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Prevalence, risk factor analysis, and hematological findings of hemoplasma infection in domestic cats from Valdivia, Southern Chile

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ABSTRACT

Four distinct cat hemoplasma species are recognized worldwide. However, this is the first study to investigate the prevalence, risk factors, and hematological findings of hemoplasmas in cats from Chile. Complete blood count and 16S rRNA real-time PCR for cat hemoplasma species were performed in 384 blood samples from domestic cats in Valdivia, Chile. Among the 384 samples the species-specific prevalence was as follows: '*Candidatus* Mycoplasma haemominutum' (7.8%), *Mycoplasma haemofelis* (4.4%), '*Candidatus* Mycoplasma turicensis' (1%), '*Ca.* M. haemominutum' + *M. haemofelis* (0.78%), '*Ca.* M. haemominutum' + '*Ca.* M. turicensis' (0.52%), '*Ca.* M. haemominutum' + '*Candidatus* Mycoplasma haematoparvum' (0.26%) and '*Ca.* M. haemominutum' + *M. haemofelis* + '*Ca.* M. haematoparvum' (0.26%). Male sex, older age, outdoor access, and FIV status were risk factors for hemoplasmosis. *Mycoplasma haemofelis*-positive cats had higher mean corpuscular volume and monocyte count. Four hemoplasma species circulate in the cat population of Valdivia. '*Candidatus* M. turicensis' and '*Ca.* M. haematoparvum' have been reported for the first time in Chilean cats.

1. Introduction

Hemotropic mycoplasmas (hemoplasmas) are wall-less bacteria, uncultivable in vitro, that parasitize erythrocytes of various mammals, including domestic cats [1–5]. Four cat hemotropic mycoplasma species have been recognized worldwide, namely *Mycoplasma haemofelis* (*Mhf*), '*Candidatus* Mycoplasma haemominutum' (*C*Mhm), '*Candidatus* Mycoplasma turicensis' (*C*Mt) and '*Candidatus* Mycoplasma haematoparvum-like' (*C*Mhp) [6–9]. These bacteria attach to the erythrocyte surface and can generate hemolytic anemia [10].

*C*Mhm is the most common type of *Mycoplasma* species detected, followed by *Mhf* and *C*Mt [11–16]. There are few reports on *C*Mhp worldwide, which shows a low prevalence in the majority of studies [7,12].

In North America, the molecular prevalence of *Mycoplasma* infection in cats from the United States and Canada ranges from 4.1% to 11% [17,18]. In South America the prevalence ranges from 9.4% to 18.4%, based on studies mostly carried out in Brazil [19–22]. The prevalence in Chile has not been investigated, although there is a report in cats of central Chile [23].

Under experimental conditions, cat hemoplasmas have been transmitted by infected blood subcutaneously, intravenously, or intraperitoneally [24]. In contrast, the natural route of transmission of hemoplasma infection between cats in the field has not yet been determined [10], but blood-sucking arthropods such as ticks and fleas may play a role in hemotropic mycoplasma transmission [25]. History of cat bite abscesses as a risk factor for hemoplasma infection [26] and detection of *C*Mt [14] and *C*Mhm in the saliva of cats suggest that hemoplasma infection might be transmitted via saliva during cat bites [27]. Nevertheless, experimental transmission studies will ultimately be the only method of confirming whether saliva acts a mode of transmission for hemoplasma infection [27].

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Regarding risk factors, there seems to be no overall consensus. Some studies agree that risk factors for acquiring hemoplasma infections are male sex, outdoor access, and old age [15,29,30]. Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) status could predispose a cat to hemoplasma infections, favoring the chronic carrier state or worsening the clinical course [7,11,31,32].

Usually, the pathogenicity of hemoplasma varies regarding the infecting species. *Mhf* is the most severe, often inducing hemolytic anemia in immunocompetent cats with acute infection [33,34]. On the other hand, *C*Mhm and *C*Mt are less pathogenic, and both can cause a drop in erythrocyte parameters without clinical anemia, except in *Mhf* coinfections or in immunosuppressed cats [10,33,34]. Although the association between anemia and hemoplasma infection is not well defined, it tends to be regenerative macrocytic and hypochromic [12,13,35]. Correlation between leukogram changes and hemoplasma infections is not reported, leukopenia, neutrophilia with a left shift, monocytosis, and a presence of activated monocytes and erythrophagocytosis being described in severely ill cats [7,36].

Little information is available about hemoplasma infection in domestic cats from Chile. One study was carried out in 30 cats, with an occurrence determined by polymerase chain reaction (PCR) of 3.3% for *Mhf*, and 10% for *C*Mhm in the city of Chillaín [23]. On Chiloeí Island, another study was performed, in 30 foxes, with the prevalence described as 56.7%; this is the first evidence that a canid can be infected by a cat hemoplasma [37]. There are no data concerning risk factors or hematological findings in this country.

The aim of the current study was to determine hemoplasma prevalence in domestic cats in southern Chile. Additionally, the present work aimed to perform a risk factor analysis and evaluate hematological parameters associated with hemoplasma PCR-positive cats.

