3 GENERAL METHODOLOGY

3.1 Cases

3.1.1 Retrospective cases

Medical records of 297 dogs diagnosed with spirocercosis at the Onderstepoort Veterinary Academic Hospital, University of Pretoria, South Africa, during 1995-2006, were retrospectively reviewed. From these records two groups of cases were selected: confirmed neoplastic cases and confirmed non-neoplastic oesophageal nodule cases.

The inclusion criteria for the non-neoplastic group were: an endoscopic diagnosis of spirocercosis with an obvious response to treatment within at least 6 weeks or a histological diagnosis of a non-neoplastic nodule after surgical resection of the whole nodule or diagnosis of non-neoplastic spirocercosis at necropsy, including histological appraisal of the entire nodule. A diagnosis of non-neoplastic nodule based only on endoscopic guided biopsy was judged to be unsatisfactory as it has been shown to be highly insensitive in a few studies (Dvir et al., 2001; Mazaki-Tovi et al., 2002; Ranen et al., 2004). The inclusion criteria for the neoplastic group were: histological diagnosis of malignancy of an oesophageal nodule obtained either by endoscopic-guided biopsy, surgical resection or necropsy. Cases with radiographic diagnosis of caudal oesophageal nodules, spondylitis and thoracic metastases with no other obvious primary neoplasm elsewhere in the body without histological analysis of the oesophageal nodule or the metastasis were also included in the neoplastic group.

Sixty-two out of 297 dogs had adequate data to fulfil the inclusion criteria. Thirty one were included in the non-neoplastic group; 19 were based on endoscopic diagnosis and response to treatment, 12 were based on histology of the entire oesophageal

nodule, ten of which were necropsy cases, and two were surgical resection cases. Thirty one dogs were included in the neoplastic group; 27 were diagnosed histologically and four cases were selected according to the combination of a caudal oesophageal mass, spondylitis and thoracic metastasis.

3.1.2 Prospective cases

The study population comprised of 103 client-owned dogs admitted to the Onderstepoort Veterinary Academic Hospital, at the Faculty of Veterinary Science, University of Pretoria between 2008 and 2011. The dogs were divided into 3 groups, One hundred and three dogs were enrolled in the study and were divided into 3 groups, non-neoplastic (n=49), neoplastic (n=29) and healthy control (n=25). The same inclusion criteria were used as for the retrospective case series. This population was used for collecting plasma to investigate the cytokine concentrations, as described in chapter 8.

3.2 Samples

3.2.1 Histopathology

Sixty two paraffin blocks containing *S. lupi*-induced non-neoplastic or neoplastic oesophageal nodules, collected between 1998-2008, were retrieved from the archives of the Section of Pathology, Faculty of Veterinary Science, University of Pretoria. Per block, one 5-µm-thick hematoxylin and eosin–stained section was examined under a light microscope. In addition, 10 sections of normal distal third of oesophagus were evaluated and compared with the *Spirocerca*-induced nodules.

Only one nodule was selected per dog and if a dog had more than one nodule, the nodule that was most mature or advanced in relation to the progression of the nodule

toward neoplasia was selected for evaluation. If a nodule was sectioned more than once, the section with the most advanced fibroplasia was selected. Once the stages within the nodule progression were established (chapter 5), additional sections per block was used to study growth factors expression (chapter 6) and to label the different inflammatory cells (chapter 7) using immunohistochemistry. For the labelling of the different inflammatory cells, an additional 9 *S. lupi*-induced oesophageal nodule cases (5 neoplastic and 4 non-neoplastic) were collected prospectively together with the draining lymph nodes of the distal oesophagus (bronchial) and remote lymph nodes (popliteal) that served as control.

3.2.2 Plasma

Blood samples were collected from the 103 dogs enrolled in the prospective study at admission by jugular venipuncture with a 21g needle and a 5 mL potassium EDTA vacutainer syringe. The samples were then immediately centrifuged, separated, aliquoted and frozen at −80°C. The samples were batched and analyzed together.

4 CLINICAL DIFFERENTIATION BETWEEN DOGS WITH BENIGN AND MALIGNANT SPIROCERCOSIS

This chapter was published as a research paper:

Clinical differentiation between dogs with benign and malignant spirocercosis

Eran Dvir[a,*], Robert M Kirberger[a], Varaidzo Mukorera[a], Liesel L van der Merwe[a], Sarah J Clift[b]

Veterinary Parasitology, 2008, 155: 80-88.

[a]*Department of Companion Animal Clinical Studies and* [b]*Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, Republic of South South Africa*

*Corresponding author. Tel.: +27 12 529 8366, Fax: +27 12 529 8308

Email address: eran.dvir@up.ac.za, edvir2000@yahoo.com (E. Dvir).

4.1 Abstract

Spirocerca lupi is a nematode infesting the canine oesophagus, where it induces the formation of a nodule that may transform into a malignant sarcoma. The current, retrospective study compared the clinical presentation, haematology, serum albumin and globulin and radiology of benign cases (n=31) and malignant cases (n=31) of spirocercosis.

