Serum C-reactive protein concentration in benign and malignant canine spirocercosis

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Abbreviations

CRP	C-reactive protein
S. lupi	*Spirocerca lupi*
TIA	Turbidometric immunoassay
ANOVA	Analysis of variance
SPSS	Statistical package for the social sciences
χ^2	Chi squared

Abstract

Background: *Spirocerca lupi* is a nematode of canids which forms a nodule in the esophagus which can undergo neoplastic transformation. C-reactive protein (CRP) is a major acute phase protein in the dog which has been used for treatment monitoring and prognostication in inflammatory and neoplastic disease.

Hypothesis/Objectives: The objectives of this study were to determine if serum CRP concentration is (1) elevated in canine spirocercosis, (2) could be used to determine neoplastic transformation and (3) could be used to monitor response to treatment in benign spirocercosis.

Animals: Forty two dogs naturally infected with *S. lupi* and 21 control dogs

Methods: A retrospective study was performed. The infected cases were divided into benign (n=28) or malignant (n=14) spirocercosis. C-Reactive protein was performed on all the spirocercosis and control cases at presentation. Statistical analysis was done using the one way ANOVA and Student's *t*-test.

Results: The mean CRP concentration of the benign cases was 60.41 ± 48.02 mg/l and that of the malignant cases was 76.52 ± 44.79 mg/l, both values were significantly higher ($p<0.001$) than the control group where the mean was 13.39 ±17.94 mg/l. The mean CRP concentration for the convalescent sera in the benign group was lower than the pretreatment values ($p=0.01$).

Conclusion and clinical importance: C-Reactive protein cannot be used to differentiate between benign and malignant spirocercosis. There is a decrease in CRP concentration in dogs with benign spirocercosis once treatment has commenced. Serial CRP measurement can be used to monitor response to treatment in benign spirocercosis.

Key words: Esophagus, Neoplasia, Osteosarcoma

C - reactive protein (CRP) is one of the major positive acute phase proteins in the dog and is produced in response to any tissue injury including immunologic, neoplastic and traumatic.[1] Serum CRP concentrations are not influenced by age and sex.[2]Serum CRP has shown potential as a prognosticator for survival in some neoplastic conditionssuch as lymphoma, ovarian cancer, thymic cell carcinoma, pancreatic cancer and colorectal cancer.[3] Serum CRP concentration has also been evaluated for monitoring response to therapy and relapse status in dogs with lymphoma.[3]

Spirocerca lupi (*S. lupi)* is a nematode of canidae which commonly infests the esophagus.[4]Dogs become infected when they ingest either the intermediate host or paratenic hosts. Following ingestion, the larvae excyst and eventually reach the caudal thoracic aorta by migrating through the gastric and coelic arteries. From the aorta, the larvae migrate to the caudal thoracic esophagus where they mature into adult worms.[4] In the esophagus, an inflammatory fibrous tissue nodule is formed around the worms.[5] The nodule may be transformed into an osteosarcoma, fibrosarcoma, or anaplastic sarcoma[4]

Benign spirocercosis can be successfully treated with doramectin[a,] at 400µg/kg every two weeks while malignant spirocercosis requires surgical excision of the esophageal sarcoma, with or without chemotherapy, and the long-term prognosis is poor.[6] Ante mortal differentiation between benign and malignant esophageal masses in canine spirocercosis is a diagnostic challenge and is currently based on histopathology of biopsy samples obtained via esophageal endoscopy or surgical excision. However, endoscopically obtained biopsy samples have been

shown to be insensitive or non-diagnostic because they often contain only superficial necrotic tissue.[6] Grossly, the benign *S. lupi* induced nodule has a typical smooth appearance and may sometimes have an operculum through which the female lays its eggs.[6] As the mass enlarges and neoplastic transformation occurs, the mass becomes cauliflower-shaped and lobular with superficial necrosis and ulceration.[6] The endoscopic appearance of the nodule, however, may be misleading since it is subjective and is not 100% accurate. In a few malignant cases, the nodule appeared smooth instead of lobulated and ulcerated as would be expected and the opposite occurred in a small percentage of benign cases.[7]

Anemia, leucocytosis, hyperproteinemia and hyperglobulinemia have been associated with neoplastic transformation in canine spirocercosis but all have poor specificity.[8] Reliable, and readily available tests are therefore warranted to help make the distinction. The aims of this study were (1) to determine if serum CRP concentration is elevated in canine spirocercosis, (2) to determine if serum CRP could be used to distinguish between benign and malignant cases and (3) to determine if serum CRP could be used to monitor response to treatment in dogs with benign spirocercosis.

