Spirocerca lupi induced oesophageal neoplasia: Predictors of surgical outcome

P. Pazzi[1*], Anastasia Kavkovsky[2], Anna Shipov[2], Gilad Segev[2], Eran Dvir[3]

[1]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.

[2] Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel.

[3] Tel Hai Academic College, Upper Galilee, Israel.

*Corresponding author: Tel: +27 12529 8096, paolo.pazzi@up.ac.za

Highlights

- No difference was identified in survival between endoscopic ablation and surgery.
- Reduced survival was documented in patients presenting with weight loss, hypochromasia or leucocytosis.
- The hospitalisation time of dogs undergoing ablation was significantly shorter than dogs undergoing surgery.

Abstract

Canine spirocercosis is caused by the nematode *Spirocerca lupi*. Migration results in oesophageal fibro-inflammatory nodules that may undergo neoplastic transformation. No studies have assessed pre- or post-surgical prognostic indicators in dogs that undergo intervention for *S. lupi* induced oesophageal neoplasia.

This observational, multi-center study aimed to assess the outcome of dogs with *Spirocerca* induced sarcoma undergoing endoscopic-guided ablation (n=12) or surgery (n=18), and identify prognostic indicators. Parameters evaluated included: age, weight, gender, presenting complaints, duration of clinical signs, complete blood count, serum biochemistry, neoplasia size, placement of

percutaneous endoscopically-placed gastrostomy tube, histopathological mitotic indices, days to discharge and chemotherapy administration.

Kaplan-Meier survival curves showed no difference in survival between ablation and surgery {(median: 73.5 days (range: 0-1511) vs. 108 days (range: 0-1550), respectively (p = 0.982)}. Reduced survival was documented in patients presenting with weight loss (P = 0.027), hypochromasia (MCHC <33 g/dL, P = 0.023) or leucocytosis (>15x10^9/L, P = 0.017) with a hazard ratio of 2.51 ($CI_{95\%}$ = 1.071-6.018, P = 0.034), 2.71 ($CI_{95\%}$ = 1.10-6.65, P = 0.03) and 4.39 ($CI_{95\%}$: 1.21-15.97, P = 0.025) respectively. In the dogs surviving more than 21 days, Ht <36% and leucocytosis >15.0x10^9/L at presentation were associated with reduced survival (p = 0.016, p = 0.021 respectively) and hazard ratio of 3.29 ($CI_{95\%}$ = 1.18-9.2, P = 0.023) and 3.81 ($CI_{95\%}$ = 1.15-12.55, P = 0.028) respectively. Intra-intervention-group survival analysis identified increased survival time in dogs receiving chemotherapy, but only within the surgical group (P = 0.02).The hospitalisation time of dogs undergoing ablation (median: 0 days, range: 0-4) was significantly shorter than dogs undergoing surgery (9 days, 1-21) (P < 0.001).

In this study, no clear benefit was identified for surgery, thus when ablation is technically possible it should be considered advantageous, as hospitalisation time is significantly shorter. Weight loss, hypochromasia and leucocytosis were identified as long-term prognostic indicators at presentation.

Keywords: Oesophageal neoplasia; sarcoma; prognosis; *Spirocerca lupi*, oesophagectomy

1. Introduction

Spirocercosis is caused by *Spirocerca lupi*, a spiurid nematode of carnivores, particularly Canidae. Spirocercosis is highly prevalent in dogs in the tropics and subtropics, including South Africa and Israel (Bailey, 1972; Mazaki-Tovi et al., 2002; van der Merwe et al., 2008). Dogs become infected through ingestion of infected intermediate hosts (i.e., coprophagous beetles) or less commonly by

preying on paratenic hosts. After ingestion, the infective (L3) larvae penetrate the gastric wall, migrate to the small gastric blood arterial vessels, travel in the gastric and celiac artery walls to the caudal thoracic aorta, and finally to the caudal thoracic oesophagus where it causes formation of fibro-inflammatory nodules (Krishna and Anantaraman, 1971). The latter, through uncharacterised mechanisms, may undergo neoplastic transformation to various types of sarcomas, in up to 25% of cases (Dvir et al., 2001; Dvir et al., 2010). Typical clinical signs relate to the presence of oesophageal nodules or neoplasia and include regurgitation, vomiting, dysphagia, weight loss, pyrexia and lethargy (Dvir et al., 2001; Mazaki-Tovi et al., 2002; Lavy et al. 2002).

Non-neoplastic nodules are responsive to avermectins (Lavy et al., 2002), however, treatment of dogs with neoplastic transformation is challenging. The tumour becomes progressively larger, eventually resulting in oesophageal obstruction (Dvir et al., 2001; Dvir et al., 2008). Histologically the neoplasms are typically classified as osteosarcoma, fibrosarcoma, or anaplastic sarcoma (Ranen et al., 2004a; Ranen et al., 2008). Oesophageal osteosarcoma, the most common form of sarcoma in spirocercosis, has been compared to appendicular osteosarcoma in which a number of prognostic indicators have been reported (Boerman et al., 2012; Romano et al., 2016). Comparing certain grading criteria of canine appendicular osteosarcoma, spirocercosis-induced sarcoma may be considered a more aggressive neoplasia (Kirpensteijn et al., 2002; Dvir et al., 2010). Although removal of the oesophageal neoplasia undoubtedly extends life expectancy, there are no studies that directly evaluate prognostic indicators in *Spirocerca* induced sarcoma.

Oesophageal neoplasia's are rare in areas not endemic to spirocercosis (Ridgway and Suter, 1979), making *Spirocerca* induced sarcoma a highly attractive model to study the prognosis and complications associated with oesophageal neoplasia and surgery. A review of dogs that underwent oesophageal surgery for various reasons found that the presence of a mass and mass size were significant independent prognostic factors for the development of delayed postoperative complications, but only seven dogs in the review had an oesophageal mass (Sutton et al., 2016).

The preferred surgical technique for *Spirocerca* induced sarcoma is a thoracotomy with a partial oesophagectomy (Ranen et al., 2004b), but the tumour is not always resectable due to infiltration into surrounding structures (Dvir et al., 2001). The procedure is considered invasive, costly, painful and is associated with prolonged hospitalisation. Recently, transendoscopic oesophageal mass ablation was described as a new mode of therapy (Yas et al., 2013; Shipov et al., 2015). Removal or debulking of the neoplasia was performed using a 9 mm video-endoscope and laser or electrocauterisation. The procedure is less invasive and can be performed on an outpatient basis (Shipov et al., 2015).

The aim of the current study was to evaluate the prognostic indicators of dogs diagnosed with spirocerca induced sarcoma and to compare the outcome of the 2 validated removal techniques: transendoscopic ablation and partial oesophagectomy.

2. Material and Methods

2.1 Animals

The medical records from 2007 to 2016 of Onderstepoort Veterinary Academic Hospital (Pretoria, South Africa) and The Hebrew University Koret School of Veterinary Medicine - Veterinary Teaching Hospital (Beit Dagan, Israel) were retrospectively reviewed for the following inclusion criteria: 1) any dog diagnosed with *Spirocerca* induced sarcoma, based on supportive clinical signs, typical cauliflower-ulcerated-necrotic appearance on endoscopy and confirmation with histopathology; 2) absence of macroscopic metastasis on radiographs or computed tomography (CT); 3) intervention with surgery (partial oesophagectomy) or endoscopic-ablation mass removal. Depending on the type of intervention, dogs were divided into the surgery or ablation group.