Dogs with spirocercosis-induced sarcoma were significantly older (6.4±1.91 years) than benign cases (4.93±2.87). In the malignant cases there were significantly (p=0.03) more sterilized females (10/31) and fewer intact males (4/31) compared to 2/31 and 13/31, respectively, in the benign cases. Hypertrophic osteopathy was observed in 38.7% of malignant cases and in none of the benign cases (p=0.0002). Common clinical signs included weight loss, regurgitation, anorexia, pyrexia

(T≥39.5°), respiratory complications and salivation but did not differ in prevalence between groups. On haematology, the malignant group had significantly ($p<0.05$) lower haematocrit (0.34±0.08 vs. 0.41±0.07) and higher white cell count (31.6±27.83 vs. 17.71±13.18 $x10^3$/µl), mature neutrophil count (26.06±26.08 vs. 12.23±9.96 $x10^3$/µl) and thrombocyte count (493.15±151.61 vs. 313.27±128.54$x10^9$/µl). There were no differences in the mean corpuscular volume and immature neutrophil count. On radiology, the mass length was not significantly different, but the height and the width of the malignant masses were significantly larger (62.59 ± 15.15 and 73.93 ± 20.94 mm) compared to the benign group (46.43 ± 23.62 and 49.29 ± 25.56, respectively). Spondylitis was more prevalent in the malignant group (67.86% vs. 38.46%, $p = 0.03$). Examining secondary pulmonary changes revealed significantly higher prevalence of bronchial displacement in the malignant group (52% vs. 17%, $p = 0.008$).

Hypertrophic osteopathy appeared to be a very specific but relatively rare (poor sensitivity) marker of malignancy. Female gender, anaemia, leukocytosis, thrombocytosis, spondylitis and bronchial displacement are significantly more common in malignant cases, but appear in benign cases as well. However, if found together in a specific case, they should increase the index of suspicion for malignancy in a diagnosed spirocercosis case.

Keywords: Spirocercosis; dog; sarcoma; oesophageal nodule; hypertrophic osteopathy.

4.2 Introduction

Spirocerca lupi (S. lupi) is a nematode of worldwide distribution, but it is most commonly found in tropical and subtropical areas (Bailey, 1972). Dogs are the definitive hosts and become infested by ingesting the coprophagous beetle intermediate hosts (Bailey, 1972). After ingestion the larvae are liberated in the gastric lumen, and migrate via the gastric mucosa, gastric arteries, and aorta and eventually through the thoracic aortic wall to the caudal oesophagus. Typically, the worms settle within the oesophageal wall, mature to adults and promote formation of a nodule (Bailey, 1963, 1972; van der Merwe et al., 2008). The nodules are often referred to as granulomatous (Bailey, 1963, 1972), but histologically this is inappropriate as the mature nodule is composed mostly of actively dividing immature fibroblasts with relatively pronounced vascularisation (van der Merwe et al., 2008). The host inflammatory reaction is commonly mild to moderate and is not characterized by a predominance of macrophages, as would be expected in granulomatous inflammation (Bailey, 1963; van der Merwe et al., 2008). Spirocercosis induces a few pathognomonic lesions; aortic scarring with aneurysm formation, thoracic vertebral spondylitis and caudal oesophageal nodule formation. The typical clinical signs associated with spirocercosis are related to the presence of oesophageal nodules and include regurgitation, vomiting, dysphagia and weight loss, together with non-specific signs like pyrexia (Dvir et al., 2001; Mazaki-Tovi et al., 2002). The clinical diagnosis of spirocercosis is largely dependant upon thoracic radiography, which demonstrates the caudal oesophageal soft tissue masses, caudal thoracic vertebral spondylitis and aortic undulation due to aneurysm formation. Faecal flotation tests are also used to detect the typical embryonated eggs. Oesophagoscopy typically demonstrates one or more nodules with a nipple-like protuberance.

The oesophageal nodule may undergo malignant neoplastic transformation (Bailey, 1963; Seibold et al., 1955). The association between *S. lupi* infection and oesophageal sarcoma was first described in 1955 (Seibold et al., 1955). This association was based on the finding of *S. lupi* worms and related oesophageal nodules close to the malignant neoplasm or the pathognomonic findings of spondylitis or aortic aneurysm in conjunction with the malignant neoplasm. Macroscopically, an increased size, and progression to cauliflower-like shape and area of necrosis in the malignant nodule compared to the smooth appearance of the benign nodule may be indicative of neoplastic transformation (Ranen et al., 2004). Histologically the malignant neoplasms are classified as fibrosarcoma, osteosarcoma or anaplastic sarcoma (Ranen et al., 2007; Ranen et al., 2004). The histopathological characteristics of the *S. lupi*-induced fibrosarcoma include interwoven bundles of tapered to plump spindle shaped cells, variable amounts intercellular collagenous matrix, and a high mitotic index (Bailey et al., 1963). Histological characteristics of the *S. lupi*-induced osteosarcoma include foci of polygonal osteoblasts, and variable numbers of osteoclasts and/or interwoven bundles of spindle cells, variable amount of osteoid matrix with or without foci of chondroid differentiation. Sometimes obvious spicules or trabeculae of bone are identified amidst solid foci of neoplastic spindle or polygonal cells (Bailey, 1963). In areas where spirocercosis does not exist, malignant neoplasms of the oesophagus are extremely rare (Ridgway and Suter, 1979), making spirocercosis the major cause of malignant oesophageal neoplasms in the dog. Spirocercosis-induced sarcoma metastasizes commonly to the lung but also to a variety of abdominal organs (Bailey, 1963; Dvir et al., 2001).