Materials and methods

Medical records of dogs diagnosed with spirocercosis, at the Onderstepoort Veterinary Academic Hospital (OVAH), University of Pretoria, were retrospectively reviewed for the period 2008-2009. The diagnosis of spirocercosis was confirmed based on various combinations of the following: thoracic radiography, endoscopy and/or fecal examination. On thoracic radiographs, a soft tissue mass in the caudodorsal mediastinum, spondylitis of the ventral bodies of the caudal

thoracic vertebrae and an undulating aorta were considered diagnostic. Only cases with confirmed benign or malignant spirocercosis which had serum collected prior to any treatment were enrolled in the study. Full haematology, serum chemistry, thoracic radiographs and abdominal ultrasound were performed on the dogs in order to exclude the presence of a concurrent inflammatory condition. Twenty eight benign and 14 malignant cases were selected. The cases were determined to be benign if they had endoscopic visualization of a typical benign nodule at presentation and regression of the nodule in response to doramectin[a] treatment as observed at follow-up endoscopy at least 6 weeks after treatment was started. Cases with malignant transformation were selected based on histopathology of endoscopically obtained biopsies or entire esophageal masses obtained through surgical excision or at necropsy. All cases had to have serum from the day of presentation that was stored at -80ºC. Analysis of the samples was done as a single batch to avoid inter-assay variation. The data retrieved from the medical records included, age, breed, and bodyweight. Six of the benign cases had follow up serum samples collected between 2 weeks and 4 weeks after treatment was commenced. Twenty one dogs that were presented for routine ovariohysterectomy were included as controls. C-Reactive protein was performed on all the spirocercosis and control cases at presentation. C-reactive protein concentrations were determined as a batch on the stored serum using an automated human CRP Turbidometric Immunoassay[b], previously validated for use in dogs.

The analysis was performed using an autoanalyser[c] according to the manufacturer's instructions. Statistical analysis was performed with SPSS software[d]. The data was checked for normal distribution using the Kolmogorov-Smirnov test. The one way ANOVA was used to compare the means of the benign, malignant and control groups. The Student's *t*-test was used as a *post hoc* test. The paired-Student's *t*-test was used to compare the serum CRP values between the pre-

treatment and convalescent sera in the six benign cases with convalescent sera available for analysis. The gender and breed distribution were compared by the χ^2 test. Statistical significance was set at $p<0.05$.

Results

Twenty eight cases with benign spirocercosis and 14 cases with malignant spirocercosis fulfilled the selection criteria. The neoplasms in the malignant cases were osteosarcoma (11), fibrosarcoma (2), and anaplastic sarcoma (1). Three of the cases with malignant spirocercosis had concurrent benign *S. lupi* nodules. The breed distribution for the benign cases was as follows: German shepherd dogs (5), Jack Russell terrier (3), Staffordshire bull terrier (3) Mixed breed (3), Bull terrier (2), Rottweiler (2), Boerboel (1), Border collie (1), Chow chow (1), Daschund (1), Fox terrier (1), Great Dane (1) Rhodesian ridgeback (1) and Scottish terrier (1). The breed distribution for the malignant cases was as follows: Staffordshire bull terrier (3), Boerboel (2), German shepherd dog (2), Labrador retriever (2), Border collie (1), Boxer (1), Mixed breed (1), Daschund (1), and Doberman pinscher (1). There was no difference in the breed distribution for both the benign and malignant cases. The mean age for the benign cases (4.3±2.9 years) was not significantly different from the mean age of the malignant cases (5.7 ±2.6 years) ($p=0.12$). There were 14 males and 14 females in the benign group of which 12 (86%) of the males and 9 (64%) of the females were intact. There were 7 males and 7 females in the group with malignant spirocercosis of which 4 (57%) of the males and females were intact.

