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Acute phase proteins response in cats naturally infected by hemotropic mycoplasmas

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ABSTRACT

Information about the acute phase proteins (APP) response in cats naturally infected with hemoplasmas and in cats co-infected with different species of hemoplasmas is lacking.

This study evaluated serum amyloid A (SAA), haptoglobin (Hp) and albumin in 48 cats naturally infected with hemoplasmas, including 25 with *Candidatus Mycoplasma haemominutum* and 23 co-infected with different hemoplasmas agents; and in 10 healthy control cats.

Infected cats had significantly higher Hp and lower albumin than controls. Symptomatic cats had significantly higher SAA and Hp, and lower albumin than asymptomatic animals, and also than controls. Asymptomatic cats had significantly higher Hp than controls. Concentrations of APP were not significantly different between single infected and co-infected cats.

According with these results, hemoplasmosis should be considered when alterations in APP are detected in diseased cats with compatible clinical signs. Furthermore, a subclinical infection should be considered in apparently healthy cats from endemic areas with increased Hp.

1. Introduction

Hemotropic mycoplasmas (hemoplasmas) are small epierythrocytic bacteria that infect a wide variety of mammalian species, including domestic cats [1]. Formerly considered as *Eperythrozoon* and *Haemobartonella* species, hemoplasmas were reclassified into the genus *Mycoplasma* [2,3]. Four hemoplasmas species are recognized to infect cats – *Mycoplasma haemofelis* (Mhf), *Candidatus Mycoplasma haemominutum* (CMhm), *Candidatus Mycoplasma turicensis* (CMt) and *Candidatus Mycoplasma haematoparvum*-like (CMhp) [2–5]. These agents have a worldwide distribution; prevalence of infection presents geographical variation, however CMhm is reported as the most prevalent agent in most studies [1,6–8]. Co-infections with two or more hemoplasmas species are frequently described [9–13].

From the four agents reported to infect cats, Mhf is considered the most pathogenic, causing hemolytic anemia in immunocompetent cats, which can be in some cases severe and life-threatening [13–15]. *Candidatus Mycoplasma haemominutum* and CMt are considered less pathogenic, but can also cause anemia in infected cats, mainly when associated with immunosuppressant conditions [16–18]. Clinical information on CMhp feline infection is lacking.

Acute phase proteins (APP), which are serum proteins whose concentrations are altered when animals are exposed to an inflammatory stimulus, are being increasingly used in human and veterinary medicine in diagnosis, prognosis, treatment monitoring, and in general health screening [19–21]. It was suggested that the APP profiles should

Abbreviations: APP, acute phase proteins; CMhm, *Candidatus Mycoplasma haemominutum*; CMhp, *Candidatus Mycoplasma haematoparvum*-like; CMt, *Candidatus Mycoplasma turicensis*; FeLV, feline leukemia vírus; FIV, feline immunodeficiency virus; Hp, haptoglobin; Mhf, *Mycoplasma haemofelis*; SAA, serum amyloid A

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include at least one positive major, one positive moderate, and one negative APP in order to better differentiate between pathological states and to provide more information on the evolution of the disease [22]. In the cat, serum amyloid A (SAA) is considered a positive major APP, haptoglobin (Hp) a positive moderate APP, and albumin the principal negative APP [19,23].

Serum concentrations of APP have proved to be useful biomarkers in several feline infectious diseases [24–29], including experimental single infections with Mhf and CMhm [30,31]. Nonetheless, to the authorś knowledge, the APP response in cats naturally infected with hemoplasmas or the APP response in cats co-infected with different species of hemotropic mycoplasmas has not been investigated.

Thus, the aim of the present study was to investigate the APP response by measuring SAA, Hp, and albumin concentrations in cats naturally infected by one or various species of hemotropic mycoplasmas (hemoplasmas).

2. Materials and methods

2.1. Cats and samples

The present study was performed using serum samples from previous research of prevalence of hemoplasmas and agents of vector-borne diseases in cats from the North and Center regions of Portugal [11,32,33], and was approved by the Scientific Council of the School of Agricultural and Veterinary Sciences of the University of Trás-os-Montes and Alto Douro, Portugal. Briefly, diseased cats that were presented to veterinary medical centers from the North and Centre regions of Portugal that required blood analyses as part of their diagnostic plan, and apparently healthy cats that required hematological analysis for elective surgical procedures, geriatric check-ups or determination of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infection status were randomly selected, without inclusion or exclusion criteria, and included in the prevalence studies [11,32,33]. Identification and medical data of each cat was collected, including gender, age, breed, living conditions, and clinical data related with owners information, clinical examination and results of complementary diagnostic exams. Blood samples were collected at admission from jugular or saphenous veins, centrifuged (10 min, 2000*g*, 4 °C) and serum was stored at −20 °C until analysis. All animals were tested by real-time PCR for Mhf, CMhm, CMt, CMhp [11], *Babesia vogeli*, *Babesia canis*, *Hepatozoon felis*, *Hepatozoon canis*, *Leishmania infantum*, *Toxoplasma gondii*, *Anaplasma* species, *Ehrlichia* species, *Rickettsia* species [32], and by ELISA for anti-*Dirofilaria immitis* and anti-*Wolbachia* antibodies [33]. Of the samples available from the prevalence studies referred above, samples from cats infected with hemoplasmas (single and co-infections) and without other concomitant diseases, including negative results to all the agents tested; and samples from cats that showed no abnormalities on clinical examination and results of complementary diagnostic exams, including negative results for all agents tested and for FIV and FeLV, were selected for the present research.