Benign spirocercosis is treated successfully with avermectins [doramectin (Dectomax, Pfizer, France) 400 µg/kg SC at 2-week intervals] (Lavy et al., 2002). However,

malignant neoplasms can only be treated by surgical excision with or without chemotherapy and the success rate is lower (Ranen et al., 2004). This difference in prognosis emphasizes the need to improve diagnostic and prognostic markers for the antemortem diagnosis of the oesophageal nodule and the need for a better understanding of the malignant transformation that may improve treatment for the malignant cases.

While spirocercosis has a few pathognomonic lesions, the ante-mortem differentiation of malignant neoplasm-bearing cases and benign cases has not been studied. The objectives of the present study were to identify clinical ante-mortem differences between malignant and benign spirocercosis cases to assist in diagnosis, treatment and prognostication.

4.3 Material and Methods

Medical records of 297 dogs diagnosed with spirocercosis at the Onderstepoort Veterinary Academic Hospital, University of Pretoria, South Africa, during 1995-2006, were retrospectively reviewed. From these records two groups of cases were selected: confirmed benign cases and confirmed malignant oesophageal nodule cases.

The inclusion criteria for the benign group were: An endoscopic diagnosis of spirocercosis with an obvious response to treatment within at least 6 weeks or a histological diagnosis of a benign nodule after surgical resection of the whole nodule or diagnosis of benign spirocercosis at necropsy, including histological appraisal of the entire nodule. A diagnosis of benign nodule based only on endoscopic guided biopsy was judged to be unsatisfactory as it has been shown to be highly insensitive in a few studies (Dvir et al., 2001; Mazaki-Tovi et al., 2002; Ranen et al., 2004). The inclusion criteria for the malignant group were: histological diagnosis of malignancy of an oesophageal nodule obtained either by endoscopic-guided biopsy, surgical

resection or necropsy. Cases with radiographic diagnosis of caudal oesophageal nodules, spondylitis and thoracic metastases with no other obvious primary neoplasm elsewhere in the body without histological analysis of the oesophageal nodule or the metastasis were also included in the malignant group.

The following clinical parameters were compared: age, breed, gender, body weight, duration of illness and the prevalence of weight loss, vomiting/regurgitation, anorexia, pyrexia (≥ 39.5 ºC), lameness, hypertrophic osteopathy (HO), respiratory signs and salivary gland enlargement. The clinicopathological parameters that were compared included: haematocrit (Ht), mean corpuscular volume (MCV), white blood cell count (WBC), mature and immature neutrophil, monocyte and eosinophil counts and serum albumin and globulin concentrations.

Thoracic radiographic evaluation and measurements were done by one board-certified radiologist (RK) on cases having at least dorsoventral and right lateral thoracic films. The following radiological parameters were compared: mass location (relative to thoracic vertebrae) and mass size (length, width, height). The presence of the following radiological findings were recorded and their prevalence was compared between the two groups: mass mineralization, oesophageal gravel sign (ingested mineralized debris) and air (indicating partial obstruction), spondylitis, pulmonary parenchymal changes, fissure lines/pleural effusion, thoracic lymph node visualization, tracheal displacement, bronchial displacement/compression and aortic changes.

Differences in the above stated categorical parameters (age, breed, gender and prevalence of clinical signs and clinicopathological and radiological abnormalities) were then compared between the two groups using the chi-square (χ^2) test. Continuous parameters (body weight, duration of illness, clinicopathological values and

radiological measurements) are presented as mean ± standard deviation and were compared between the two groups using the t-test. The level of significance for both tests was determined as $p < 0.05$.

4.4 Results

Sixty-two dogs had adequate data to fulfil the inclusion criteria. Thirty one were included in the benign group; 19 were based on endoscopic diagnosis and response to treatment, 12 were based on histology of the entire oesophageal nodule, ten of which were necropsy cases, and two were surgical resection cases. Thirty one dogs were included in the malignant group; 27 were diagnosed histologically and four cases were selected according to the combination of a caudal oesophageal mass, spondylitis and thoracic metastasis.

4.4.1 Signalment

There was a significant difference in the gender distribution between the groups ($p=0.03$) with more females, especially sterilised ones, in the malignant group and more males, especially intact ones, in the benign group (Table 1). The age of the dogs differed significantly between the two groups ($p=0.02$) being 4.93 ± 2.87 years in the benign group and 6.40 ± 1.91 years in the malignant group. There were no significant differences between the average body weight of the two groups (23.61 ± 9.90 kg vs. 26.27 ± 11.10 kg in the benign and malignant group, respectively).

4.4.2 Clinical presentation

Hypertrophic osteopathy was the only clinical sign with a significantly different prevalence between the two groups (Fig. 1), as it presented only in the malignant group (38.7% prevalence $p= 0.0001$). The prevalence of the remaining clinical signs:

weight loss, vomiting/ regurgitation, anorexia, pyrexia, lameness, respiratory signs and salivary gland enlargement, did not differ significantly between groups (Table 2). There were also no significant group differences on average body temperature (39.13 ± 0.73ºC vs. 39.19 ± 0.69ºC) and average duration of illness (7.89 ± 7.92 weeks vs. 8.83 ± 20.58 weeks) between the benign and malignant groups, respectively.

