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Short communication

Serum amyloid A concentration in naturally infected cats with *Hepatozoon felis* alone or coinfected with other pathogens

D. Marteles[1,2], M.E. Lebrero[1], P. Quilez[1,2], A. Gómez[1], A. Fernández[1,2], S. Villanueva-Saz[1,2]

[1] Department of Animal Pathology, Faculty of Veterinary Medicine, University of Zaragoza, Miguel Servet 177, 50013, Zaragoza, Spain
[2] Laboratory of Clinical Immunology, Faculty of Veterinary Medicine, University of Zaragoza, Miguel Servet 177, 50013, Zaragoza, Spain

Correspondence to: S. Villanueva-Saz, e-mail: svs@unizar.es
D. Marteles, e-mail: diana.martelesaragues@gmail.com tel.: +34 876554288

Abstract

Feline hepatozoonosis, caused by *Hepatozoon felis* and occasionally other *Hepatozoon* species, is a vector-borne disease transmitted through the ingestion of infected arthropods, primarily ticks. This study investigates serum amyloid A, a major acute-phase protein, in cats naturally infected with *H. felis* or co-infected with other vector-borne pathogens. A total of 41 asymptomatic, infected cats were categorized into single or co-infection groups, alongside 12 healthy controls. Serum amyloid A levels were measured using enzyme-linked immunosorbent assay and analyzed statistically. Results showed no significant association between serum amyloid A concentrations and variables such as co-infection status or pathogen load. Although levels of this acute phase proteins were slightly lower in co-infected cats, findings suggest that serum amyloid A alone may have limited diagnostic utility in distinguishing these co-infections.

Keywords: acute phase protein, cat, enzyme-linked immunosorbent assay, serum amyloid A

Introduction

Feline hepatozoonosis is a vector-borne disease caused by protozoan parasites from the family Hepatozoidae and transmitted through the ingestion of infected arthropods, particularly ticks. In cats, most of the infections are caused by *Hepatozoon felis* (Baneth et al. 2013); however other species such as *Hepatozoon canis* can infect also cats. *Hepatozoon* species commonly parasitize leukocytes, and in some cases, such as *Hepatozoon americanum*, they form true cysts within skeletal muscle. In comparison, *H. felis* predominantly infects myocardial and skeletal muscle tissues (Klopfer et al. 1973, Beaufils et al. 1998). Moreover, meronts have been detected in various other organs, including the lungs, liver, pancreas, bone marrow, lymph nodes, placenta and amniotic fluid (Baneth et al. 2013).

Although hepatozoonosis in cats is less documented compared to dogs, its prevalence in regions with high tick activity underscores its clinical relevance. From an immunological point of view, immune response against vector-borne pathogens is crucial to control/dissemination the infection. Information about the role of co-infections in cats infected with pathogens of vector-borne disease is lacking (Baneth and Vincent-Johnson 2016). However, in the case of dogs, animals with clinical leishmaniosis have a higher rate of co-infections with other vector-borne pathogens when compared with healthy controls, in some cases with more marked clinicopathological abnormalities and severity (Baxarias et al. 2018). Moreover, some laboratory abnormalities such as polyclonal gammopathy in dogs with vector-borne co-infections, could be the consequence of a chronic antigenic stimulation caused either by pathogen or the combination of several pathogen (De Tommasi et al. 2013).

Acute phase proteins have been used as tools for the diagnosis, monitoring, and prognosis of several diseases in pets. Acute-phase proteins (APPs) are a class of biomarkers synthesized by the liver in response to inflammatory stimuli, including infections. In cats, the major APP is serum amyloid A (SAA), followed by α-1-acid glycoprotein (AGP) and haptoglobin, which are classified as moderate APPs. Finally, the minor APPs include C-reactive protein (CRP) and ceruloplasmin (Kajikawa et al. 1999, Paltrinieri 2008).

