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Serum protein profile of cats as biomarker of *Aelurostrongylus abstrusus* invasion

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Abstract

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Lung strongyloidoses in cats are caused by members of superfamily Metastrongyloidea. The most frequently encountered species *Aelurostrongylus abstrusus* (Railliet, 1898) (Rhabditida; Angiostrongylidae) parasitises in the lung tissue of both domestic and wild cats. They become infected by eating paratenic hosts or food contaminated by snail slime. The present study aims to establish the changes in circulating blood proteins: total protein, albumin, globulins and fibrinogen, as well as albumin/globulin (A/G), fibrinogen/albumin (F/A) and fibrinogen/total protein (F/TP) ratios in cats with chronic *A. abstrusus* infection. The research was performed in cats from both sexes, 4 months to 8 years of age, regardless of the breed. All animals were reared outdoor and had a history of respiratory illness. The diagnosis was made on the basis of analysis by the method of Baermann funnel technique. Statistically significant ($P < 0.05$) increase in total protein (0.048), fibrinogen (0.032), F/TP (0.048) and F/A (0.019), as well as a statistically significant ($P= 0.017$) reduction in albumin were found compared to the control group.

Keywords: cats; lungworm; fibrinogen; protein; *Aelurostrongylus abstrusus*

Introduction

Lung strongyloidiases in cats are nematodiases caused by members of suborder Metastrongyloidea. The most frequently encountered species *Aelurostrongylus abstrusus* parasitises in the lung tissue of both domestic and wild cats. Infected cats shed first-stage larvae (L1), which are being ingested by intermediate hosts – slugs and snails (Morelli et al., 2021). Cats become infected by eating paratenic hosts or food contaminated by snail slime. In the different endemic regions, prevalence of invasion in hunting cats varies from 1% to 50% (Knaus et al., 2011; Giannelli et al., 2017). In the past, the pathogenic impact of *A. Abstrusus* was substantially underestimated. During the 1980s however, it was proved that the infection with *A. Abstrusus* has caused 10% of fatal outcomes of inhalational anaesthesia complications (Gerdin et al., 2011). Subsequent studies have shown that strongylids induce obstruction and inflammation of airways. Furthermore, they provoke immune-mediated type III and type IV hypersensitivity, associated with alveolar, peribronchial, vascular and interstitial lesions (Pennisi, 2015). Laid eggs and lar-

vae induce thrombosis of pulmonary capillaries. Massive invasions with lung strongylids produce lung oedema, airways' obstruction, multifocal haemorrhages and diffuse hepatisation (Naylor et al., 1984). Adult stages of *A. Abstrusus* move through the lung parenchyma and females lay their eggs. This way, even a few parasites may cause extensive lung damage (Olsen et al., 2015). Massive egg production and the migration of L1 in the upper respiratory tract (when they are ingested) generates a strong inflammatory response (Grandi et al., 2005; Morelli et al., 2021), which alters liver protein synthesis. Three main proteins are encountered in blood circulation: albumin, globulin and fibrinogen. All they make up the total protein. The amount of fibrinogen is the smallest – about 5% of total protein content. In daily practice, it is used as indicator of early systemic inflammatory response. In all species, fibrinogen increase is initiated within 24 hours after pathological injuries. The magnitude of fibrinogen response differs among species, but is generally proportional to the extent of inflammation. If disease course is favourable, fibrinogen concentrations rapidly return to the normal range. In chronic disease they increase and remain elevated while disease persists. At the same time, a balance between the production and destruction of leukocytes during the prolonged course of the pathological process is established and white blood cell counts are already normalised. The increase in plasma fibrinogen levels in response to systemic inflammation and tissue damage is moderate (2-10 times), which classifies it in the group of moderate positive acute-phase proteins (Murata al., 2004; Van den Bossche et al., 2010). Similarly to other plasma proteins except for immunoglobulins, albumin is also synthesised in the liver and is catabolised by all metabolically active tissues. It belongs to negative acute-phase proteins, whose concentrations decrease during the course of infection or inflammation. Reduced albumin levels during inflammation are probably associated with the effect of cytokines such as interleukin-6 (IL-6) and tumour necrosis factor -α (TNF-α). It is evidenced that serum albumin has protective properties comprising maintenance of physiological homeostasis, antioxidant activity, anti-inflammatory effects and prevention of apoptosis (Seo et al., 2016). Albumin to globulin ratio is often deemed a more informative parameter than albumin and globulin absolute concentrations, especially in diseases that cannot be diagnosed only through total protein estimation (Zapryanova et al., 2017). In available literature, there is no information on the diagnostic and prognostic value of the fibrinogen/albumin ratio in animals. Recent reports demonstrated that this ratio was correlated with patient's prognosis in various human oncological diseases (Tan et al., 2017; Xu et al., 2018).

