BRIEF COMMUNICATION

Serum alkaline phosphatase activity is not a marker for neoplastic transformation of esophageal nodules in canine spirocercosis

Varaidzo Mukorera[1], Liesel L. van der Merwe[1], Eran Lavy[2], Itamar Aroch[2], Eran Dvir[1]

[1]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa; and [2]Koret School of Veterinary Medicine and Hebrew University Teaching Hospital, Hebrew University of Jerusalem, Jerusalem, Israel

Key Words

Anaplastic sarcoma, esophagus, fibrosarcoma, malignant neoplasia, osteosarcoma, *Spirocerca lupi*

Correspondence

Varaidzo Mukorera, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, 0110, South Africa

E-mail: vari.mukorera@up.ac.za

Running short title: Total serum ALP in canine spirocercosis

Running authors: Mukorera et al

Background: *Spirocerca lupi* is a nematode of Canidae that matures within the esophageal wall to form fibroblastic nodules with potential for malignant transformation. Diagnosis is based on histopathologic examination, but false negative results may be obtained from samples collected by endoscopy. Serum alkaline phosphatase activity (ALP), frequently increased in hepatobiliary disease, is also increased in a variety of neoplastic conditions in dogs, including appendicular osteosarcoma, and has also been reported to be increased in dogs with spirocercosis.

Objective: The aim of this study was to evaluate serum ALP activity as a marker for malignant transformation of esophageal nodules in *S. lupi*-infected dogs.

Methods: In this retrospective study, medical records of dogs diagnosed with spirocercosis from 1991-2008 were reviewed, and serum ALP activity determined at presentation was compared between dogs with non-neoplastic and neoplastic nodules. Owing to use of multiple analyzers, ratios of ALP activity to the upper reference interval for ALP were calcuated and compared.

Results: Median ALP activity ratios were 0.65 (0.07-4.00) and 0.86 (0.10-3.40) for dogs with non-neoplastic (n = 88) and neoplastic (n = 32) nodules, respectively, with no significant difference (P = .18) and substantial overlap between groups. Tumors included osteosarcoma (15 dogs), fibrosarcoma (15 dogs), and anaplastic sarcoma (2 dogs) there was no difference in ALP activity between the dogs with osteosarcoma and fibrosarcoma

Conclusion: ALP is a poor marker of malignant transformation in canine spirocercosis.

Spirocerca lupi is a nematode of Canidae that matures to the adult stage within the esophageal wall, where the nematodes form fibroblastic nodules that have potential to undergo malignant transformation to sarcoma in about 25% of cases.[1, 2] The initial diagnosis of canine spirocercosis is made using a combination of thoracic radiography, fecal analysis, and esophageal endoscopy. Determination of malignant transformation is the next important step as it affects prognosis for individual dogs. Currently, antemortem diagnosis of malignancy is based on surgical biopsies obtained by endoscopy.[2-4] This is an invasive expensive procedure that requires general anesthesia and is often non-diagnostic because specimens commonly contain only superficial necrotic tissue.[4-6]

Alkaline phosphatase (ALP) is a membrane-bound glycoprotein present in various tissues, including liver, bone, intestine, kidney, mammary gland, and placenta, and in leukocytes. [7] Total activity of canine ALP measured in serum includes liver, bone, and corticosteroid-induced ALP isoforms. [7] Increase ALP activity may occur in dogs with diseases involving tissues that express ALP, dogs receiving exogenous corticosteroids, and young growing dogs, which have higher ALP activity compared with adults owing to bone growth. [7] In canine osteosarcoma, increased total ALP has been associated with a poorer prognosis,[8-11] and measurement of ALP has been proposed as a method to assess the efficacy of chemotherapy. A variety of malignant tumors in people have been shown to express a heat-stable placental ALP isoform. [12]In seminoma, placental ALP along with human chorionic gonadotrophin and lactate dehydrogenase were shown to be predictive of relapse. [12,13] In human patients with prostate cancer, total serum ALP activity combined with tartrate-resistant acid phosphatase and prostate-specific antigen were shown to be predictive of bone metastasis in up to 70% of cases without performing a bone scan. [14]

In a previous study of spirocercosis, 23% of cases had increased ALP activity; however; differentiation between non-neoplastic and malignant nodules was not made.[15] In another study,