The serum CRP concentration values at presentation had a normal distribution. There was an overall difference in the CRP concentrations of the malignant, benign and control groups. The

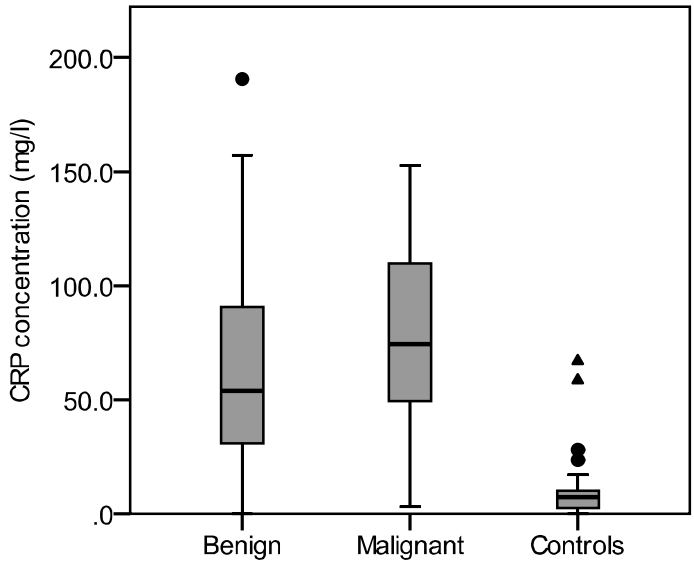

Fig 1. Box plot showing serum C-reactive protein (CRP) concentrations in the benign (n528), malignant (n514), and control (n5 21) cases at presentation. The box incorporates the middle 50% of the bservations with the line inside the box as the median. The whiskers extend to the smallest (25th percentile) and largest (75th percentile) observations indicating the range of the data. Outliers, values that are 1.5 times removed from the interquartile range, are plotted as dots. Extreme outlier values that are 3 times removed from the interquartile range are represented by a triangle.

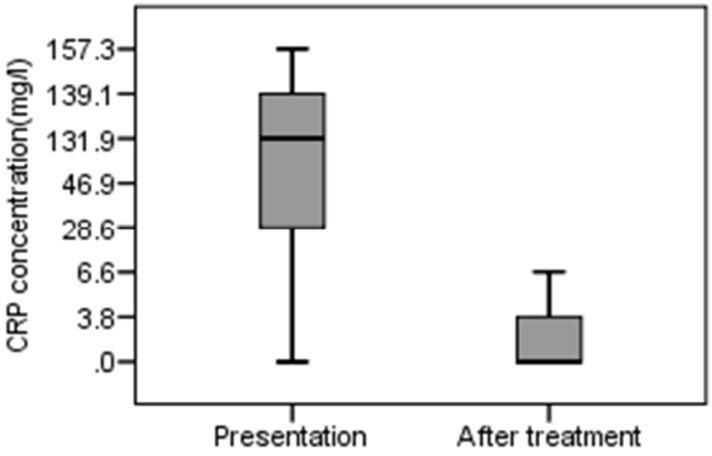

Fig 2. Box plot representing serum C-reactive protein (CRP) concentrations of the 6 benign cases with follow-up serum samples. The samples were collected at presentation and between 2 and 4 weeks after treatment had started. The box incorporates the middle 50% of the observations with the line inside the box as the median. The whiskers extend to the smallest (25th percentile) and largest (75th percentile) observations indicating the range of the data.

mean CRP concentration of the benign cases was 60.41 ± 48.02 mg/l (mean ± S.D) l and that of the malignant cases was 76.52 ± 44.79 mg/l both values were significantly higher ($p<0.001$) than the control group where the mean was 13.39 ± 17.94 mg/l. There was no significant difference in the serum CRP concentrations between malignant and benign cases ($p=0.3$) (Fig. 1). Three cases in the malignant group had concurrent benign nodules. Excluding these cases did not change the results of the statistical analysis (data not shown). The mean serum CRP concentration for the six follow-up sera was 1.76 ± 2.8 mg/l compared to 86.96 ± 66.6 mg/l before treatment. The convalescent sera had significantly lower concentrations of CRP compared to the concentrations at presentation ($p=0.01$) (Fig. 2).