The present study aimed to establish the changes in circulating blood proteins: total protein, albumin, globulins and fibrinogen, as well as albumin/globulin, fibrinogen/albumin and fibrinogen/total protein ratios in cats with chronic *A. Abstrusus* infection.

Material and Methods

The present study was performed in cats from both sexes, 4 months to 8 years of age, mixed breed from the Stara Zagora region, referred for diagnostics and treatment to the University Veterinary Hospital of the Trakia University. All animals were reared outdoor and had a history of respiratory illness. The diagnosis was made on the basis of analysis by the method of Baermann (Zajac & Conboy, 2012). L1 had a mean length of 407.9±27.7 μm (360.0÷475.0 μm), a terminally located mouth opening and a forked tail with a curve in the shape of the Latin letter "S", with a short dorsal spine.

For blood analyses, 2 ml blood were collected from *vena cephalica antebrahii* using sterile G20 needles and disposable 2 ml syringes. Immediately after collection, blood was transferred in containers with lithium heparin. Heparinised blood was centrifuged (600g, 10 min, 4 C) within 30 minutes from collection. Total protein and albumin were analysed within an hour from blood collection on an automated biochemical analyzer (Mindray BS-120, China).

Fibrinogen concentration was determined by the nephelometric method of Podmore (Todorov, 1972) with 10% Na_2SO_4 at λ 570 nm within 2 hours after blood sampling. To 0.25 mL of plasma, 2.5 mL of 10.5% Na_2SO_4 was added; a control sample of 0.25 mL of plasma and 2.5 mL of 0.9% solution of NaCl was also prepared. The absorbance was measured after 3 min at λ=570 nm, and the result was calculated by multiplication of absorbance value by 15.5.

Globulins were determined as the difference between total protein minus albumin and fibrinogen values.

Only cats (n=6) positive for A. abstrusus larvae and no history of previous illness, comorbidity, organ or traumatic injury were chosen. The control group (n=6) was constitute from cats, free of internal and external parasites.

Statistical analysis of data was done in Microsoft Excel (ToolPack) using descriptive statistics functions. Values are presented as means and standard error of mean (Mean±SE). The group means were compared using IBM® SPSS® Statistics software platform. Differences with $P < 0.05$ were considered statistically significant.

Results

The analysis of results from blood biochemical analysis showed substantial between-group differences in values of most analysed parameters. The most prominent difference was observed between fibrinogen concentrations. In infected cats, the average fibrinogen value (14.4 g/L) was more than twice higher compared to values in controls (6.3 g/L). The highest individual measurement (21.6 g/L) was from an infected cat. The highest level of significance (P=0.017) between the groups was observed for albumin values, which were lower in the group infected with *A. Abstrusus* (29.0 g/L) than in controls (41.0 g/L). Blood total protein was also statistically significantly different (P<0.05). Higher average concentrations (80.3 g/L) were detected in infected cats, whereas mean total protein in controls was 75.0 g/L. For globulins, no statistically significant difference between both groups was demonstrated.

Changes were detected also for ratios of total protein and protein fractions. The highest level of statistical significance was found out for fibrinogen/albumin ratio (P=0.019). In cats infected with *A. Abstrusus* the average ratio was 0.56 vs 0.16 in controls. A similar substantial difference (P<0.05) was evidenced for fibrinogen/total protein ratio: 0.17 in lungworm-infected and 0.08 in healthy cats. The differences between albumin/globulin and fibrinogen/globulin ratios in both groups were not relevant.

All results with P values in the two groups are presented in Table 1.

Discussion

According to obtained results, serum protein profile in cats with chronic *A. Abstrusus* invasion was altered compared to healthy controls. The observed changes in concentrations of studied parameters were statistically significant regardless of statements that the infection with *A. Abstrusus* generally leads to mild to moderate clinical signs (Traversa et al., 2014). We rather agree

Table 1. Blood total protein, protein fractions and their ratios in cats with natural *A. abstrusus* invasion.