APPs have been less extensively studied in feline infectious diseases in comparison to canine infectious disease. Nevertheless, cats infected by different pathogens such as haemotropic mycoplasmal bacteria including *Mycoplasma haemofelis*, *Mycoplasma haemominutum*, *Mycoplasma turicensis* (Vilhena et al. 2018), Feline immunodeficiency virus (FeLV) (Korman et al. 2012), *Hepatozoon felis*, *Babesia vogeli* (Vilhena et al. 2017), *Anaplasma phagocytophilum* (Geisen et al. 2024), feline panleukopenia (Petini et al. 2020, Yanar et al. 2024), feline infectious peritonitis (Tecles et al. 2015), *Leishmania infantum* (Savioli et al. 2021) and *Dirofilaria immitis* (Silvestre-Ferreira et al. 2017) have been evaluated.

The available information on SAA in relation to *H. felis* infection in cats is limited. To date, it has primarily been investigated in a single study that analyzed SAA concentrations in symptomatic and asymptomatic cats infected with *H. felis*, reporting higher SAA levels in symptomatic cats (Vilhena et al. 2017). The aim of the present study was to investigate the SSA in cats naturally infected with *Hepatozoon felis* or co-infected with other blood pathogens.

Materials and Methods

Samples were obtained in Spain as part of prevalence studies on feline vector-borne pathogens and had been previously collected for a published epidemiological investigation (Villanueva-Saz et al. 2023). A total of 41 naturally infected cats were included in the study. All cats were in good condition, active and alert, normothermic and properly hydrated. In all of these cats, cardiac auscultation was within normal limits and respiratory sounds were also normal. None of the cats had evidence of inadequate body condition score, signs of dehydration or showed any signs of disease such as lymph node enlargement, ocular or skin lesions. All animals were tested by quantitative polymerase chain reaction (PCR) for the presence of *Anaplasma phagocytophilum*, *Anaplasma platys*, *Bartonella henselae*, *Ehrlichia canis*, *Rickettsia* spp., *Mycoplasma haemofelis*, *Mycoplasma haemominutum*, *Mycoplasma turicensis*, *L. infantum*, piroplasms, microfilariae, leukaemia virus and feline immunodeficiency virus in a private laboratory. The qPCR was considered positive for each pathogen when the cycle threshold (Ct) was lower than 35 and when the amplification was detected in all the replicates.

Molecular analysis of *Hepatozoon* was performed as previously described by Villanueva-Saz et al. (2023) at a private laboratory, using 18S rRNA TaqMan® real-time PCR. The primer sequences used were 5′-AACACGGGAAAACTCACCAG-3′ and 5′-CCT CAAACTTCCTCGCGTTA-3′. In addition, a separate PCR protocol was employed for sequence analysis of *Hepatozoon* spp. Specifically, a 779-bp fragment of the 18S rRNA gene was amplified using the primers Hep18S-F74 (5′-CAGTAAAACTGCAAATGGCT CAT-3′) and Hep18S-R853 (5′-CCAATAATG TAGAACCAAAATCCT-3′). The 25 µl PCR mixture

contained 15.25 µl of PCR-grade water, 5 µl of 5× reaction buffer (Biozym, Oldendorf, Germany), 1 µl of each primer (10 pmol), 0.25 µl of Biozym HS Taq DNA Polymerase, and 2.5 µl of template DNA. The cycling conditions consisted of an initial denaturation at 98°C for 2 minutes, followed by 40 cycles of 98°C for 15 seconds, 55.5°C for 15 seconds, and 72°C for 15 seconds. The *Hepatozoon* 18S rRNA amplicons were then Sanger-sequenced by LGC Genomics.

Additionally, an enzyme-linked immunosorbent assay (ELISA) for anti-*Leishmania infantum* (Marteles et al. 2024) and anti-*Dirofilaria immitis* antibodies were also performed (Villanueva-Saz et al. 2021). Routine laboratory tests, such as a complete blood count and biochemistry profile, were not performed, as the samples had originally been collected for a previously study (Villanueva-Saz et al. 2023).