ALP activity was increased in 5 of 7 dogs with neoplasms secondary to spirocercosis.[4] Neither study compared ALP activity between dogs with nonneoplastic and malignant lesions or determined if the high frequency of osteosarcoma reported to occur secondary to spirocercosis could have been a contributing factor to the increased activity. Differentiating non-neoplastic and neoplastic lesions in spirocercosis has implications for treatment and prognosis. The former can be successfully treated with doramectin,[3] but medical treatment is ineffective in dogs with malignancies in which surgical excision, with or without chemotherapy, is required and in which the long-term prognosis is poor.[4,5] Response to appropriate anthelminthic treatment can be used to differentiate between non-neoplastic and neoplastic esophageal nodules, but monitoring for response to treatment is expensive, time-consuming, and often impractical. Reliable and readily available tests would be beneficial in identifying malignant nodules. The objective of this study was to determine if ALP activity could be used as a marker for malignant transformation in dogs with spirocercosis.

The dogs included in this retrospective study were from 2 referral centers, Onderstepoort Veterinary Academic Hospital (OVAH), University of Pretoria, South Africa and the Koret School of Veterinary Medicine (KSVM), Hebrew University of Jerusalem, Israel. Medical records of dogs admitted to these centers during the period of January 1991 to December 2008 were reviewed for the diagnosis of spirocercosis. Diagnosis of spirocercosis was confirmed by thoracic radiography, endoscopy, fecal examination, or a combination of these tests. Dogs were excluded if they were < 1 year of age, had been previously treated with corticosteroids, or had been diagnosed with other diseases, especially hepatobilliary disease (based on history, clinical signs, serum biochemical analysis, and abdominal ultrasonography), bone disease, hyperadrenocorticism, or neoplasia unrelated to spirocercosis.

Dogs were included in the study if spirocercosis had been confirmed and ALP activity had been measured at presentation. Data retrieved from the medical records included breed, sex,

age, weight, and ALP activity at presentation. Esophageal nodules were designated as non-neoplastic based on endoscopic visualization of a typical nodule at presentation and regression of the nodule in response to doramectin treatment (Dectomax, Pfizer, City, France, 400 μg/kg subcutaneously every 14 days) with at least one 6-week follow-up endoscopic evaluation. Nodules were designated as malignant based on histopathologic evaluation of either endoscopic biopsy samples or esophageal masses excised surgically or at necropsy. Whole blood was collected in plain serum tubes, allowed to clot, and centrifuged to separate RBCs from serum. Three different analyzers (ACE-aleral Alfawasserman, Siemens, South Africa; Cobas-Mira, Rottkereutz, Switzerland; Konelab, Kone, Espoo, Finalnd) were used to determine ALP activity by the same colorimetric kinetic method based on formation of p-nitrophenol. The 2 medical centers had different reference intervals (RIs). During the study period, both institutions changed analyzers, chamber temperatures, and reagents, resulting in establishment of new RIs. The RI at KSVM was established by analyzing serum ALP activity of 67 healthy dogs belonging to the hospital staff members and Israeli defense force army dogs; ALP activity had a normal distribution, and the RI was based on the 2.5^{th}-97.5^{th} percentiles. The RI at OVAH was established by analyzing the serum ALP activity of 78 healthy dogs belonging to the South African defense forces, South African police service, and hospital staff members. The RI was based on the 2.5^{th}-97.5^{th} percentiles. To permit comparison of ALP activities across these variables, for each dog ALP activity was calculated as a ratio between the measured result and the upper limit of the RI for the individual medical center and instrument. Thus, dogs with ALP activity above the RI had ratios > 1, whereas dogs with ALP activity within or below the RI had ratios ≤ 1.

Data were entered into an Excel spreadsheet (Microsoft Office Excel 2003 for Windows, Microsoft Inc., Redmond, WA, USA), and statistical analysis was performed using SPSS software (SPSS 17.0 for Microsoft Windows, SPSS Inc., Chicago, IL). The data did not have a

normal distribution based on the Kolmogorov-Smirnov test, and ALP activity ratios of the 2 groups with non-neoplastic or neoplastic lesions were compared using the Mann-Whitney *U* test. The effect of sex on the proportion of cases with increased ALP activity ratios between groups was compared by the χ^2 test. The Kruskal-Wallis test was used to analyze differences in median ALP activity among the types of neoplasia in dogs with malignancies. Fisher's exact test was used to analyze differences in ALP ratios between breeds in both groups. All tests were 2-tailed and a P value $\leq .05$ was considered significant.

