical records

A total of 347 EDTA-anticoagulated canine blood samples were collected from September 2008 to October 2009 at the veterinary hospital of Universidade de Passo Fundo (UPF), Rio Grande do Sul, Brazil. The samples were sent out to the Laboratory of Veterinary Clinical Analyses of UPF, where they were submitted to complete blood count, serum biochemistry and storage in Eppendorf tubes at −20 °C until molecular analysis. The study protocol was approved by the local Research Ethics Committee.

All of the medical records ($n = 347$) were collected from the dogs sampled at the animal hospital. The individuals were sampled by convenience sampling from the only veterinary hospital in the region. All dog owners agreed to participate in this study. The following variables were recorded: age (old and young), ectoparasites (ticks and/or fleas), contact with other animals (dogs, cats, cows, and horses), outdoor access, living condition (private homes, farm, and kennel), dog bite wounds, breed, gender, reproduction (intact or neutered), clinical condition (unhealthy, healthy) and neoplastic disease.

2.2. DNA extraction and control for inhibitors

On a weekly basis, DNA samples were extracted from 200 μl of total blood (Generation Capture Column, Qiagen, West Sussex, United Kingdom) and stored at −20 °C for 6 months until their analyses. To check for the presence of inhibitors, a conventional PCR assay of canine GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was performed in all extracted samples [33] and those that turned out to be negative were excluded from the study.

2.3. Conventional PCR for the detection of M. haemocanis and 'Candidatus M. turicencis'

The PCR assays for canine hemoplasmas were performed at Purdue University (School of Veterinary Medicine, Department of Comparative Pathobiology) in all GAPDH positive samples. Initially, the samples were tested for *M. haemocanis* using Mhf F1: 5′-GACTTTGGTTTCGGCCAAGG-3′ and Mhf R3: 5′-CGAAGTACTATCATAATTATCCCT-3′, as previously described for the detection of *M. haemofelis* [30]. The PCR carried out on a total volume of 25 μl contained autoclaved ultrafiltered water, 1× Green GoTaq® Flexi Buffer (pH 8.5), 1.5 mM $MgCl_2$, deoxynucleoside triphosphates at a concentration of 0.2 μM, 1.25 U of GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA) forward and reverse primers at a concentration of 10 μM each and template DNA (5 μl). Cycling conditions were as follows: 95 °C for 2 min, 34 cycles of amplification (45 s at 94 °C, 30 s at 53 °C and 30 s at 72 °C) and final extension of 5 min at 72 °C in an Eppendorf Mastercycler gradient thermocycler (Eppendorf Scientific, Inc., Westbury, NY).