Infected cats were divided into two groups according to the identified infectious agents. Group 1 consisted of 27 asymptomatic cats infected solely with *H. felis* (11 males and 16 females). Additionally, 14 asymptomatic cats naturally co-infected with *H. felis* and other pathogens were analyzed, including *B. henselae* (n=5; 2 males, 3 females), *M. haemofelis* (n=3; 2 males, 1 female), *M. haemominutum* (n=1; female), *M. turicensis* (n=1; male), and co-infection with *M. haemofelis* and *M. haemominutum* (n=4; 3 males, 1 female). In addition, 12 samples from healthy cats (6 males and 6 females) were examined. All animals tested negative for the remaining pathogens screened by both molecular and serological methods. Samples were collected between June and December 2023 and stored at −20°C until analysis. This study was conducted under Project Licence PI75/20, approved by the Ethics Committee for Animal Experiments of the University of Zaragoza. The care and use of animals were performed according with the Spanish Policy for Animal Protection RD 53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

SAA concentrations were determined by the use of a commercial ELISA kit (Tridelta Development Ltd., Brey, Ireland) previously validated for use in cats. Absorbance values were read at 450 nm (reference wavelength) in an automatic microELISA reader (ELISA Reader Labsystems Multiskan, Midland, Canada). A calibration curve was obtained for the assay using standards within a concentration ranging between 100 to 6.25 ng/ml. The standard curve was calculated using a computer generated polynomic curve. A R2-value of standard of 0.995 was obtained. All samples and standards were analyzed in duplicate on each plate.

Results are shown as mean and standard deviation using routine descriptive statistical procedures and software (Statistical analysis was performed using MedCalc® Statistical Software version 20.118 (Ostend, Belgium; https://www.medcalc.org; 2022). 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, a Kruskal-Wallis test followed by the Dunn's multiple comparison test were used to compare variables between groups. The Spearman correlation coefficient was used to determine correlations between concentration of SAA, and the number of pathogens. A value of $p \leq 0.05$ was considered significant.

Results and Discussion

SAA concentration (mean±standard deviation) of the group infected by *H. felis* alone was 2.86±8.74 µg/ml in comparison to group co-infected with a concentration of 2.40±7.45 µg/ml and control cats with a concentration of 1.64±5.27 µg/ml. Finally, no significant association ($p>0.05$) was detected between SAA concentration and the related variables included (gender, number of pathogens and presence/absence of co-infection and control samples). Our findings suggest that the acute-phase response, as indicated by SAA levels, was lower in the co-infected group compared to cats infected with *H. felis* alone; however, in both groups, SAA concentrations remained within the reference range for this acute-phase protein. For comparison, a previous study reported SAA concentrations ranging from 0 to 5.4 µg/mL in 54 clinically healthy cats (Waugh et al. 2022). Serum amyloid A is a sensitive marker that typically increases rapidly in response to inflammation. Nevertheless, Baneth et al. (2013) observed no inflammatory cell infiltration in muscle tissue containing *H. felis* meronts, particularly within the capsule, and gamonts were primarily found circulating in neutrophils. These findings, together with our results, suggest that the early or asymptomatic stages of *H. felis* infection may not elicit a strong acute-phase response, potentially explaining the low SAA concentrations observed. In our study, most co-infected cats with *Hepatozoon* were also infected with *Mycoplasma* spp. However, no significant differences in SAA concentrations were observed between cats infected with *Mycoplasma* alone and those with co-infections involving multiple *Mycoplasma* species in either asymptomatic or symptomatic cats (Vilhena et al. 2018). In contrast, another study found that cats infected with *M. haemofelis* had higher SAA concentra-

tions than those infected with *M. haemominutum*, although both species induced an acute-phase response. Moreover, while the presence of other factors, such as FIV infection, did not alter SAA concentrations, other acute-phase proteins, including α-1-acid glycoprotein, were affected (Korman et al. 2012). Among *Mycoplasma* species, *M. haemofelis* is recognized as the most pathogenic, capable of causing hemolytic anemia in immunocompetent cats. On the other hand, *M. haemominutum* and *M. turicensis* are considered less pathogenic, although they can still lead to anemia in immunosuppressed cats (Tasker et al. 2009, Weingart et al. 2016, Willi et al. 2006).

For other pathogens, such as *L. infantum*, no significant differences were observed between cats with positive serology but negative molecular test results, cats with positive PCR and serology results, and healthy control cats. Similarly, no differences were detected between seropositive but uninfected cats and seropositive infected cats (Savioli et al. 2021). In the case of feline heartworm infection, no significant correlation was found between SAA levels and antibody titres for *D. immitis* or *Wolbachia* species. However, elevated SAA levels were associated with the presence of clinical signs caused by *D. immitis* (Silvestre-Ferreira et al. 2017).