The study population included 125 dogs, with >25 breeds represented; 88 dogs had non-neoplastic nodules and 32 had malignant lesions. Median ages of dogs were 6 (range, 1-16) and 6.5 (range, 2-13) years for dogs with non-neoplastic and neoplastic nodules, respectively. The median ALP ratios were 0.65 (range, 0.07-4.00) and 0.78 (range, 0.10-3.40) for dogs with non-neoplastic and neoplastic nodules, respectively, with no significant difference between groups (P = .18) (Figure 1). There was substantial overlap in ALP ratios between groups, and most dogs in the study had ALP activities within the RI. ALP activity was > RI in 14/88 (18%) and in 6/32 (16%) of dogs with non-neoplastic and neoplastic nodules, respectively. In dogs with neoplastic nodules, the specific tumor types were osteosarcoma (15 dogs, median ALP ratio of 0.64, range 0.09-1.88), fibrosarcoma (15 dogs, median of ALP ratio = 0.5, range 0.09-3.31), and anaplastic sarcoma (2 dogs, ALP ratios of 0.08 and 0.39); there was no significant difference between the osteosarcoma and fibrosarcoma tumor types (P = .47).

The proportion of males and females in the groups was not different (P = .67), with 49 females and 39 males in the non-neoplastic group compared with 20 females and 12 males in the group with neoplastic disease. In the latter group, the ALP ratio was significantly higher in intact compared with spayed females (P = .03), but median values for both were still < 1.

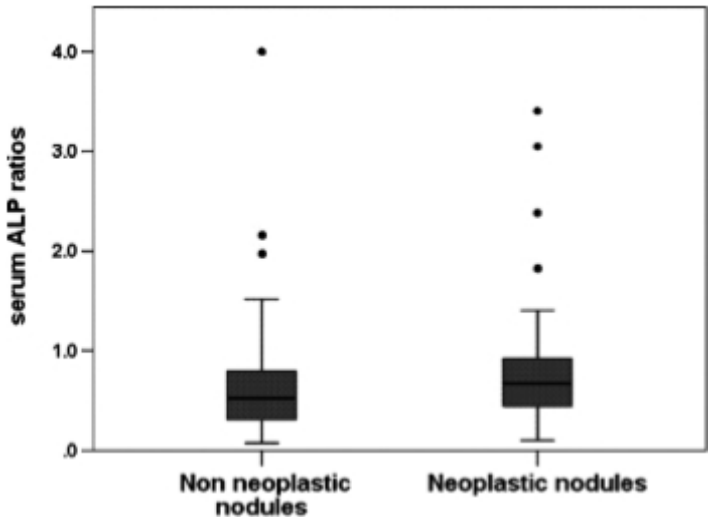

Figure 1. Box plot of alkaline phosphatase (ALP) ratios in *Spirocerca lupi*-infected dogs with non-neoplastic and neoplastic nodules. The box incorporates the middle 50% of the ratios; the line inside the box is the median value; the whiskers indicate the range from the 25th to75th percentiles; outliers are values that were 1.5 and 3 times above or belowe the interquartile range and are plotted as dots. The difference between the 2 groups was not significant ($P = .18$).

Discussion

This is the first study to evaluate ALP activity in canine spirocercosis in which clear inclusion and exclusion criteria were established and in which non-neoplastic and malignant nodules in dogs with spirocercosis were compared using stricter criteria for differentiating non-neoplastic and neoplastic disease than reported previously.[4,15] The exclusion criteria in the present study were specifically designed to avoid selecting cases with disease processes that might contribute to high ALP activity, whereas in previous studies of spirocercosis, other concurrent diseases unrelated to spirocercosis may have contributed to high ALP activity.