Discussion

The high serum CRP levels that were observed in the canine spirocercosis cases indicate a marked inflammatory response. Severe inflammation has been described in the histopathology of benign and malignant nodules in canine spirocercosis.[5] The inflammatory reaction in the benign nodule is predominantly lymphoplasmacytic, but pockets of neutrophilic infiltrates around the worms, eggs and migratory tracts are very common. The malignant nodules have a predominantly neutrophilic infiltrate which is associated with areas of ulceration and necrosis.[5] The neutrophilic infiltration seen on histopathology in the malignant nodules is an indicator of an active inflammatory reaction. The presence of inflammation, especially neutrophilic response in both the benign and malignant nodules possibly explains the similarities between the serum CRP concentrations in the dogs with benign and malignant spirocercosis at presentation. Elevated CRP levels have been associated with several neoplasms namely lymphoma, pancreatic cancer,

squamous cell carcinoma and colorectal cancer.[3] The reason for the high CRP concentrations in the malignant spirocercosis cases might be as a result of direct inflammatory properties of the neoplasm and not only the ulceration and necrosis.

C-reactive protein concentrations in the dog have been demonstrated to peak rapidly,[1] The half life of CRP is 19 hours and relatively constant such that levels fall sharply after initiation unless the plasma level is maintained by continued CRP production in response to continued antigen exposure and inflammation.[9] The rapid decrease of CRP concentrations in the absence of continued inflammation makes it an ideal tool for monitoring response to treatment in inflammatory conditions.[9] In this study, six of the benign cases had follow up convalescent sera available for analysis. The CRP levels obtained showed that all six cases had significantly reduced CRP concentrations 2-4 weeks after treatment had been initiated. Follow up endoscopy on the six benign cases correlated well with this result as the nodules were regressing in all cases. A decrease in serum CRP levels once treatment has commenced is therefore consistent with a positive response to treatment.

C-reactive protein has been shown to have no value in the prognostication of disease outcome as a once off measurement. However, serial CRP measurements have the potential to provide valuable prognostic information.[10] While CRP concentrations at admission in dogs with *S. lupi* would not be able to differentiate between benign and malignant cases, serial measurements can give valuable prognostic information. Our assumption is that CRP levels will remain elevated in dogs with neoplastic spirocercosis despite treatment because of the ongoing tissue necrosis and ulceration as well as the neoplasia itself. A sustained elevation in serum CRP levels would therefore be likely to be associated with neoplastic transformation and hence carry a poorer prognosis if no other source of inflammation can be identified. A decrease in CRP concentrations

would be likely to be associated with response to therapy in benign spirocercosis. More research is warranted with regards to the use of serum CRP concentrations as a tool for monitoring and prognostication in benign and malignant *S. lupi* cases. The current methods for the determination of neoplastic transformation and hence prognostication in *S. lupi* are time consuming, expensive and potentially insensitive. Measurement of CRP on the other hand is relatively cheap and easily available.

The major limitation of this study is the small number of benign cases with follow up serum samples that were available for analysis. In addition, possible concurrent infection and reaction that may continue when a worm is dead may limit the usefulness of CRP. In addition, there was no follow up sera from neoplastic cases available for analysis. C-reactive protein is a non-specific acute phase protein and as such it is essential to assess patients for concurrent inflammation and or infection which could elevate the CRP. C-reactive protein shows substantial variation between patients. It may be important to establish a baseline for a particular patient at the beginning of treatment in order to have an objective starting point.[3]

Conclusion

Serum CRP concentration is elevated in both the benign and malignant forms of canine spirocercosis which is a direct reflection of the inflammation observed on histopathology. Single serum CRP measurements cannot, however, be used to differentiate between benign and malignant cases. Serial CRP measurements could potentially be used to differentiate neoplastic from non neoplastic cases and to monitor response to treatment in benign spirocercosis cases in the absence of other causes of an acute phase response.

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Footnotes

a. Dectomax, Pfizer, France

b. Bayer CRP TIA, Newbury, United Kingdom

c. Nexct, Alfa Wasserman, Bayer, South Africa

d. SPSS Statistics 17.0, SPSS Inc, Chicago

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