Finally, SAA concentration serves as a valuable marker for identifying the presence or absence of inflammation in diseased cats. However, this molecule is unable to differentiate between septic and non-septic inflammation (Troìa et al. 2017). In this context, the clinical applications of APPs in veterinary medicine are expected to align closely with those in human medicine, potentially utilizing a panel of different APPs to enhance diagnostic accuracy across various clinical settings.

The main limitations of this study stem from its retrospective design, particularly the lack of detailed anamnesis and clinical information. Although the cats were classified as apparently healthy based on general physical examination, haematological, and biochemical results, comprehensive clinical histories were not available. However, a strength of this study is the exclusion of other pathogens that could have contributed to increased serum amyloid A levels.

In conclusion, this study highlights the role of SAA in evaluating the acute-phase response in cats naturally infected with *H. felis* and co-infected with other pathogens. Although SAA levels were slightly lower in co-infected cats, no significant differences were detected between groups. These findings underscore the complexity of immune responses in feline vector-borne diseases, emphasizing the need for further research to better understand APP dynamics and the impacts of co-infections.

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References

Baneth G, Sheiner A, Eyal O, Hahn S, Beaufils JP, Anug Y, Talmi-Frank D **(2013)** Redescription of Hepatozoon felis (Apicomplexa: Hepatozoidae) based on phylogenetic analysis, tissue and blood form morphology, and possible transplacental transmission. Parasit Vectors 6: 102.

Baneth G, Vincent-Johnson N **(2016)** Hepatozoonosis; In: Day MJ (ed), Arthropod-borne infections diseases of the dog and cat. CRC Press Taylor & Francis Group, pp 109-124.

Baxarias M, Álvarez-Fernández A, Martínez-Orellana P, Montserrat-Sangrà S, Ordeix L, Rojas A, Nachum-Biala Y, Baneth G, Solano-Gallego L **(2018)** Does co-infection with vector-borne pathogens play a role in clinical canine leishmaniosis?. Parasit Vectors 11: 135.

Beaufils JP, Martin-Granel J, Jumelle P **(1998)** Hepatozoon spp. parasitemia and feline leukemia virus infection in two cats. Feline Pract 26: 10–13.

De Tommasi AS, Otranto D, Dantas-Torres F, Capelli G, Breitschwerdt EB, de Caprariis D **(2013)** Are vector-borne pathogen co-infections complicating the clinical presentation in dogs?. Parasit Vectors 6: 97.

Geisen V, Pantchev N, Wuelfing K, Würthner C, Gierschner K, Urban C, Lambach Y, Hartmann K, Bergmann M **(2024)** Anaplasma phagocytophilum infection associated with strong inflammatory response in 3 cats. Tierarztl Prax Ausg K Kleintiere Heimtiere 52: 40–47.

Kajikawa T, Furuta A, Onishi T, Tajima T, Sugii S **(1999)** Changes in concentrations of serum amyloid A protein, alpha 1-acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery. Vet Immunol Immunopathol 68: 91–98.

Klopfer U, Nobel TA, Neumann, F **(1973)** Hepatozoon-like parasite (schizonts) in the myocardium of the domestic cat. Vet Pathol 10: 185–190.

Korman RM, Cerón JJ, Knowles TG, Barker EN, Eckersall PD, Tasker S **(2012)** Acute phase response to Mycoplasma haemofelis and 'Candidatus Mycoplasma haemominutum' infection in FIV-infected and non-FIV-infected cats. Vet J 193: 433-438.

Marteles D, Martínez MV, Fernández A, Riera C, Fisa R, Roca-Geronès X, Chavez-Fisa S, Castañeda S, Ramírez JD, Davis JE, Sumova P, Volf P, Verde M, González A, Alcover MM, Villanueva-Saz, S. **(2024)** Assessment of the exposure to Phlebotomus perniciosus and the presence of anti-Leishmania infantum antibodies in stray cats in an endemic region of Spain, and their potential correlation with environmental factors. Vet Q 44: 1-8.

Paltrinieri S **(2008)** The feline acute phase reaction. Vet J 177: 26-35.