2.2 Procedures

Surgery was performed via thoracotomy with full thickness partial esophagectomy, ideally achieving 1 cm margins from the neoplasia borders as previously described (Ranen et al., 2004b). Transendoscopic oesophageal mass ablation was performed using a 9 mm video-endoscope and laser or electrocauterization as previously described (Shipov et al., 2015).

2.3 Data

The following clinical parameters were collected for all cases where available: age, gender, weight, presenting complaint and duration of clinical signs (based on the owner first noticing any sign attributable to spirocercosis). If weight loss was part of the presenting complaint, it was based on the owner's subjective perception of weight loss. The following clinical-pathological parameters were recorded and evaluated: haematocrit (Ht), mean corpuscular volume , mean corpuscular haemoglobin content (MCHC), total white cell count (WCC), total neutrophil, lymphocyte, monocyte, eosinophil and platelet counts; serum total protein, albumin, globulin, alanine aminotransferase , alkaline phosphatase (ALP), urea, creatinine, total calcium, amylase, glucose, phosphate, sodium, potassium and chloride. Additionally, the following parameters were recorded: the size of the mass on radiographs or CT and placement of percutaneous endoscopically placed gastrotomy (PEG) tube. Post-surgical parameters evaluated included histopathological mitotic indices (low, moderate or high), days to discharge, inclusion of chemotherapy and drug used as well as survival time after surgery.

2.4 Statistical analyses

All continuous variables were tested for normal distribution using a Shapiro-Wilks test. Values are reported as median (and range) unless specified otherwise. Difference in continuous variables between groups was analysed using the Mann-Whitney U test. The Kaplan-Meier method (log-rank test) was used to generate survival curves, and COX regression analysis used to generate hazard ratios (with 95% confidence interval ($CI_{95\%}$)) in all dogs, dogs surviving longer than 21 days

after intervention (long-term survival group), as well as intragroup intervention analysis. Dogs that died <21 days after intervention were classified as having a short-term survival. Dogs still alive at the time of analysis were censored. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS® 24.

3. Results

3.1 Animals

Thirty client-owned dogs met the inclusion criteria. Twelve dogs underwent endoscopic-guided laser ablation and 18 underwent surgery. One dog from Onderstepoort Veterinary Academic Hospital underwent ablation and fifteen underwent surgery, while eleven dogs from The Hebrew University Koret School of Veterinary Medicine underwent ablation and three underwent surgery. The age range was 3–12 years with a median of 6.5 years (mean 6.8 years). Twenty-one (70%) of the dogs were females (19 spayed), and 9 (30%) dogs were male (3 neutered). The median weight was 22.2 kg (range 4–40 kg; mean 20.3 kg). Breeds included 4 German shepherds, 3 Labrador retrievers, 2 Boxers, 2 Dachshund, 2 Siberian huskies, and one each of the following: Jack Russel Terrier, Fox terrier, Basset hound, Border Collie, Golden retriever, Pug, American Pitbull terrier and Cavalier King Charles spaniel and 9 mix-breeds. There was no significant difference between intervention groups regarding age, breed or sex.

3.2 Presenting complaint and duration of clinical signs

The most common presenting complaint of all dogs included gastrointestinal signs (26/30, 87%) and weight loss (10/30, 33%), with the following individual categories: regurgitation only (10/30, 33%); regurgitation and vomiting (4/30, 13%); regurgitation, vomiting and weight loss (4/30, 13%), regurgitation & weight loss (3/30, 10%), vomiting only (3/30, 10%), vomiting & weight loss (2/30, 7%), regurgitation and melena (1/30, 3%), weight loss only (3%), coughing only (3%) and melena only (3%). Survival analysis showed decreased survival times if a patient presented with

weight loss (P = 0.027, Fig 1.) with a hazard ratio of 2.51 ($CI_{95\%}$ = 1.071-6.018, P = 0.034) compared to dogs without weight loss. Weight loss did not remain significant in the long-term survival group (dogs surviving >21 days) (P = 0.068). The duration of clinical signs before presentation ranged from 1 to 170 days (median 23 days) and was not associated with survival (P = 0.162).

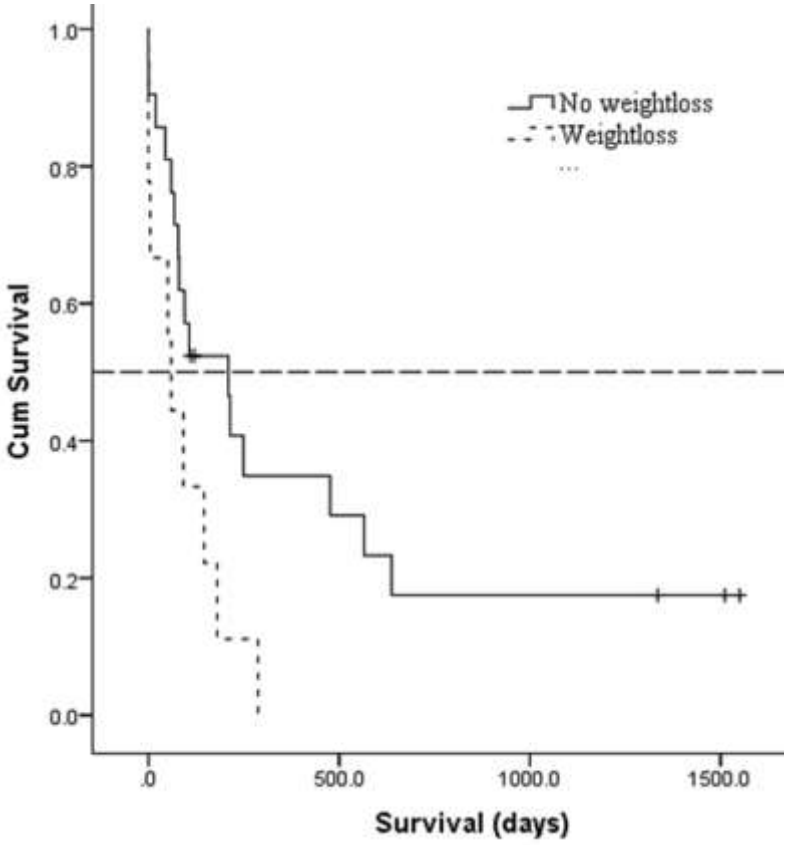


Fig 1. Kaplan-Meier survival curves for dogs presenting with or without weight loss. Weight loss as part of the presenting complaint was associated with reduced survival (P = 0.027). ┼ Indicates censored dogs alive at the time of analysis; —— indicates 50% cumulative (cum) survival.