2. Materials and methods

2.1. Animals

In order to accurately determine the prevalence of hemoplasmas in Valdivia, in southern Chile, the sample size required was estimated according to Thrusfield [38], considering a prevalence of 50%, which fits the criteria when the prevalence is not known, with a sample of 384 cats. A precision of 5% was used, with a 95% confidence interval [39]. The study was approved by the Universidad Austral de Chile (UACh) bioethics committee, under the protocol number UACh 142/2013. Over a 15-month period (August 2013 to November 2014), 384 client-owned cats had their blood sampled by a veterinary team. The cats came from eight Valdivia locations throughout the city in order to acquire a balanced and representative sampling. Samples were taken from (1) home visits to pet-owning households by a veterinary team and (2) cats that presented to the Veterinary Hospital of UACh, Valdivia.

Cats were sampled regardless of age, sex, health, and reproductive status. Data obtained at the time of presentation included age, sex, lifestyle, health, and reproductive status and were recorded for potential risk factors. Each owner signed a consent form before sampling.

2.2. Hematological and serological analysis

Blood samples were collected aseptically by cephalic or jugular venipuncture, aliquoted in two EDTA collecting plastic tubes (Vacutainer®), and submitted to the Veterinary Clinical Pathology Laboratory at UACh. One EDTA anticoagulated blood sample was stored at −20 °C until real-time PCR testing. The other EDTA anticoagulated blood was used to perform a complete blood count (CBC); red blood cell (RBC), white blood cell (WBC), and platelet (PLT) counts; hemoglobin concentration (HB); packed red cell volume (PCV); mean corpuscular volume (MCV); and mean corpuscular hemoglobin concentration (MCHC). For evaluation of these hematologic parameters, an automated hematology analyzer, KX-21N (Sysmex©, Japan), was used. The blood smears were stained with rapid staining (Hemacolor®, Merck) for a differential WBC count. Later, plasma was separated, and the serological detection of FIV antibody and FeLV antigen was determined by commercial ELISA Anigen Rapid FIV Ab/FeLV Ag Test Kit® (Bionote, Inc.).

2.3. DNA extraction/purification

Frozen EDTA blood samples were thawed at room temperature and vortexed. DNA extraction and purification from 100 μL of blood were performed using a DNeasy® Blood & Tissue Kit (Qiagen, USA) and was eluted with 100 μL of elution buffer, according to the manufacturer's instructions. Concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific©, USA). The ratio between absorbance at 260 and 280 nm (OD_{260}:OD_{280}) provided an estimate of sample purity, accepting a ratio of 1.8 ± 0.2 as "pure." DNA was stored at −80 °C prior to performing the real-time PCR assay.

2.4. PCR assays

Previously described [25] real-time PCR amplifications of the partial 16S rRNA gene of hemoplasma, based on the SYBR green PCR principle, were performed for the detection of *Mhf*, *C*Mhm, and *C*Mt through melting temperature. The reaction mixture was composed of 12.5 μL of Maxima® SYBR Green/Rox Master Mix (Thermo Scientific©, USA) 300 nM concentration of the forward and reverse primers (MY16SF, MY16SR1, MY16SR2), and 5 μL of DNA template brought to a total volume of 25 μL with water nuclease free (Thermo Scientific©, USA). The protocol was 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The thermal profile of the dissociation curve was 95 °C for 15 s, 60 °C for 20 s and an increase from 60 °C to 95 °C for 20 min and finally 95 °C for 15 s. All co-positive samples were submitted to a second real-time PCR specific for *Mhf*, *C*Mhm, *C*Mt, and *C*Mhp, as previously described [12] to identify the *Mycoplasma* species present. The amplifications were carried out in a final volume of 20 μL using Maxima® SYBR Green/Rox Master Mix (Thermo Scientific©, USA), 300 nM of each primer (Mysp_F, Myhf_R, CMyh_R, and CMyhp_R), with the exception of the reverse primer of *C*Mt at 500 nM (CMyt_R) and 4 μL of DNA. The thermal cycling profile was 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. All real-time PCR reactions were triplexed, and the 28S rDNA gene was used as an internal control assay for feline genomic DNA [40] with the same thermic protocol of the first real-time PCR. All PCR runs were performed with nuclease-free water as a negative control. Genomic blood DNA from naturally infected *Mhf*, *C*Mhm, and *C*Mt from cats and *C*Mhp from a dog were provided by Dr. Severine Tasker (Bristol University) and used as positive controls. All the reactions were performed in a Stratagene Mx3000P™ (Agilent Technologies), and the amplification efficiency (E) was calculated from the slope of the DNA-positive control diluted (10-fold serial dilution) in each run as $E = 10^{1/-\text{slope}} - 1$. The sequences of the primers used and assay temperatures are shown in Table 1.

2.5. Statistical analysis

For determining prevalence, PCR-positive cats were divided by the total number of cats sampled and multiplied by 100. The