Petini M, Drigo M, Zoia A **(2020)** Prognostic value of systemic inflammatory response syndrome and serum concentrations of acute phase proteins, cholesterol, and total thyroxine in cats with panleukopenia. J Vet Intern Med 34: 719-724.

Savioli G, Archer J, Brianti E, Benelli G, Schnyder M, Iatta R, Otranto D, Cantacessi C **(2021)** Serum amyloid A levels and alpha 2 and gamma globulins on serum protein electrophoresis in cats exposed to and infected with Leishmania infantum. Parasit Vectors 14: 217.

Silvestre-Ferreira AC, Vieira L, Vilhena H, Cerón JJ, Tvarijonaviciute A, Montoya-Alonso J A, Carretón E, Pastor J **(2017)** Serum acute phase proteins in Dirofilaria immitis and Wolbachia seropositive cats. J Feline Med Surg 19: 693-696.

Tasker S, Peters IR, Papasouliotis K, Cue SM, Willi B, Hofmann-Lehmann R, Gruffydd-Jones TJ, Knowles TG, Day MJ, Helps CR **(2009)** Description of outcomes of experimental infection with feline haemoplasmas: copy numbers, haematology, Coombs' testing and blood glucose concentrations. Vet Microbiol 139: 323-332.

Tecles F, Caldín M, Tvarijonaviciute A, Escribano D, Martínez--Subiela S, Cerón JJ **(2015)** Serum biomarkers of oxidative stress in cats with feline infectious peritonitis. Res Vet Sci 100: 12-17.

Troìa R, Gruarin M, Foglia A, Agnoli C, Dondi F, Giunti M **(2017)** Serum amyloid A in the diagnosis of feline sepsis. J Vet Diagn Invest 29: 856–859.

Vilhena H, Tvarijonaviciute A, Cerón JJ, Vieira L, Pastor J, Silvestre-Ferreira AC **(2017)** Acute phase proteins response in cats naturally infected with Hepatozoon felis and Babesia vogeli. Vet Clin Pathol 46: 72–76.

Vilhena H, Tvarijonaviciute A, Cerón JJ, Pastorinho MR, Martinez-Subiela S, Pastor J, Silvestre-Ferreira AC **(2018)** Acute phase proteins response in cats naturally infected by hemotropic mycoplasmas. Comp Immunol Microbiol Infect Dis 56: 1-5.

Villanueva-Saz S, Giner J, Verde M, Yzuel A, González A, Lacasta D, Marteles D, Fernández A **(2021)** Prevalence of microfilariae, antigen and antibodies of feline dirofilariosis infection (Dirofilaria immitis) in the Zaragoza metropolitan area, Spain. Vet Parasitol Reg Stud Reports 23: 100541.

Villanueva-Saz S, Martínez M, Nijhof AM, Gerst B, Gentil M, Müller E, Fernández A, González A, Yusuf MS, Greco G, Verde M, Sgroi G, Lacasta D, Marteles D, Trotta M, Schäfer I **(2023)** Molecular survey on vector-borne pathogens in clinically healthy stray cats in Zaragoza (Spain). Parasit Vectors 16: 428.

Waugh EM, Haining H, Harvie J, Ridyard AE, Eckersall PD **(2022)** Validation of an automated immunoturbidimetric assay for feline serum amyloid A. BMC Vet Res 18: 359.

Weingart C, Tasker S, Kohn B **(2016)** Infection with haemoplasma species in 22 cats with anaemia. J Feline Med Surg 18: 129-136.

Willi B, Boretti FS, Baumgartner C, Tasker S, Wenger B, Cattori V, Meli ML, Reusch CE, Lutz H, Hofmann--Lehmann R **(2006)** Prevalence, risk factor analysis, and follow-up of infections caused by three feline hemoplasma species in cats in Switzerland. J Clin Microbiol 44: 961-969.

Yanar KE, Baysal S, Ulaş N, Aktaş MS, Timurkan MÖ, Aydın H **(2024)** Prognostic potential of copper, zinc, copper/zinc ratio, cobalamin, and serum amyloid A in cats with panleukopenia. J Vet Intern Med 38: 1535-1541.