3.3 Complete blood count

There were no significant differences in complete blood count parameters between dogs that underwent the ablation or surgical procedure (Table 1). The most common haematological abnormalities in all dogs were leucocytosis (>15x10^9/L; 79%), neutrophilia (>12x10^9/L; 71%), thrombocytosis (>500x10^9/L; 50%), anaemia (Ht <37%; 45%), monocytosis (>1.35x10^9/L; 44%) and eosinophilia (>1.25x10^9/L; 26%). For all dogs, hypochromasia (MCHC <33 g/dL) was associated with decreased survival time (P = 0.023, Fig 2) and a hazard ratio of 2.71 ($CI_{95\%}$ = 1.10-6.65, P = 0.03),

Table 1. Complete blood count and biochemistry results for all dogs in this study.

Parameter (normal range)	Number of cases	Median (range)	Number and percentage below normal range	Number and percentage above normal range
Haematocrit (37-55 L/L)	29	38.9 (12-62)	13 (45%)	1 (4%)
Mean corpuscular volume (60-77 fL)	27	65.8 (49.7-78.5)	4 (15%)	1 (4%)
Mean corpuscular haemoglobin content (32-36 g/dL)	27	33.3 (24.2-35.8)	8 (30%)	0
Total white cell count (6-15x10^9/L)	29	20.4 (8.2-77.8)	0	23 (79%)
Total neutrophils (3.5-12x10^9/L)	28	17 (5.7-69.2)	0	20 (71%)
Lymphocytes (1-4.8x10^9/L)	27	1.8 (0.3-5.1)	3 (11%)	2 (7%)
Monocytes (0.15-1.35x10^9/L)	27	1.2 (0.1-3.5)	9 (33%)	12 (44%)
Eosinophils (0.1-1.25x10^9/L)	26	0.4 (0-2.3)	4 (15%)	5 (19%)
Platelets (200-500x10^9/L)	28	513 (133-1060)	3 (11%)	14 (50%)
Total serum protein (56-73 g/L)	28	62.9 (38-74.5)	8 (29%)	1 (4%)
Albumin (28-41 g/L)	27	25 (11.7-43.9)	17 (63%)	1 (4%)
Globulin (20-41 g/L)	26	33.5 (17.8-50)	1 (4%)	7 (27%)
Alanine transaminase (9-73 U/L)	23	23 (13-3925)	0	2 (9%)
Alkaline phosphatase (20-165 U/L)	26	61.5 (14-1019)	4 (15%)	4 (15%)
Urea (2.3-8.9 mmol/L)	19	3 (1.4-17.3)	3 (16%)	1 (5%)
Creatinine (59-109 µmol/L)	28	67 (28-106)	8 (29%)	0
Total calcium (2.2-2.9 mmol/L)	15	2,3 (1,4-3,1)	5 (33%)	1 (7%)
Amylase (290-1125 U/L)	11	860 409-1700)	0	2 (18%)
Glucose (3.3-5.5 mmol/L)	11	5.6 (4-7.7)	0	6 (55%)
Phosphate (2.3-6.5 mg/dL)	10	1.52 (1-3.4)	0	1 (10%)
Sodium (142-151 mmol/L)	22	146.5 (140-154)	2/22 (9%)	3/22 (14%)
Potassium (3.6-5.1 mmol/L)	23	4.1 (3.4-5.77)	3/23 (13%)	2/23 (9%)
Chloride (99-110 mmol/L)	9	111 (106-117)	0/9	5/9 (56%)

while total WCC >15x10^9/L was associated with decreased survival (P = 0.017, Fig 3) and a hazard ratio of 4.39 ($CI_{95\%}$: 1.21-15.97, P = 0.025). The WCC remained statistically significant when the long-term survival group was analysed (P = 0.021, supplementary material (S1-Fig 1) and hazard ratio of 3.81 ($CI_{95\%}$ = 1.15-12.55, P = 0.028), additionally Ht <36% in the long-term survival group was associated with decreased survival time (P = 0.016, S2-Fig 2) and hazard ratio of 3.29 ($CI_{95\%}$ = 1.18-9.2, P = 0.023).

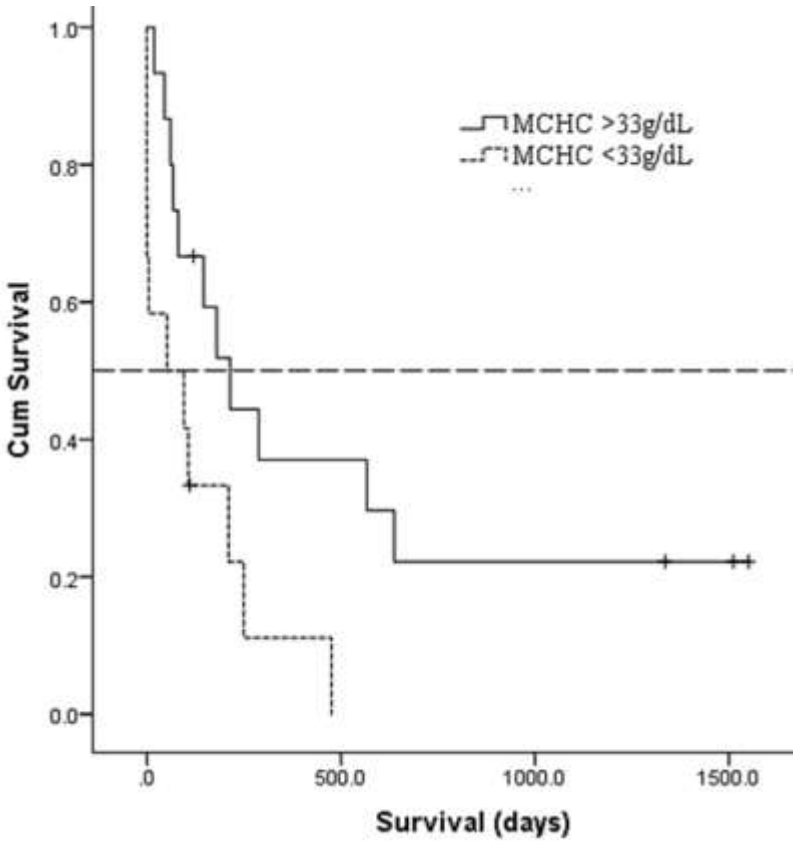


Fig 2. Kaplan-Meier survival curves for dogs' mean corpuscular haemoglobin content (MCHC). Hypochromasia (MCHC <33 g/dL) was associated with a reduced survival (P = 0.023). ┼ Indicates censored dogs alive at the time of analysis; —— indicates 50% cumulative (cum) survival.