4.4.3 Haematology

Complete blood counts were available for 27 dogs in each group (Table 3). Although the haematocrit was significantly lower in the malignant group (p=0.002), there was a substantial overlap in the range of both groups. Anaemia, defined as a haematocrit < 37l/l, was diagnosed with significantly higher prevalence in the malignant group (p=0.003) and was normocytic in most cases (50% vs. 64.71 % in the benign and malignant groups, respectively). The prevalence of leukocytosis was significantly higher in the malignant group (p=0.03), but there was a substantial overlap in the range of the counts. The increase in cells consisted of mature neutrophils and monocytes. Eosinophil counts were significantly higher in the benign group (p=0.04), however the overlap in the range between the two groups was marked. The thrombocyte count and prevalence of thrombocytosis was significantly higher in the malignant group (p<0.001 and p=0.002, respectively).

4.4.4 Serum proteins

Serum protein concentrations were available for 19 cases in each group. Serum albumin was higher in the benign group compared to the malignant group (2. 87 ± 0.77 vs. 2.5 ± 0.53 mg/dl, normal range 2.7-3.5 mg/dl), but there was a marked overlap in the range and the difference between the groups was not significant (p=0.09). Serum globulin was significantly higher in the benign group (5.09 ± 1.60 vs.

4.11 ± 0.91 mg/dl in the benign and malignant group, respectively, p=0.03, normal range 2–3.7 mg/dl) but again, the overlap was substantial.

4.4.5 Radiology

Thoracic radiographs were available for 25 dogs from the benign group and for 28 dogs in the malignant group (Table 4).

In the benign group, 24 dogs had a confirmed oesophageal mass diagnosed on endoscopy or necropsy, but the mass was only radiologically visible in 21 dogs. In the malignant group 27/28 masses were visible radiologically (Fig. 2). In both groups the masses were located between T5 and T12. Comparing the length, height and width of the oesophageal masses between the groups revealed significant differences in the height and width only (higher values, p = 0.006 and 0.0006, respectively) with substantial overlap. Mass mineralization was a relatively rare sign, more likely to be seen with malignant transformation. Bronchial displacement was significantly more common in the malignant group (p=0.008), as was spondylitis (p=0.03).

4.5 Discussion

Previous studies that discussed spirocercosis-associated malignancy hypothesized that genetic and environmental factors may play a role in carcinogenesis (Bailey, 1972). In the current study, the number of sterilised females with malignant transformation was significantly higher compared to those with benign disease. Intact males were more prevalent in the benign group. This higher prevalence of malignant transformation in sterilised females has also been described in a previous study evaluating only malignant *S. lupi* cases (Ranen et al., 2007; Ranen et al., 2004). This might indicate a predisposition of sterilised female dogs with spirocercosis to undergo

malignant transformation of the oesophageal nodule and a possible resistance in intact males. This may indicate protective effect of sex steroids, especially androgens.

The mean age of the group with malignant transformation is similar to the mean age previously described in *S. lupi*-induced sarcoma cases (Ranen et al., 2004). The difference in age between the two groups, observed in the current study, may be partially explained by the time taken for the malignant neoplasm to develop and be diagnosed, but may also indicate an increase predisposition to malignant transformation with advanced age.

A comparison of the clinical presentation of the two groups revealed only one clinical sign which appeared to be highly specific for malignant cases, namely HO. Hypertrophic osteopathy is often reported in conjunction with *S. lupi*-induced sarcomas (Bailey, 1963; Brodey et al., 1977). It is characterised by irregular thickening of the parosteal tissue and exostotic proliferation of bone and cartilage in the distal limbs (phalanges, metatarsal and metacarpal) and vascularisation (Brodey, 1979; Seibold et al., 1955). Radiologically, HO is described as periosteal new bone formation (Brodey, 1971). Hypertrophic osteopathy has been linked to intrathoracic, especially pulmonary, masses (Brodey, 1971). Pulmonary neoplasia is the most common cause of HO. However, before 1944 the most common aetiology was tuberculosis, indicating that inflammatory–induced masses can also cause HO (Brodey, 1971). It is not clear from the literature if only spirocercosis-induced malignant neoplasms can induce HO or if benign nodules can also induce it (Brodey, 1971). In the current study, with its limited case numbers, only malignant spirocercosis-induced nodules were associated with HO. We therefore encourage clinicians to look for signs of HO on those parts of the thoracic limb seen on thoracic

radiographs as a possible clue for malignancy and to perform distal limb radiographs in any suspected swollen limbs.