The overall population of hemoplasmas infected cats was grouped according with the infectious agent(s) (Table 1). In addition, the overall population of infected cats, of cats mono-infected with CMhm, of cats co-infected with CMhm and Mhf, and of cats co-infected with CMhm and CMhp, were further subdivided into two subgroups: asymptomatic cats, and cats with clinical signs and clinicopathological abnormalities compatible with hemoplasmosis (Table 1). These subgroups were made based on owner information, clinical examination and results of complementary diagnostic exams. Clinical signs and clinicopathological abnormalities considered compatible with hemoplasmosis included anemia (considered when the hematocrit < 25%) and its clinical manifestations such as mucosal pallor, weakness, depression, tachypnea and tachycardia; and anorexia, weight loss, dehydration, fever, icterus, splenomegaly, leucocytosis, leucopenia, hyperbilirrubinemia, prerenal azotaemia and elevated serum concentration of alanine aminotransferase [1,7,34]. Due to the low number of animals included in the study, only alterations in APP concentrations are presented for cats infected with CMt, co-infected with CMhm, Mhf and CMhp and co-infected with CMhm, Mhf, CMt and CMhp. Information about presence or absence of clinical signs in these cats is presented in Table 1.

Table 1
Number of cats infected with hemoplasmas (single and co-infections) included in the study.

	Total (n)	Asymptomatic (n)	Symptomatic (n)
Single infections			
CMhm	25	19	6
CMt	1	1	–
Co-infections			
CMhm + Mhf	12	8	4
CMhm + CMhp	7	–	7
CMhm + Mhf + CMhp	2	1	1
CMhm + Mhf + CMt + CMhp	1	1	–
Overall population	48	30	18

CMhm – *Candidatus Mycoplasma haemominutum*; CMhp – *Candidatus Mycoplasma haematoparvum*-like; CMt – *Candidatus Mycoplasma turicensis*; Mhf – *Mycoplasma haemofelis*.

2.2. Acute phase proteins assays

Acute phase proteins determinations were performed within two years after collection in all cases, at the Interdisciplinary Laboratory of Clinical Analysis Interlab-UMU, University of Murcia, Spain. Serum concentration of SAA, Hp and albumin were determined in all samples.

Serum amyloid A concentrations were determined by a human turbidimetric immunoassay (LZ-SAA; Eiken Chemical Co., Tokyo, Japan) previously validated for use in cats [35]. Serum concentrations lower than 5.0 μg/ml were considered normal for cats; limit of detection was set at 0.38 μg/ml [35]. Serum Hp concentrations were determined by use of the hemoglobin-binding method with the use of a commercial kit (Tridelta Development Ltd., Brey, Ireland) previously validated for use in cats [36]. Serum concentrations lower than 3.0 g/l were considered normal; limit of detection considered was 0.0088 g/l [36]. Serum albumin was performed using a commercially available kit (Albumin OSR 6102; Olympus Life and Material Science Europe GmbH, Irish branch) following instructions of the manufacturer. Serum albumin concentrations ranging from 2.5 to 3.6 g/dl were considered normal for the species.

All analyses were performed on an automated biochemistry analyzer (Olympus AU600, Olympus Diagnostica, GmbH, Freiburg, Germany), and showed an inter- and intra-assay imprecision lower than 15%, and the dilution of the samples resulted in linear regression equations with correlation coefficients close to one.

2.3. Statistical analysis

Results are shown as medians and inter quartile range (IQR) unless otherwise stated and were calculated using routine descriptive statistical procedures and software (Graph Pad Prism, Version 6). Concentrations with results lower than the detection limit were set as equal to the detection limit for further statistical analysis. Normality of distribution for each group was assessed by the Shapiro-Wilk test. As data were not normally distributed, the Mann-Whitney *U* Test or the Kruskal-Wallis one-way Analysis of Variance on Ranks, followed by Dunn's multiple comparison test, were used to compare groups of samples. P values < 0.05 were considered significant.

3. Results

Serum samples from a total of 58 cats were included in the present