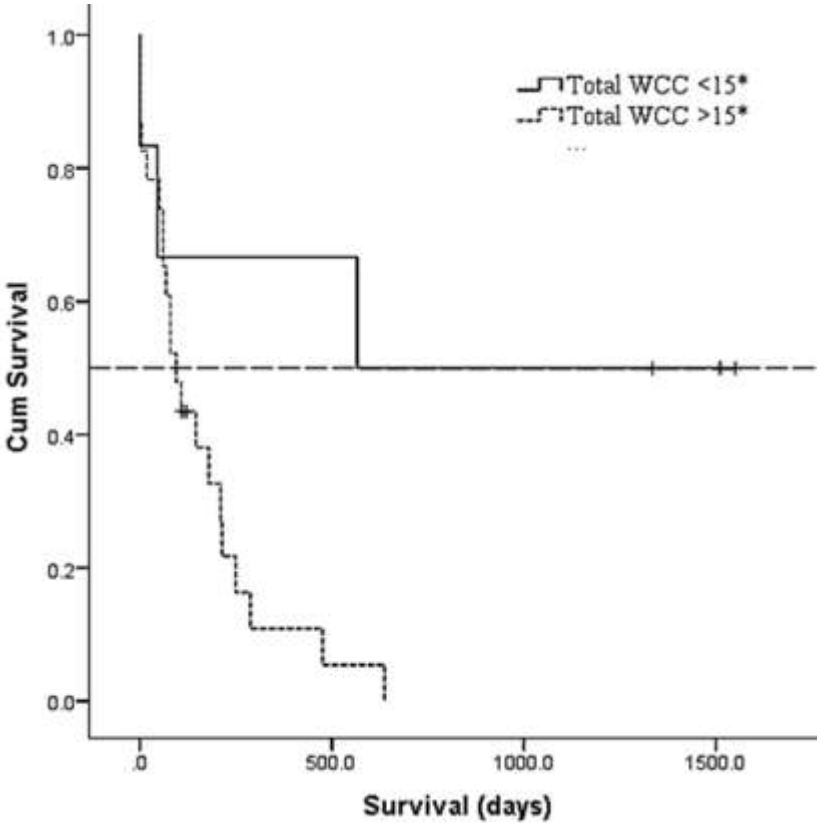


Fig 3. Kaplan-Meier survival curves for total white cell count (WCC). A WCC of *>15x10^{9}/L was associated with reduced survival (P = 0.017). ┼ Indicates censored dogs alive at the time of analysis; ——indicates 50% cumulative (cum) survival.

3.4 Biochemistry

There were no significant differences in biochemistry parameters between groups (Table 1). The most common biochemical abnormalities in all dogs were hypoalbuminaemia (<28 g/L; 63%), hypoproteinaemia (<56 g/L; 29%) and hyperglobulinaemia (>41 g/L; 27%) of cases. Of all the biochemical parameters evaluated, only TSP (<56 g/L) was associated with decreased survival time in the long-term survival group (P = 0.049, S3-Fig 3)

3.5 Diagnostic tests

The size of the neoplastic mass was measured on radiographs (17/30) and/or CT (16/30). The approximate area of the neoplastic masses was calculated based on the largest values on either radiographs or CT. Although area of the mass was not statistically different between the surgery (median: 319 cm, range: 143-996) and ablation group (median: 223 cm, range: 88-536), there was a

trend to larger masses in the surgery group (median of all masses: 280 cm, range: 88-996) and there was no significant association with survival (P = 0.35).

Sixty-six percent (19/29) of dogs were diagnosed with an osteosarcoma only. Other types of neoplasia included undifferentiated sarcoma (3/29, 10%), fibrosarcoma (3/29, 10%), anaplastic sarcoma (2/29, 7%), rhabdomyosarcoma (1/29, 3.5%), osteosarcoma and angioleiomyosarcoma (1/29, 3.5%). Mitotic index was reported as low (1-4 mitotic figures) in 9/17 (53%), moderate (5-10 mitotic figures) in 4/17 (23.5%) and high (>10 mitotic figures) in 4/17 (23.6%). Neither neoplastic type or mitotic index were significant for survival (P = 0.603 and P = 0.282, respectively).

3.6 Treatment and outcomes

Chemotherapy was instituted in 33% (10/30) of dogs. Doxorubicin as sole chemotherapeutic was used in 5/10, carboplatin as a sole chemotherapeutic in 3/10, doxorubicin and carboplatin combination were used in 1/10 and carboplatin intralesionally in one patient. Neither the use, nor the type of drug used were significant for survival (P = 0.242 and P = 0.965 respectively).

PEG tube placement was performed in 17/30 (57%), of which 15/18 (83%) in the surgery group and 2/12 (17%) in the laser ablation group (P = 0.001). Two minor complications (one infected insertion site and one pre-mature removal by patient without further complications) and one major complication (death after PEG tube removal due to septic peritonitis) were documented. PEG tube placement was not associated with improved survival (P = 0.21).

Hospitalisation time of dogs undergoing ablation (median: 0 days, range 0-4) was significantly shorter than dogs undergoing surgery (median: 9 days, range: 1-21; P<0.001).

Endoscopic ablation was performed in 12/30 (40%) dogs while surgery was performed in 18/30 (60%) of dogs. Median survival time in the ablation group was 73.5 days (range: 0-1511, mean: 310 days), and median survival in the surgical group was 108 days (range: 0-1550, mean: 257 days), with no significant difference in survival times between the two procedures (P = 0.662). No survival advantage to either intervention (P = 0.982, Fig 4) nor associated hazard was found. The

long-term survival group also showed no significance in survival after ablation or surgery {79 days (45-1550) vs. 250 days (80-1511), respectively, P = 0.082}.

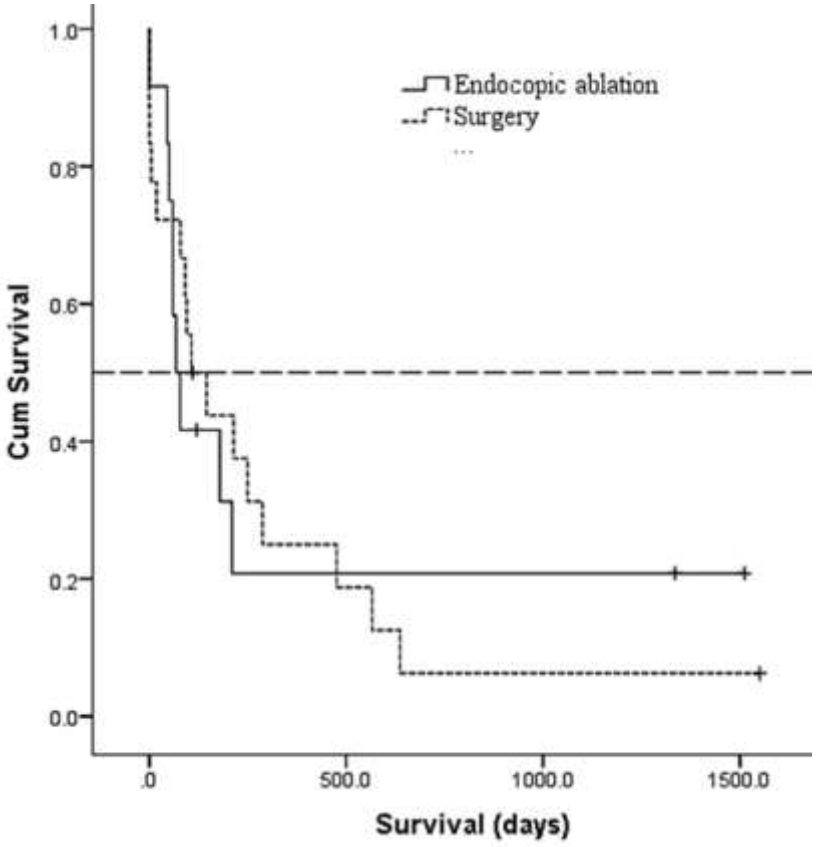


Fig 4. Kaplan-Meier survival curve of dogs undergoing endoscopic ablation or surgery. No difference in survival was identified between the two intervention methods (P = 0.982). ┼ Indicates censored dogs alive at the time of analysis; —— indicates 50% survival.