Four theories have been proposed for the pathogenesis of HO: vagal nerve stimulation, pulmonary arterio-venous shunting, the production of a humeral substance by neoplastic cells and factors secreted by megakaryocytes or platelet clumps lodged in blood vessels of distal limbs (Dunn et al., 2007). Vagotomy caused dramatic reversal of HO (Brodey, 1979). This finding formed the basis of the theory that vagal stimulation is responsible for the development of HO. Bailey observed involvement of the vagus nerve within some of the malignant *S. lupi*-induced neoplasms (Bailey, 1963), and mass infiltration within the oesophageal vagus has been postulated as the major cause of HO in spirocercosis. Increased limb blood flow was proposed as a major contributor to the development of HO. It might be induced by the vagal stimulation and can be reduced by resection of the affected lung lobe and vagotomy (Brodey, 1971). Experimentally created right-to-left shunts can cause HO (Brodey, 1979). It was proposed that shunting allows humoral substances that are normally inactivated by the lungs to escape and reach the distal limbs (Martinez-Lavin, 1992). Later it was shown that platelet clumps are commonly lodged in the blood vessels of the extremities in HO cases, and it was hypothesized that megakaryocytes that escape the pulmonary capillary beds via the shunts facilitate the production of the platelet clumps that induce HO (Atkinson and Fox, 2004). The same might happen in diseases that release platelet aggregates from the left side of the heart, as happens in mitral or aortic vegetative endocarditis (Dunn et al., 2007). In the early form of HO in humans, also called digital clubbing, increased platelet derived growth factor and vascular endothelial growth factor expression was observed in tissue samples from digits stained immunohistochemically (Atkinson and Fox, 2004).

These growth factors were postulated to be released from the platelet clumps, inducing the tissue proliferation, increased vascularity and capillary permeability seen in HO (Atkinson and Fox, 2004). In a unifying theory for the pathogenesis of HO, platelet derived growth factor was proposed as the humeral substance inducing HO (Martinez-Lavin, 1992). Vagal stimulation was suggested to be only a contributing factor, which presumably causes increased blood flow in distal limbs and therefore facilitate platelets lodging and increased circulation of the humeral agent, which would explain the improvement in some patients following vagotomy. The concept of a humeral factor causing HO in spirocercosis seems very attractive. Spirocercosis appears to induce connective tissue proliferation throughout the course of the disease (spondylitis, oesophageal nodule, sarcoma and HO). It may be postulated that a circulating oncogenic or growth factor induced by the worm infection, which induces connective tissue proliferation, might provide a unifying pathogenesis for the development of the different forms of connective tissue proliferation in spirocercosis.

A few reports have claimed that spondylitis is more common in malignant cases (Brodey et al., 1977; Ranen et al., 2004), as shown in the current study. However, the prevalence of spondylitis in the benign cases was 38.26%, indicating that spondylitis starts early in the disease process and is progressive. The finding of larvae in the muscles between the aorta and the spine led to the theory that aberrant migration may be responsible for the spondylitis (Bailey, 1972). This explanation is under investigation in our institute and it appears to be at best only partially true, because often the malignancy appears to progress long after the worms have disappeared and they therefore can no longer play a role in the development of more overt spondylitis. It can be postulated that the worm may induce the spondylitis by stimulating changes

that later become independent of its presence or further changes could be induced by the malignant tumour.

Other than HO, no other presenting clinical signs or complications, or period of illness before presentation occurred significantly more frequently in either group. In a recent publication describing only spirocercosis associated oesophageal sarcoma cases (Ranen et al., 2004), the prevalence of vomiting and/or regurgitation (94%) was higher than what we observed in any of the groups and was reported in prior spirocercosis studies that investigated malignant and benign cases together (Dvir et al., 2001; Mazaki-Tovi et al., 2002). Thus, an increased prevalence of vomiting or regurgitation might be more frequent in malignant cases but we cannot support it comparing the 2 groups.

Anaemia related to spirocercosis has been described in benign (Brodey et al., 1977) as well as malignant cases (Ranen et al., 2004). In the current study, anaemia was proved to be more severe and more prevalent in malignant cases, but the overlapping range was quite broad. Comparing our results with those of the other study of *S. lupi*-induced sarcoma (Ranen et al., 2004), the current study showed fewer microcytic anaemia cases (35% vs. 63% in Ranen's study) and the current study also revealed no statistical difference from the benign group. Comparing the mean MCV in both studies reveals similar results, making the differences between the studies negligible. The most common explanation for microcytic anaemia is chronic blood loss, which can easily be explained by the predisposition of the *S. lupi* nodule to ulcerate. However, considering the high prevalence of dogs with normocytic anaemia, there are probably other factors that play a role in *S. lupi*-associated anaemia, such as anaemia of chronic disease or possibly paraneoplastic effects.

In the current study, leukocytosis was significantly more severe and prevalent in the malignant cases, as has been reported in another study (Ranen et al., 2004). Leukocytosis and eosinopenia has also been reported in malignant spirocercosis cases in an earlier study based only on 3 dogs (Brodey et al., 1977). In the current study, we confirmed these observations using a larger number of cases and by comparing values between malignant and benign cases. In the current study, thrombocytosis was also more common in the malignant group. This set of abnormalities, anaemia, leukocytosis and thrombocytosis, could be caused by continuous oesophageal irritation and blood loss from the malignant neoplasm. However, these haematological abnormalities may have a paraneoplastic origin and may provide a hint about the role that thrombocytes and leukocytes may play in the malignant transformation of the oesophageal nodule. Further research is required to explore this issue.