Our results indicate that ALP ratio is not a useful discriminatory biomarker of malignant transformation of esophageal nodules in canine spirocercosis. Furthermore, the overall proportion of dogs with increased ALP activity was lower compared with that previously

reported.[4,15] There was substantial overlap in ALP activity between the 2 groups, and in most dogs, regardless of group, ALP activity was within the RI. It is unclear why ALP activity was not increased in the dogs with osteosarcoma; however, in most reports of increased ALP activity in dogs with osteosarcoma, the animals had appendicular osteosarcoma.[8-11] *S lupi*-induced osteosarcoma is not appendicular, and perhaps pathophysiologic mechanisms are different. Ccytochemical staining of tissue samples for ALP could help determine if it is indeed expressed by *S lupi*-induced osteosarcomas; if so, expression may be too low to contribute to measurable ALP activity. Alternatively, the half-life of the isoform elaborated by the neoplastic cells might be too short to contribute significantly to total ALP. Measurement of the activity of the different ALP isoforms, specifically the bone isoform, may be useful.

Disclosure: The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

References

1. Bailey WS. Spirocerca-associated esophageal sarcomas. *J Am Vet Med Assoc.* 1979;175:148-150.
2. Dvir E, Kirberger RM, Mukorera V, van der Merwe LL, Clift SJ. Clinical differentiation between dogs with benign and malignant spirocercosis. *Vet Parasitol.* 2008;155:80-88.
3. Lavy E, Aroch I, Bark H, et al. Evaluation of doramectin for the treatment of experimental canine spirocercosis. *Vet Parasitol.* 2002;109:65-73.
4. Ranen E, Lavy E, Aizenberg I, Perl S, Harrus S. Spirocercosis-associated esophageal sarcomas in dogs. A retrospective study of 17 cases (1997-2003). *Vet Parasitol.* 2004;119:209-221.

5. van der Merwe LL, Kirberger RM, Clift S, Williams M, Keller N, Naidoo V. Spirocerca lupi infection in the dog: a review. *Vet J.* 2008;176:294-309.
6. Dvir E, Kirberger RM, Malleckzek D. Radiographic and computed tomographic changes and clinical presentation of spirocercosis in the dog. *Vet Rad and Ultrasound.* 2001;42:119-129
7. Fernandez NJ, Kidney BA. Alkaline phosphatase: beyond the liver. *Vet Clin Pathol.* 2007;36:223-233.
8. Garzotto CK, Berg J, Hoffmann WE, Rand WM. Prognostic significance of serum alkaline phosphatase activity in canine appendicular osteosarcoma. *J Vet Intern Med.* 2000;14:587-592.
9. Ehrhart N, Dernell WS, Hoffmann WE, Weigel RM, Powers BE, Withrow SJ. Prognostic importance of alkaline phosphatase activity in serum from dogs with appendicular osteosarcoma: 75 cases (1990-1996). *J Am Vet Med Assoc.* 1998;213:1002-1006.
10. Kirpensteijn J, Kik M, Rutteman GR, Teske E. Prognostic significance of a new histologic grading system for canine osteosarcoma. *Vet Pathol.* 2002;39:240-246.
11. Liptak JM, Kamstock DA, Dernell WS, Monteith GJ, Rizzo SA, Withrow SJ. Oncologic outcome after curative-intent treatment in 39 dogs with primary chest wall tumors (1992-2005). *Vet Surg.* 2008;37:488-496.
12. Lange PH, Millan JL Stugbrand T, Wessella RL, Ruoslahti E, Fishman WH. Placental alkaline phosphatase as a tumor marker for seminoma. *Cancer Res.* 1982;42:3244-3247.
13. Koshida K, Yokoyama K, Imao T, et al. Immunolocalization of anti-placental alkaline phosphatase monoclonal antibody in mice with testicular tumors and lymph nodes metastasis. *Urol Res.* 1998;26:23-28.
14. Ozu C, Nakashima J, Horiguchi Y, Oya M, Ohigashi T, Murai M. Prediction of bone metastases by combination of tartrate-resistant acid phosphatase, alkaline phosphatase

and prostate specific antigen in patients with prostate cancer. *Int J Urol.* 2008;15:419-422.

15. Mylonakis ME, Rallis T, Koutinas AF, et al. Clinical signs and clinicopathologic abnormalities in dogs with clinical spirocercosis: 39 cases (1996-2004). *J Am Vet Med Assoc.* 2006;228:1063-1067.