The most common cause of death was metastatic disease (1/12 in the ablation group and 11/18 of the surgical group), occurring at a median of 197 days (range: 80-637 days). Two of the dogs in the surgical group were euthanized during surgery due to non-resectable infiltrative masses and one dog in the ablation group was euthanized during the procedure due to oesophageal perforation. Other causes of death in the surgical group included suspected pulmonary thromboembolism (1 dog, 12 h after surgery), ventilator induced acute respiratory distress syndrome (1 dog, 5 days after surgery), septic peritonitis after PEG tube removal (1 dog, 19 days after surgery). In the ablation group, two dogs were euthanized due to anorexia (days 60 and 79 after ablation), one dog due to persistent vomiting/regurgitation (45 days post ablation), one dog to

bleeding of a gastric mass (51 days post ablation) and the reason was unknown in 3 dogs (days 60, 68, 210 after ablation). Five dogs were still alive at the time of manuscript submission (120, 1335, 1511 days after ablation and 110, 1550 after surgery).

3.7 Intragroup survival and hazard ratio analysis

Intragroup analysis showed the use of chemotherapy in the surgical group was the only parameter associated with survival benefit (P = 0.02), but this became insignificant in the long-term survival group (P = 0.43). Chemotherapy was associated with a reduced hazard ratio in the surgical group (hazard ratio = 0.282, $CI_{95\%}$: 0.90-0.887, P = 0.03), but again became insignificant in the long-term survival group (P = 0.436). Only Ht (>36%) in the ablation group was associated with a survival advantage (P = 0.031), and remained significant in the ablation-long-term survival group (P = 0.006).

Discussion

This retrospective study describes the signalment, clinicopathological findings, outcome and identifies prognostic indicators in 30 dogs diagnosed with *Spirocerca* induced sarcoma, undergoing endoscopic-guided ablation or partial oesophagostomy. The results of this study may assist in decision making regarding the type of intervention and possible outcome in dogs diagnosed with *Spirocerca* induced sarcoma.

In previous studies the age of dogs diagnosed with *Spirocerca* induced sarcoma ranged from 6.4 to 8.7 years (Ranen et al., 2004a; Dvir et al., 2008; Ranen et al., 2008; Shipov et al., 2015), which is comparable to the age of dogs in this study. Dogs diagnosed with *Spirocerca* induced sarcoma are typically older compared to dogs diagnosed with benign spirocercosis (Dvir et al., 2008), but the age of diagnosis did not affect outcome in this study. Despite 70% of dogs with neoplasia being female, consistent with previous studies (Ranen et al., 2004a; Dvir et al., 2008; Ranen et al., 2008; Pazzi et al., 2014), sex did not affect intervention outcome. Androgens may be responsible for a protective effect against development of neoplasia in males, although other unknown factors may also play a role (Dvir et al., 2008).

The most common presenting complaints were gastrointestinal signs (89%) and weight loss (33%), consistent with previous reports (30-94% and 10-77% respectively) (Dvir et al., 2001; Mazaki-Tovi et al., 2002; Ranen et al., 2004a; Dvir et al., 2008). The prevalence of respiratory signs as a presenting complaint (3%) was much lower in this study compared to other studies (18-60%)(Ranen et al., 2004a; Dvir et al., 2008). Respiratory signs have been previously attributed to the mass size resulting in bronchial displacement (53% in one study) (Dvir et al., 2008), and metastasis (Dvir et al., 2001). It is also possible that aspiration pneumonia might have contributed to respiratory signs in some of the dogs. The degree of bronchial displacement was not routinely recorded in this cohort however metastasis and aspiration pneumonia were not documented in any of the dogs which could be the reason for reduced prevalence of respiratory signs.

The reduced survival associated with weight loss as a presenting sign was most likely related to the chronicity of disease before presentation, mass size, cachexia and subsequent associated decreased appetite, increased frequency of regurgitation or vomiting, reduction of lean muscle mass, and increased inflammation (Freeman, 2012). In the long-term survival group (surviving >21 days) weight loss was not a predictor of survival, likely since animals excluded from this group died of procedure associated complications. Weight loss, if part of the presenting complaint, may therefore be considered a negative predictor of immediate post-interventional survival and while specific information in veterinary medicine is lacking, it is well established that nutritional status is an important determinant of surgical outcome in human patients (Stratton and Elia, 2007). The duration of clinical signs was not associated with survival, potentially due to poor owner observation of their dogs or the mass growing to a particular size (particularly in large breeds) before clinical signs become apparent. This suggests weight loss is a more accurate chronic marker of disease and immediate post-intervention survival than the duration of clinical signs.

The mild normocytic anaemia identified in this study is a common finding in *Spirocerca* induced sarcoma (Mazaki-Tovi et al., 2002; Dvir et al., 2008). Anaemia was a risk factor for shorter survival in the following groups: ablation group at presentation, ablation-long-term survival and all

dogs in the long-term survival group. Anaemia was likely a combination of inflammatory processes and blood loss from ulcerated areas in the mass (Ranen et al., 2004a). The reason anaemia primarily affected long-term survival may be related to the mild degree of anaemia, the mass size and likelihood of recurrence of the mass as opposed to the short-term complications of intervention. The reduced survival and increased hazard ratio associated with hypochromasia (taken from the lower limit of normal) described in this study is most likely a result of the same reasons as the anaemia that is present in some cases, as well as decreased serum iron levels secondary to anaemia of inflammatory disease and blood loss (Naigamwalla et al., 2012). Anaemia of inflammatory disease results in reduced iron absorption and iron sequestration in the liver, spleen, and bone marrow and a functional iron deficiency, defective heme synthesis, and the formation of some microcytic, and hypochromic erythrocytes despite adequate body iron stores (Naigamwalla et al., 2012).

Animals presented with advanced *Spirocerca* induced sarcoma are more likely to show leucocytosis (Dvir et al., 2008). In previous studies of oesophageal osteosarcomas, 82% of cases presented with leukocytosis, compared to only 32% of patients with non-neoplastic spirocecercosis (Mazaki-Tovi et al., 2002; Ranen et al., 2004a). Increase total WCC is likely associated with the necrosis and inflammation of large neoplastic masses. This is supported by a recent study evaluating serum acute phase protein concentrations and total WCC in dogs with *Spirocerca* induced sarcoma, which demonstrated that inflammatory response and increased total WCC is common in these dogs (Nivy et al., 2014). Moreover, total WCC was one of the most accurate predictors of *Spirocerca* induced sarcoma (Nivy et al., 2014). It is possible that the degree of inflammation is associated with the mass size. The association in this study with short and long-term survival and leucocytosis is likely related to the inflammation and necrosis associated with neoplastic transformation although 6/29 dogs had leukocyte counts within normal range. Leukocyte count has been previously correlated to hypercoaguability as defined by maximum amplitude on tissue-factor activated thromboelastography, but no association with survival or development of thromboembolism was evaluated (Pazzi et al., 2014). In this study, only one dog died secondary to suspected

thromboembolism and one due to acute respiratory distress syndrome. The overall effect of inflammation on survival could not be determined from this study and requires further investigation.