The search for radiological parameters to differentiate malignant from benign cases revealed substantial overlap in most measurements excluding HO. It may seem surprising that the mass length was similar for benign and malignant nodules, but this is probably due to the number of nodules, which can range from 1 to 8 and may coalesce longitudinally on radiographs. The height and width of the nodules are more reliable parameters, reflecting the larger size of malignant nodules. Bronchial displacement was also more common in the malignant group, and is probably secondary to the mass size.

Mass mineralization was assumed to be a relatively rare but specific marker for malignant transformation; however we did detect 1 benign case with mineralization on radiographic examination and 2 additional benign cases with foci of osseous metaplasia within a nodule (seen histologically), which could provide a pathophysiological mechanism for the presence of mineralization in benign nodules.

The presence of ingested mineralised debris should not be mistaken for mineralization of the nodule. The presence of osseous metaplasia may be another indication of the slow progression from benign to malignant nodules in spirocercosis. The gradual transition to sarcoma and the evidence of large numbers of embryonic fibroblasts in early benign nodules (Bailey, 1972), may be at least partially responsible for the big overlap or lack of significant differences in most of the parameters compared between benign and malignant groups. Computed tomography with its greater sensitivity to detect mineralization and to assess nodule perfusion after contrast medium administration may provide more clues in future about nodule characteristics and is currently being investigated by our institution.

Endoscopy was reported to be the most sensitive tool for spirocercosis diagnosis (Dvir et al., 2001; Mazaki-Tovi et al., 2002) and Ranen and others (2004) reported that they were able to make a tentative diagnosis of *S. lupi* – induced sarcoma on all the 15 cases they have scoped. We did not perform a detailed retrospective evaluation of our endoscopic and gross pathology changes as no consistent descriptions were used. However, in going through the available material various descriptions of irregularity, proliferation, necrosis and ulceration were common in the malignant cases (Fig. 3A), but 2 cases were described as smooth, which was an unexpected finding in malignant nodules. Benign nodules were very often described as typical (with a nipple-like protuberance), small and smooth (Fig. 3B); however in few cases inflammation, ulceration and necrosis were reported, which could raise the index of suspicion for malignant transformation. These lesions could be secondary to mechanical irritation of the partially obstructed oesophagus. Currently we are prospectively investigating the predictability of endoscopy to determine malignancy. Endoscopy-guided biopsy can only help if it is positive for malignancy as it is specific

but not sensitive (Dvir et al., 2001). Therefore, although we find endoscopy a reliable tool, equivocal cases need to be monitored carefully for their response to treatment. In case of uncertainty following biopsy and a short treatment course, resection is indicated for both treatment and diagnosis of malignant vs. benign nodule.

The search for antemortem indicators of malignant transformation of the oesophageal nodules did not yield any highly sensitive and specific marker. Hypertropic osteopathy was highly specific for malignancy but it is a relatively rare finding (38.7%). Female gender, anaemia, leukocytosis, thrombocytosis, spondylitis and bronchial displacement are more sensitive and less specific parameters that should be evaluated as a constellation of parameters, and, if found together in a specific case, should increase the index of suspicion for malignancy in a diagnosed spirocercosis case. They may also provide clues about the pathogenesis of the malignant transformation, which requires further investigation.

4.6 Tables

Table 1

Gender differences between benign and malignant groups (p=0.03, chi-square test)

Gender	Benign group *n*=31	Malignant group *n*=31
Intact female	38.7%	41.9%
Sterilized female	9.7%	32.3%
Intact male	41.9%	12.9%
Sterilized male	9.7%	12.9%

Table 2

Prevalence differences of clinical signs between the benign and malignant groups

Sign	Prevalence (%)	
	Benign group *n*=31	Malignant group *n*=31
Weight loss	58.06%	77.42%
Vomiting / regurgitation	67.74%	67.74%
Anorexia	45.16%	48.39%
Pyrexia	32.26%	41.94%
Lameness	12.9%	19.35%
Respiratory signs	33.26%	35.48%
*Hypertrophic osteopathy	0%	38.71%
Salivary glands enlargement	22.58%	25.81%

* p = 0.0001 (chi-square test)

Table 3

Haematology differences between the benign and malignant groups

Parameter	Benign group *n*=27	Malignant group *n*=27	Reference Interval
*Haematocrit (l/l)	0.41 ± 0.07	0.34 ± 0.08	0.37-0.55
*Prevalence of anaemia	22.22%	62.96%	
Mean corpuscular volume (fl)	63.67 ± 6.01	61.11 ± 7.62	60-77
Prevalence of microcytosis within the anaemic cases	50%	35.29%	
*White blood cell count ($x10^3/\mu l$)	18.03 ± 12.71	31.60 ± 27.84	6-15
*Prevalence of leukocytosis	44.44%	81.48%	
*Mature neutrophil count ($x10^3/\mu l$)	12.16 ± 9.81	26.06 ± 26.08	3-11.5
*Prevalence of mature neutrophilia	33.33%	70.37%	
Immature neutrophil count ($x10^3/\mu l$)	1.35 ± 3.04	1.06 ± 2.46	0-0.5
Prevalence of immature neutrophilia	29.63%	33.33%	
Monocyte count ($x10^3/\mu l$)	1.49 ± 1.47	2.20 ± 1.35	0.15-1.35
*Prevalence of monocytosis	33.33%	66.67%	
*Eosinophil count ($x10^3/\mu l$)	0.86 ± 0.79	0.49 ± 0.50	0.1-1.25
Prevalence of eosinophilia	25.93%	11.11%	
Prevalence of eosinopaenia	14.81%	29.63%	
*Thrombocyte count ($x10^9/\mu l$)	313.27 ± 128.54	493.15 ± 151.61	200-500
*Prevalence of thrombocytosis	7.41%	37.04%	