	Total protein (g/l)	Albumin (g/l)	Globulins (g/l)	Fibrinogen (g/l)	A/G	F/G	F/A	F/TP
Infected (n = 6)	80.3* ±16.32	29.0* ±6.68	36.9 ±12.31	14.4* ±7.02	1.21 ±0.99	0.35 ±0.16	0.56* ±0.32	0.17* ±0.08
Controls (n = 6)	75.0 ±6.10	41.0 ±6.64	27.6 ±8.33	6.3 ±1.87	1.77 ±1.06	0.26 ±0.13	0.16 ±0.06	0.08 ±0.03
P value	0.048*	0.017*	0.193	0.032*	0.410	0.389	0.019*	0.048*

** P<0.05.*

with suggestions of Grandi et al. (2005) and Genchi et al. (2014), affirming that the massive production of eggs and the migration of larvae to the upper respiratory tract generates a strong inflammatory response. On the other hand, the complex association between local inflammation and structural lung damage could be possibly bidirectional. Published research data prove that systemic or local inflammation may induce pathological remodelling, including apoptosis, maladaptive hypertrophy, endothelial dysfunction etc., resulting in inflammation through release of proinflammatory cytokines or through haemodynamic response (Dick & Epelman, 2016). Coagulation, fibrinolysis and complement cascade pathways perform a key role in inflammatory response. Increased concentrations of proinflammatory cytokines in the course of the pathological process alter the synthesis of proteins in the liver – acute-phase response (Eckersall & Bell, 2010).

Fibrinogen is one of main "positive" acute-phase proteins, encountered in all vertebrates (Cerón et al. 2005). It is involved in the ultimate stage of blood coagulation as substrate for formation of fibrin and serves as a matrix for migration of cells of inflammation (Thomas, 2008). Fibrin mediates aggregation of the platelets, endothelial cells proliferation, tissue fibroblast proliferation, formation of capillaries and angiogenesis, thus enhancing tissue revascularisation and wound healing. Apart its role in fibrin formation, fibrinogen promotes blood coagulation via activation of platelets (Zapryanova et al., 2013). The life cycle of *A. Abstrusus* may provide a logical explanation of the established marked increase in blood levels of this parameter. Our results support the data reported by Murata (2004) and Van den Bossche (2010) for 2- to 10-fold increase in fibrinogen values in response of systemic inflammation and tissue damage. The present study confirmed that high fibrinogen concentrations allowed identifying a non-specific inflammation at the background of normal results from all other routine haematological and blood biochemical assays.

Albumin is the most abundant protein in plasma representing about 50% of total protein (Seo et al., 2016). Its low value may reflect a poor nutritional status or massive inflammatory response. The observed significant reduction of albumin values correlates with the fact that albumin is a negative acute-phase protein and its concentrations decrease during infection or inflammation (Murata al., 2004; Eckersall & Bell, 2010). Most probably, the decrease in albumin is associated with its protective properties and depletion of available amounts for maintenance of homeostasis through its anti-inflammatory effects and apoptosis prevention (Seo et al., 2016). The obvious change in the levels of the two acute-phase proteins (fibrinogen and albumin) demonstrates the presence of inflammatory process that cannot be classified as chronic at the background of chronic parasitic infection.

The divergent trends observed for blood fibrinogen and albumin determines the change of their ratio during the course of inflammation. At this moment, the determination of this ratio is not a part of routine laboratory practice. Usually, the albumin/globulin ratio is calculated. Our results indicate that the difference between the two studied groups was far more significant with respect to the F/A ratio ($P < 0.05$), whereas the between-group difference in the A/G ratio was not consistent. This findings agrees with the studies of Tan et al. (2017), affirming the fibrinogen/albumin ratio as a promising new predictor of human oesophageal squamous cell carcinoma. Another research team also pointed at the fibrinogen/albumin ratio as a reliable and potentially useful serum prognostic biomarker of hepatocellular carcinoma in men (Qiaodong et al., 2016). According to these authors, the parameter may be also alternatively used to predict recurrence. In both cases, inflammation is the common element of the pathogenetic mechanism. Numerous clinical and experimental studies provide convincing proofs that inflammation is an important component of tumour progression (Coussens & Werb, 2002; Grivennikov et al., 2010). Increased F/A has been established and recommended for use in order to diagnose human ankylosing spondylitis (Meng Liu et al., 2020). We affirm that the inflammation is at the background of observed alterations in serum proteinogram in the course

of the parasitic infection. The determination of the F/A ratio is not yet part of veterinary medical diagnostics. To the best of our knowledge, no data are reported on the diagnostic and prognostic value of blood F/A ratio in animals. At the same time, the determination of F/A ration is based on routine laboratory assays of fibrinogen and albumin, which are not expensive and routinely done in the clinical practice. Therefore, it may become a reliable and convenient diagnostic and prognostic biomarker.

The changes in the F/TP ratio are largely similar to those of F/A. As serum globulin concentrations between the two groups were not statistically significant, it was assumed that the difference in total protein values was due to changes in A and F (Table 1).