Biochemical parameters were of little prognostic value in this study, although reduced survival was documented in the long-term survival group presenting with hypoproteinaemia. Hypoalbumiaemia, a common finding in *Spirocerca* induced sarcoma (Mazaki-Tovi et al., 2002; Dvir et al., 2008; Nivy et al., 2014) was present in half of the cases and was most likely due to albumin being a negative acute phase protein and as a consequence of blood loss from the necrotic neoplastic masses. Previously, increased ALP, creatine kinase, amylase and lactate dehydrogenase have been documented once neoplastic transformation has occurred (Ranen et al., 2004a). In this study ALP was above the reference in only 2/27 (7%) of dogs, markedly lower than previous studies (Mazaki-Tovi et al., 2002; Mylonakis et al., 2006) while creatine kinase and lactate dehydrogenase were not consistently measured. The low prevalence of elevated might result from differences between the types of tumours.

The size (height and width) of the mass on thoracic radiographs may give an indication of neoplastic transformation, although significant overlap exists between non-neoplastic and neoplastic spirocercosis (Dvir et al., 2008). Due to the retrospective nature of the study, either radiographic and/or CT values were available. As a means of standardisation, area of the mass was calculated based on the largest values (length, height or width) from radiographs or CT. There was no significant survival effect that could have been attributed to area of the mass, thus mass size should not be a reason for the euthanasia (although some dogs may not have reached the intervention stage as the mass may have been considered too large for intervention). It is more likely that the size of attachment of the mass to the oesophageal mucosa or infiltration into normal tissue is a more important parameter for success rather than the size itself. Indeed, the reason for intra-operative euthanasia was due to the infiltrative nature of the mass during surgery or oesophageal perforation during ablation. Similarly, in cases of ablation, mass removal is more challenging, and in times impossible when the mass is wide based (Shipov et al., 2015).

Osteosarcoma is the most commonly reported form of neoplastic transformation and the 69.5% prevalence in this study is in accordance with previously reported prevalence of 60 to 93% (Ranen et al., 2004a; Ranen et al., 2008; Dvir et al., 2010; Pazzi et al., 2014). Although the grading criteria of canine appendicular osteosarcoma compared to *Spirocerca* induced sarcoma considers *Spirocerca* induced sarcoma a more aggressive neoplasia (Kirpensteijn et al., 2002; Dvir et al., 2010), the biological nature of oesophageal osteosarcomas are thought to be less aggressive/metastatic than appendicular osteosarcomas. The biological behaviour of oesophageal osteosarcomas in this study shows a high prevalence of metastasis after intervention. Of the 12/21 (57%) dogs with known cause of death in the long-term survival group, 10 (83%) had osteosarcoma, equating to metastasis as a reason for euthanasia in 83% of dogs. Interestingly osteosarcoma was not associated with a worse outcome, but this finding may be related to inadequate sample size due to the low numbers in the non-osteosarcoma group.

The use of an oesophageal sarcoma-grading scheme has been suggested in a previous study and included, amongst other parameters, mitotic index (Ranen et al., 2008). No survival advantage was found between survival and the type of neoplasia or neoplasia grade in that study. The current study also did not identify survival differences based on mitotic index. Interestingly, although the majority of dogs had a low mitotic index, most dogs died due to metastatic disease within 215 days, reflecting the likelihood that biological behaviour does not necessarily follow the measured histopathological indices.

The use of chemotherapy after surgery is a well-established means of palliative or life-extending therapy in dogs with skeletal osteosarcoma as well as soft tissue sarcomas (Selmic et al., 2014). There is no evidence suggesting the use of chemotherapy in spirocercosis-induced oesophageal osteosarcoma extends disease-free-interval or survival (Ranen et al., 2008). Current protocols extrapolated from the treatment of appendicular osteosarcoma were used for spirocercosis-induced oesophageal osteosarcoma or sarcomas in this study (Selmic et al., 2014). Neither the use, nor the type of drug used were associated with survival, but the number of treated

patients was small and there was a lack of consistently used protocols. Additionally, dogs receiving chemotherapy were the ones most likely to have recovered well from surgery. Although there was a survival benefit of chemotherapy in dogs receiving surgery, the lack of survival advantage of chemotherapy beyond 21 days indicates chemotherapy makes no difference to survival prediction at presentation and was only significant in the surgical group, as a result of the death of patients, within 21 days, that did not receive chemotherapy. The use of chemotherapy in these patients requires further investigation. Interestingly, 2/3 dogs that lived past 1000 days did not receive chemotherapy.

The success of ablation or the true presence of metastasises (confirmed in only one dog) was difficult to determine as a large proportion (50%) of ablation dogs were euthanased for unknown reasons, at the owner's request or due to persistent vomiting/regurgitation. Sixty one percent of surgical dogs were euthanased due to metastasis, with these dogs showing a large range in survival time (80 to 637 days).

Partial oesophagectomy results in direct disruption of the oesophageal wall layers and therefore has a greater risk of dehiscence or stricture formation compared to ablation. A PEG tube is used to facilitate enteral nutrition following oesophageal surgery and is thought to reduce the risk of dehiscence and strictures, although no studies have shown a correlation between PEG tube placement and reduced risk of stricture formation. Oesophageal damage during laser ablation is likely dependant on operator experience and the nature of the attachment of the mass to the oesophageal wall. The complication rate of PEG tube placement in this cohort of dogs was 11% in the minor category and 6% in the moderate to severe category, lower in the latter category compared with previous reports (Salinardi et al., 2006). The majority of PEG tube placements were in the surgery group and reflects the invasive nature of the surgery compared to the lack of wall invasion during ablation. The only major complication of PEG tube placement was septic peritonitis after removal of the PEG tube by the dog's private veterinarian. No oesophageal strictures were

reported in any dogs, possibly due to the already distended oesophagus around the mass after ablation or resection, but follow-up endoscopy was not routinely performed in dogs.

No significant difference in survival was found between the two interventions, implying that either technique is acceptable depending on equipment available, owner finances and case specifics such as the size, position or invasiveness of the mass. The minimally invasive technique of ablation results in a significantly shorter hospitalisation time compared to a surgery. The reduced hospitalisation time results in reduced expenses for the owner and possibly reduced complications, but the technique is limited with regard to the size and invasive nature of the mass and may result in oesophageal perforation (Shipov et al., 2015).

Limitations of the study include its retrospective nature, procedures were performed in two different institutions resulting in variable surgical and operator experience increasing variability, lack of uniform interpretation of histopathology and mitotic index and low case numbers hampering survival analysis within groups. Weight loss, a significant finding in this study, was based on subjective owner assessment. Inflammatory markers as well as coagulation parameters were not recorded for all dogs. Patients were presented at various stages of disease and although this is expected in a clinical setting, the variable clinical state does not allow for evaluation of a standardised treatment of disease. Inconsistent measurements of the masses on radiographs or CT affected the ability to relate to survival.