* $p < 0.05$ (t-test for absolute values and chi square test for prevalence)

Table 4

Radiological differences between the benign and malignant groups

Thoracic radiological findings	Benign group *n*=25	Malignant group *n*=28
Oesophageal mass length (mm)	81.90 ± 41.79	91.67 ± 28.86
*Oesophageal mass height (mm)	46.43 ± 23.62	62.59 ± 15.15
*Oesophageal mass width (mm)	49.29 ± 25.56	73.93 ± 20.94
Prevalence of oesophageal mass mineralization	4.76%	22.22%
Prevalence of oesophageal gravel sign	0%	11.11%
Prevalence of oesophageal air	52.38%	48.15
Prevalence of pulmonary parenchyma changes	16.00%	14.29%
Prevalence of fissure lines / pleural effusion	12.00%	21.43%
Prevalence of lymph nodes visualization	8.00%	0%
Prevalence of tracheal displacement	8.00%	17.86%
*Prevalence of bronchial displacement	16.00%	53.57%
Prevalence of aortic changes	48.00%	42.86%
Spinal radiological findings	*n*=26	*n*=28
*Prevalence of spondylitis	38.46%	67.86%
Number of spondylitis vertebrae	3.40 ± 1.78	3.55 ± 1.83

* $p < 0.05$ (t-test for absolute values and chi square test for prevalence).

4.7 Figures

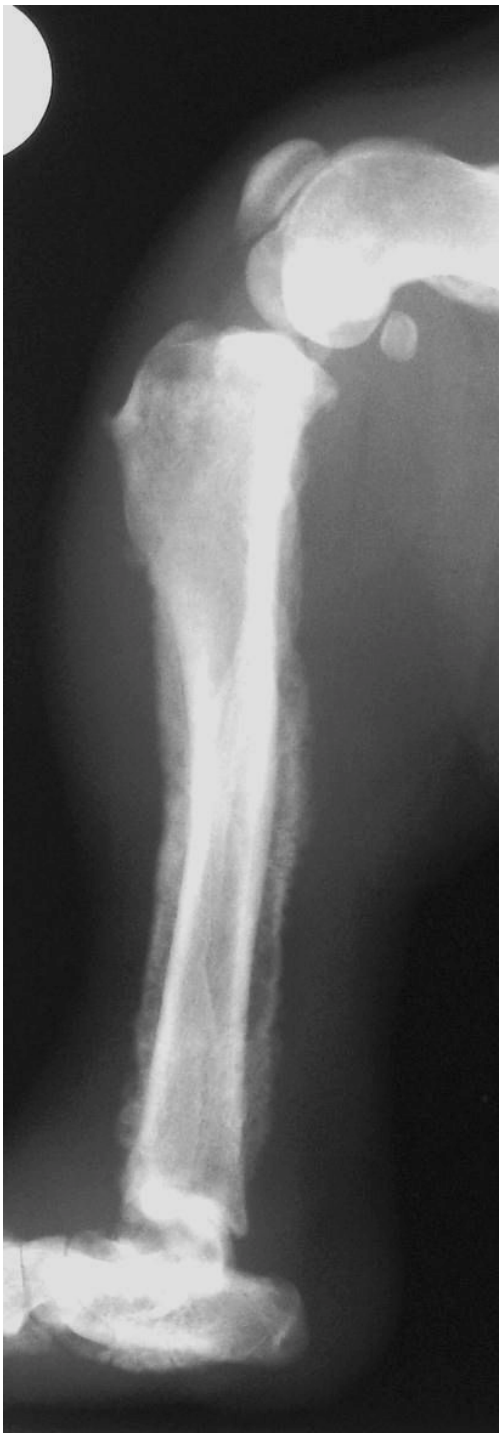

Figure 1: Mediolateral view of the tibia of a six year old Staffordshire bull terrier with hypertrophic osteopathy. Note the thick brush-like periosteal reaction on the tibia, distal femur and caudal aspect of calcaneus. This reaction was present extensively on all the limbs.