This study has some weaknesses that should be commented. First, the study is retrospective, not experimental, so the sample size is relatively small. A large-scale study with higher number of patients is therefore advised. Second, the retrospective study design did not allow monitoring of dynamics of studied parameters. The analysis of the same patients after treatment would give the opportunity to evaluate the prognostic value of the different parameters. Third, other factors as occult infections and haematological diseases that may influence the serum protein profile and compromise the correct interpretation of study results.

Conclusion

Our study demonstrated that the serum protein profile in cats was altered in cats with natural *A. Abstrusus* invasion compared to controls. This study describing the diagnostic relevance of the fibrinogen/albumin ratio in animal pathology. Indeed more research is needed before it may be used as a diagnostic and prognostic biomarker in veterinary medicine.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

Ceron, J. J., Eckersall, P. D. & Martinez, S. (2005). Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Vet. Clin. Path*., *34*, 85-99. doi: 10.1111/j.1939-165x.2005.tb00019.x.

Coussens, L. M. & Werb, Z. (2002). Inflammation and cancer. *Nature*, *420*(6917), 860–867. DOI: 10.1038/nature01322.

Dick, S. A. & Epelman, S. (2016). Chronic heart failure and inflammation: What do we really know? *Circ. Res*., *119*, 159–176. DOI: 10.1161/circresaha.116.308030.

Eckersall, P. D. & Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal*, *185*, 23–27.

Genchi, M., Ferrari, N., Fonti, P., De Francesc, I., Piazza, C. & Viglietti, A. (2014). Relation between *Aelurostrongylus abstrusus* larvae excretion, respiratory and radiographic signs in naturally infected cats. *Veterinary parasitology*, *206*(3-4), 182-187. ISSN 0304-4017. doi: 10.1016/j.vetpar.2014.10.030.

Gerdin, J. A., Slater, M. R., Makolinski, K. V., Looney, A. L., Appel, L. D., Martin, N. M. & McDonough, S. P. (2011). Post-Mortem Findings in 54 Cases of Anesthetic Associated Death in Cats from Two Spay—Neuter Programs in New York State. *Journal of feline medicine and surgery*, *13*(12), 959-966. 10.1016/j.jfms.2011.07.021.

Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., René-martellet, M., Papadopoulos, E., Farkas, R., Napoli, E., Brianti, E., Tamponi, C., Varcasia, A., Alho, A. M., Carvalho, L. M., Cardoso, L., Maia, C., Mircean, V., Mihalca, A. D., Miró, G., Schnyder, M., Cantacessi, C., Colella, V., Cavalera, M., Latrofa, M. S., Annoscia, G., Knaus, M., Halos, L., Beugnet, F. & Otranto, D. (2017). Lungworms and gastrointestinal parasites of domestic cats: a European perspective. *International journal for parasitology*, *47*(9), 517-528. doi: 10.1016/j.ijpara.2017.02.003.

Grandi, G., Calvi, L. E., Venco, L., Paratici, C., Genchi, C., Memmi, D. & Kramer, L. (2005). Aelurostrongylus abstrusus (cat lungworm) infection in five cats from Italy. *Veterinary parasitology*, *134*(1-2), 177-182. doi: 10.1016/j.vetpar.2005.06.015.

Grivennikov, S. I., Greten, F. R. & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell*, *140*(6), 883–899. doi: 10.1016/j.cell.2010.01.025.

Knaus, M,. Kusi, I., Rapti, D., Xhaxhiu, D., Winter, R., Visser., M. & Rehbein, S. (2011). Endoparasites of cats from the Tirana area and the first report on Aelurostrongylus abstrusus (Railliet, 1898) in Albania. *Wien Klin Wochenschr.*, *123* (Suppl. 1), 31-35. doi: 10.1007/s00508-011-1588-1. Epub 2011 Jun 3. PMID: 21633814.

Meng, L., Yukai, H., Zhengping, H., Zheng, Zh., Weiming, D., Zhixiang, H., Qidang, H. & Tianwang, L. (2020). The role of fibrinogen to albumin ratio in ankylosing spondylitis: Correlation with disease activity, *Clinica Chimica Acta*, *505*, 136-140. ISSN 0009-8981, https://doi.org/10.1016/j.cca.2020.02.029.

Morelli, S., Diakou, A., Colombo, M., Di Cesare, A., Barlaam, A., Dimzas, D. & Traversa, D. (2021). Cat Respiratory Nematodes: Current Knowledge, Novel Data and Warranted Studies on Clinical Features, Treatment and Control. *Pathogens*, *10*(4), 454. doi: 10.3390/pathogens10040454.