In conclusion, this retrospective study found weight loss, hypochromasia, and leucocytosis at presentation to be useful prognostic indicators in dogs with *Spirocerca* induced sarcoma. Although there was no statistically significant difference in survival between ablation or surgery, ablation resulted in shorter hospitalisation, while surgery, possibly more appropriate for larger masses, offered no benefit if ablation is technically possible. The use of chemotherapy post-intervention requires further investigation.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Bailey, W. S., 1972. *Spirocerca lupi*: a continuing inquiry. J. Parasitol. 58, 3-22.

Boerman, I., Selvarajah, G. T., Nielen, M., Kirpensteijn, J., 2012. Prognostic factors in canine appendicular osteosarcoma--a meta-analysis. BMC Vet. Res. 8, 56-67.

Dvir, E., Clift, S. J., Williams, M. C., 2010. Proposed histological progression of the *Spirocerca lupi*-induced oesophageal lesion in dogs. Vet. Parasitol. 168, 71-77.

Dvir, E., Kirberger, R. M., Malleczek, D., 2001. Radiographic and computed tomographic changes and clinical presentation of spirocercosis in the dog. Vet. Radiol & Ultrasound. 42, 119-129.

Dvir, E., Kirberger, R. M., Mukorera, V., van der Merwe, L. L., Clift, S. J., 2008. Clinical differentiation between dogs with benign and malignant spirocercosis. Vet. Parasitol. 155, 80-88.

Freeman, L. M., 2012. Cachexia and Sarcopenia: Emerging Syndromes of Importance in Dogs and Cats. J. Vet Intern. Med. 26, 3-17.

Kirpensteijn, J., Kik, M., Rutteman, G. R., Teske, E., 2002. Prognostic Significance of a New Histologic Grading System for Canine Osteosarcoma. Vet. Pathol. 39, 240-246.

Krishna, S., Anantaraman M., 1971. Some observations on the development of *Spirocercu lupi* in its intermediate and definitive hosts. J Helminthol. XLV: 123-131.

Lavy, E., Aroch, I., Bark, H., Markovics, A., Aizenberg, I., Mazaki-Tovi, M., Hagag, A., Harrus, S., 2002. Evaluation of doramectin for the treatment of experimental canine spirocercosis. Vet. Parasitol. 109, 65-73.

Mazaki-Tovi, M., Baneth, G., Aroch, I., Harrus, S., Kass, P. H., Ben-Ari, T., Zur, G., Aizenberg, I., Bark, H., Lavy, E., 2002. Canine spirocercosis: clinical, diagnostic, pathologic, and epidemiologic characteristics. Vet. Parasitol. 107, 235-250.

Mylonakis, M. E., Rallis, T., Koutinas, A. F., Leontides, L. S., Patsikas, M., Florou, M., Papadopoulos, E., Fytianou, A., 2006. Clinical signs and clinicopathologic abnormalities in dogs with clinical spirocercosis: 39 cases (1996-2004). J. Am. Vet. Med. Assoc. 228, 1063-1067.

Naigamwalla, D. Z., Webb, J. A., Giger, U., 2012. Iron deficiency anemia. Can. Vet. J. 53, 250–256

Nivy, R., Caldin, M., Lavy, E., Shaabon, K., Segev, G., Aroch, I., 2014. Serum acute phase protein concentrations in dogs with spirocercosis and their association with esophageal neoplasia – A prospective cohort study. Vet Parasitol. 203, 153-159.

Pazzi, P., Goddard, A., Kristensen, A. T., Dvir, E., 2014. Evaluation of Hemostatic Abnormalities in Canine Spirocercosis and Its Association with Systemic Inflammation. J. Vet. Intern. Med. 28, 21-29.

Ranen, E., Dank, G., Lavy, E., Perl, S., Lahav, D., Orgad, U., 2008. Oesophageal sarcomas in dogs: histological and clinical evaluation. Vet. J. 178, 78-84.

Ranen, E., Lavy, E., Aizenberg, I., Perl, S., Harrus, S., 2004a. Spirocercosis-associated esophageal sarcomas in dogs: A retrospective study of 17 cases (1997-2003). Vet. Parasitol. 119, 209-221.

Ranen, E., Shamir, M. H., Shahar, R., Johnston, D. E., 2004b. Partial Esophagectomy with Single Layer Closure for Treatment of Esophageal Sarcomas in 6 Dogs. Vet Surg. 33, 428-434.

Ridgway, R. L., Suter, P. F., 1979. Clinical and radiographic signs in primary and metastatic esophageal neoplasms of the dog. J. Am. Vet. Med. Assoc. 174, 700-704.

Romano, F. R., Heinze, C. R., Barber, L. G., Mason, J. B., Freeman, L. M., 2016. Association between Body Condition Score and Cancer Prognosis in Dogs with Lymphoma and Osteosarcoma. J. Vet. Intern. Med. 30, 1179-1186.

Salinardi, B. J., Harkin, K. R., Bulmer, B. J., Roush, J. K., 2006. Comparison of Complications of Percutaneous Endoscopic Versus Surgically Placed Gastrostomy Tubes in 42 Dogs and 52 Cats. J. Am. Anim. Hosp. Assoc. 42, 51-56.

Selmic, L. E., Burton, J. H., Thamm, D. H., Withrow, S. J., Lana, S. E., 2014. Comparison of Carboplatin and Doxorubicin-Based Chemotherapy Protocols in 470 Dogs after Amputation for Treatment of Appendicular Osteosarcoma. J. Vet. Intern. Med. 28, 554-563.

Shipov, A., Kelmer, G., Lavy, E., Milgram, J., Aroch, I., Segev, G., 2015. Long-term outcome of transendoscopic oesophageal mass ablation in dogs with *Spirocerca lupi*-associated oesophageal sarcoma. Vet. Rec. 177, 365-365.

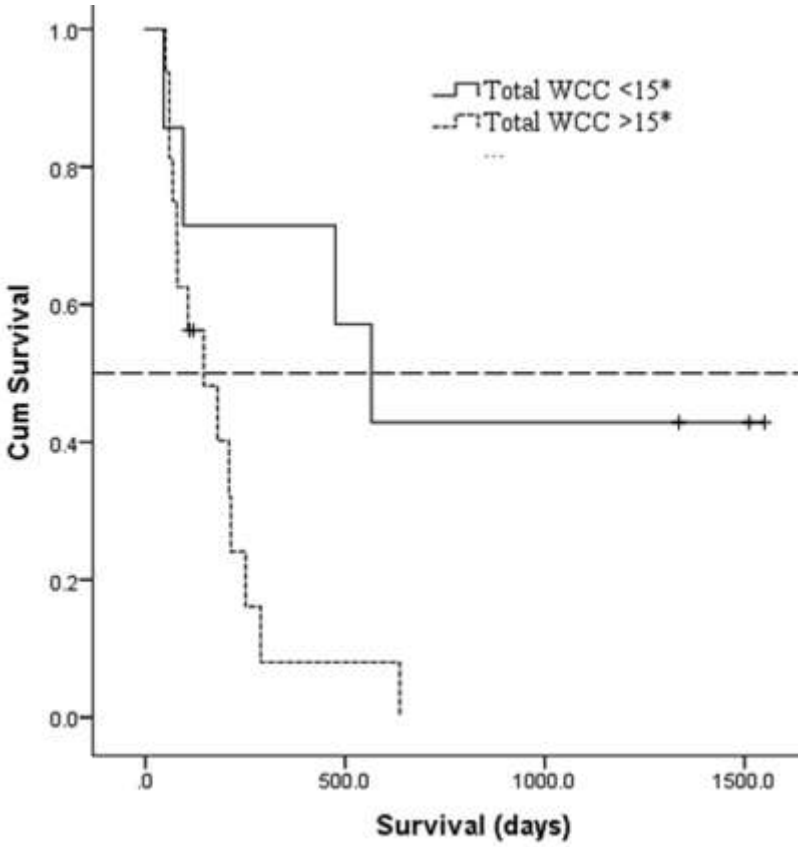
Stratton, R.J., Elia, M., 2007. Who benefits from nutritional support: what is the evidence? Europ. J. Gastroenterol. Hepatol. 19, 353-8.