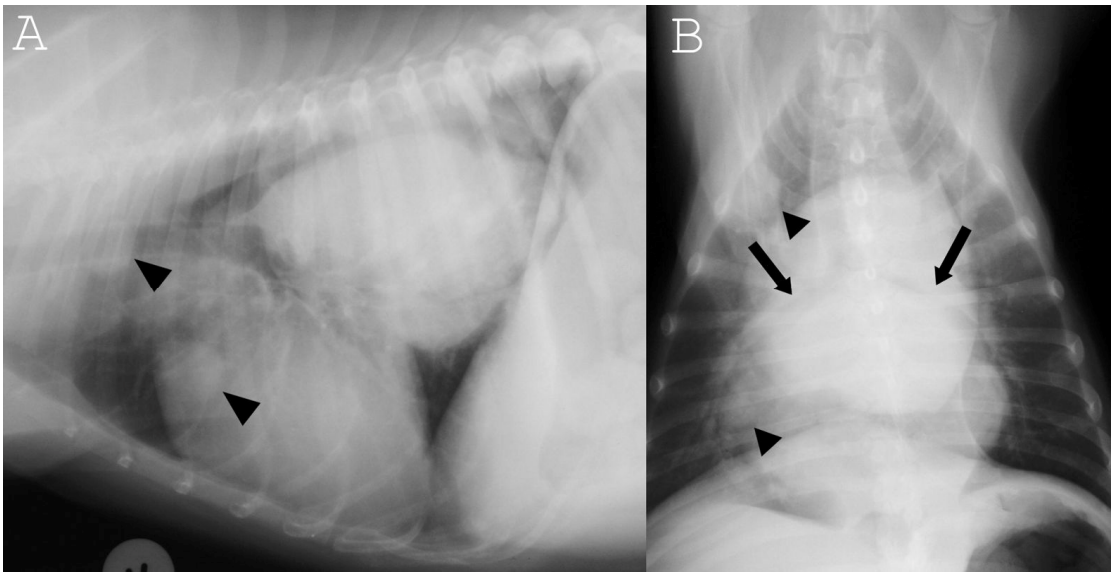

Figure 2: Medial-lateral (A) and ventro-dorsal (B) thoracic radiographs of the same dog as in Fig. 1. Note the large soft tissue mass superimposing on the caudal cardiac silhouette and cranial diaphragm. The mass displaces and compresses the main stem bronchi (arrows). Poorly defined metastatic nodules are visible (arrow heads).

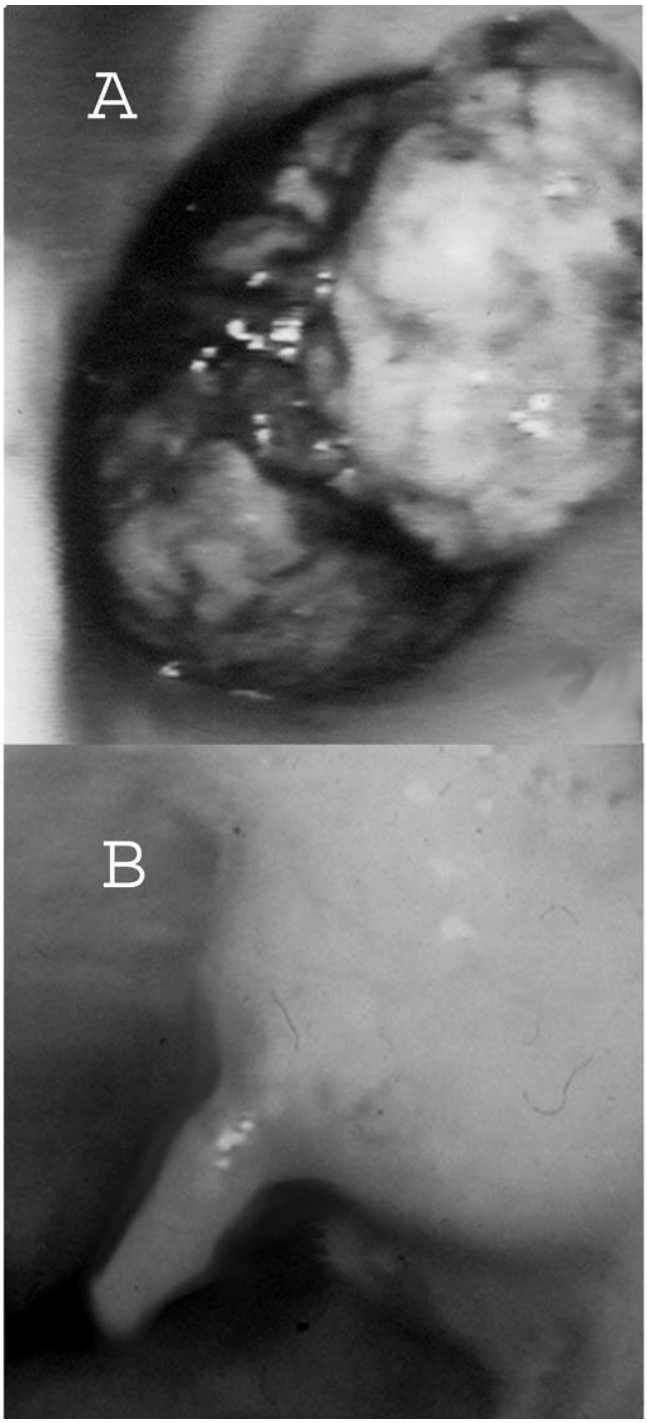

Figure 3: Oesophageal endoscopic pictures of neoplastic nodule (A) and benign nodule (B). In the neoplastic nodule, note the lobulated proliferation, area of black colouration indicating ulceration and necrosis and the size of the nodule occupying most of the lumen. In the benign nodule, note the smooth, round appearance with typical protuberance, the healthy mucosa surrounding it and the small size in relation to the oesophageal lumen.