Murata, H., Shimada, N. & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal*, *168*, 28-40.

Naylor, J. R., Hamilton, J. M. & Weathereley, A. J. (1984). Changes in the ultrastructure of feline pulmonary arteries following in infection with the lungworm Aelurostrongylus abstrusus. *Br Vet J*., *140*, 181–190. doi: 10.1016/0007-1935(84)90079-4.

Olsen, C. S., Willesen, J. L., Pipper, C. B. & Mejer, H. (2015). Occurrence of Aelurostrongylus abstrusus in Danish cats: A modified lung digestion method for isolating adult worms. *Vet Parasitol*, *210*(1-2), 32-39. doi: 10.1016/j.vetpar.2015.03.016.

Pennisi, M. G., Hartmann, K., Addie, D. D., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Horzinek, M. C., Hosie, M. J., Lloret, A., Lutz, H., Marsilio, F., Radford, A. D., Thiry, E., Truyen, U. & Möstl, K. (2015). European Advisory Board on Cat Diseases. Lungworm disease in cats: ABCD guidelines on prevention and management. *J. Feline Med Surg*., *17*(7), 626-636. doi: 10.1177/1098612X15588455.

Qiaodong, X., Yongcong, Ya., Songgang, G., Kai, M., Jianlong, Zh., Pinbo, H., Zhenyu, Zh., Zheng, Ch., Shaodong, Zh., Jiahong, L., Zhihua, L., Jie, W., Jiang, Ya. & Zhiyu X. (2018). A Novel Inflammation-Based Prognostic Score: The Fibrinogen/Albumin Ratio Predicts Prognoses of Patients after Curative Resection for Hepatocellular Carcinoma. *Journal of Immunology Research*, Article ID 4925498. https://doi.org/10.1155/2018/4925498.

Seo, M. H., Choa, M., You, J. S., Lee, H. S., Hong, J. H., Park, Y. S., Chung, S. P. & Park, I. (2016). Hypoalbuminemia, low base excess values, and tachypnea predict 28-day mortality in severe sepsis and septic shock patients in the emergency department. *Yonsei Medical Journal*, *57*(6), 1361–1369. doi: 10.3349/ymj.2016.57.6.1361.

Stief, T. W. (2008). The Fibrinogen Functional Turbidimetric Assay. *Clinical and Applied Thrombosis/Hemostasis*,*14*(1), 84-96. doi: 10.1177/1076029607308031.

Tan, Z., Zhang, M., Han, Q., Wen, J., Luo, K., Lin, P., Zhang, L., Yang, H. & Fu, J. (2017). A novel blood tool of cancer prognosis in esophageal squamous cell carcinoma: the fibrinogen/albumin ratio. *Journal of Cancer, 8*(6), 1025–1029. doi: 10.7150/jca.16491.

Todorov, J. (1972). Nephelometric determination of fibrinogen (method of Podmore). *In*: Clinical Laboratory Technics, Todorov, J. (ed.), Medizina and Fizkultura, Sofia, 250 (Bg).

Van den Bossche, I., Paepe, D. & Daminet, S. (2010). Acute pancreatitis in dogs and cats: pathogenesis, clinical signs and clinicopathologic findings. *Vlaams Diergeneeskundig Tijdschrift*., *79*, 13-22. DOI: 10.21825/vdt.87424.

Zajac, A. M. & Conboy, G. A. (2012). Veterinary clinical parasitology. 8 th. ed. Ames, Iowa: Willey-Blackwell, 5-28. ISBN: 978-1-119-30077-9.

Zapryanova, D., Mircheva, T., Hristov, T., Lazarov, L., Atanasov, A., Petrova, Y. & Lalev, D. (2017). Changes in the Concentrations of Some Plasma Proteins During Acute Inflammation in Dogs. *Advanced research in life sciences, 1*(1), 85-89. DOI: 10.1515/arls-2017-0015.

Zapryanova, D., Lazarov, L., Mircheva, T., Nikolov, J., Goranov, N., Dinev, I. & Stoycheva, I. (2011). Markers of inflammation in experimentally induced pancreatitis in dogs (Part II): Blood plasma protein profiles and fibrinogen. *Rev. Méd. Vét*., *162*(10), 449-453.

Zapryanova, D., Mircheva, T. & Denev, S. A. (2013). Plasma protein profiles and fibrinogen concentrations in dogs with experimentally induced Staphylococcus aureus infection. *Revue Méd. Vét*., *164*, 150-155.

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