Sutton, J.S., Culp, W. T. N., Scotti, K., Seibert, R. L., Lux, C. N., 2016. Perioperative morbidity and outcome of esophageal surgery in dogs and cats: 72 cases (1993-2013). J. Am. Vet. Med. Assoc. 249, 787-93.

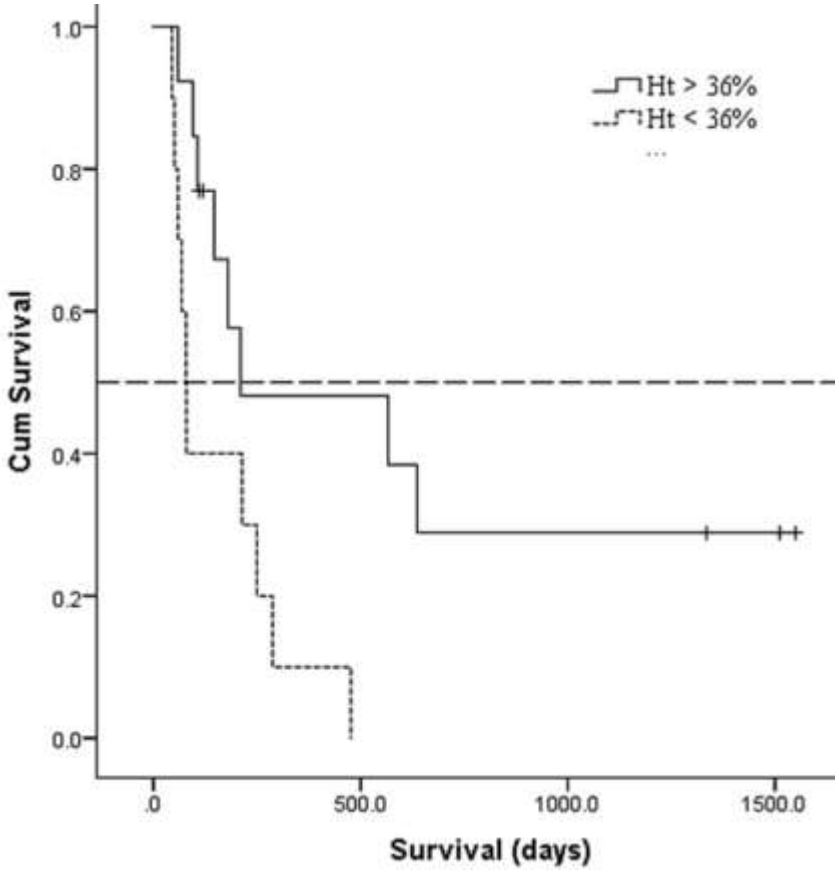
Van der Merwe, L. L., Kirberger, R. M., Clift, S., Williams, M., Keller, N., Naidoo, V., 2008. *Spirocerca lupi* infection in the dog: A review. Vet. J. 176, 294-309.

Yas, E., Kelmer, G., Shipov, A., Ben-Oz, J., Segev, G., 2013. Successful transendoscopic oesophageal mass ablation in two dogs with *Spirocerca lupi* associated oesophageal sarcoma. J. Small Anim Pract. 54, 495-498.

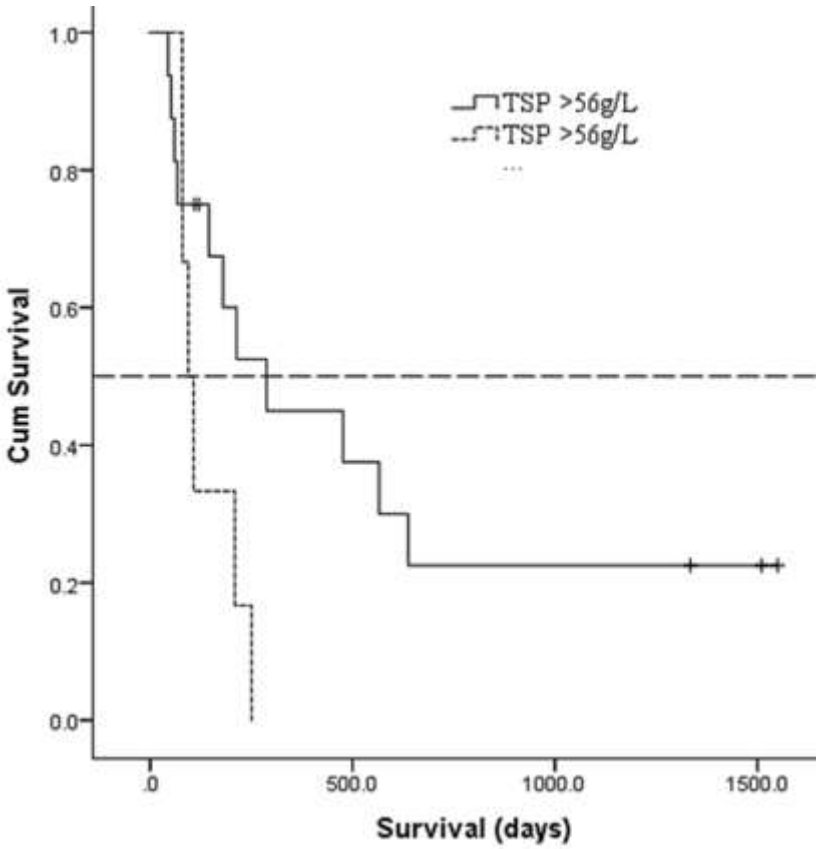
Supplementary material



S1-Fig 1. Kaplan-Meier survival curves for the long-term survival group: a total white cell count (WCC) *>15x10^9/L was associated with reduced survival (P = 0.021). ┼ Indicates censored dogs alive at the time of analysis; ——indicates 50% cumulative (cum) survival.



S2-Fig 2. Kaplan-Meier survival curves for the long-term survival group: haematocrit <36% was associated with a reduced survival (P = 0.016). ┼ Indicates censored dogs alive at the time of analysis; ——indicates 50% cumulative (cum) survival.



S3-Fig 3. Kaplan-Meier survival curves for the long-term survival group: total serum protein (TSP) was <56 g/L at presentation was associated with reduced survival (P = 0.049). ┼ Indicates censored dogs alive at the time of analysis; —— indicates 50% cumulative (cum) survival.