5 PROPOSED HISTOLOGICAL PROGRESSION OF THE *SPIROCERCA LUPI*-INDUCED OESOPHAGEAL LESION IN DOGS

This chapter was published as a research paper:

Proposed histological progression of the Spirocerca lupi-induced oesophageal lesion in dogs

Eran Dvir[a, *], Sarah J Clift[b] and Mark C Williams[b]

Veterinary Parasitology, 2010, 168:71-77.

[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 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).

5.1 Abstract

This study aims to outline the histological progression of the *Spirocerca lupi* nodule from infection to neoplastic transformation. Sixty two spirocercosis-induced nodules, 42 non-neoplastic and 20 neoplastic, were stained with HE. Ten non-overlapping high power fields per nodule were examined and non-neoplastic and neoplastic nodules were compared. Inflammation was scored 0-3 and revealed a score of 1.91±0.52 in the non-neoplastic and 0.97±0.5 in the neoplastic cases ($p<0.01$). In most non-neoplastic cases the inflammatory infiltrate was lymphoplasmacytic and in the neoplastic cases neutrophils predominated. Necrosis was scored 0-3 and revealed a score of 0.88±0.41

in the non-neoplastic and 1.47±0.5 in the neoplastic cases ($p<0.01$). The average number of mitoses over 10 high power fields per nodule was 1.31±1.55 in the non-neoplastic compared to 42.85±30.79 in the neoplastic cases ($p<0.01$). The average number of multinucleated giant cells over 10 high power fields per nodule was 0.9±1.45 in the non-neoplastic compared to 13.9±14.66 in the neoplastic cases ($p<0.01$). In the non-neoplastic cases, collagen, immature fibroblasts and fibroblast activation (excessively plump euchromatic nuclei with single or multiple prominent nucleoli) were scored 0-3 and a combined score, fibroblasts + activation score - collagen was calculated. The non-neoplastic cases were divided into a combined score of ≤1 (n=15) or >1 (n=27). The 2 groups had similar scores for inflammation and necrosis, but were significantly different ($p<0.01$) in mitotic index (0.26±0.46 vs. 1.89±1.65) and number of multinucleated cells (0 vs. 1.4±1.6). These results indicate 2 stages in the non-neoplastic nodules: early inflammation, characterized by fibrocytes and abundant collagen, and a pre-neoplastic stage, characterized by activated fibroblasts and reduced collagen.

Keywords: Spirocercosis, fibrosarcoma, osteosarcoma, undifferentiated sarcoma, oesophageal nodule

5.2 Introduction

Spirocerca lupi (S. lupi) is a spiurid nematode of carnivores, particularly Canidae, of worldwide distribution but most prevalent in the tropics and subtropics. Spirocercosis in dogs was comprehensively reviewed recently (van der Merwe et al., 2008). Dogs become infested with the worm when they ingest either the coprophagous beetle intermediate hosts or a paratenic host. Following ingestion of the intermediate or paratenic host *S. lupi* L3 larvae are liberated in the gastric lumen. Larvae penetrate the

gastric mucosa and migrate in the wall of the gastric and coeliac arteries to the caudal thoracic aorta. Larvae spend up to three months in small nodules in the aortic wall, where they moult to L4 and finally to adults. Young adult worms then migrate from the aorta to the oesophagus below. Groups of from three to six worms cluster together in the oesopageal submucosa and induce the formation of nodules. Over time, some of the oesophageal nodules may undergo malignant neoplastic transformation, with subsequent metastasis to other sites.

Spirocerca lupi-induced oesophageal nodules are invariably incorrectly referred to as granulomas in the literature (Bailey, 1972; Stephens et al., 1983). Initially the worms are surrounded by highly vascularised loose connective tissue, which contains an exudate consisting of fibrin-rich fluid and neutrophils and foci of necrotic tissue. Bailey (1963) likens it to granulation (repair) tissue. Later, the tissue surrounding the worms is composed mostly of actively proliferating fibroblasts. These fibroblasts often have an embryonal appearance, sometimes resembling those seen in fibrosarcomas (Bailey, 1963; Hu and Hoeppli, 1936). At no stage of the genesis of the nodule is there a predominance of macrophages, which is the hallmark of granulomatous inflammation (Dvir et al., 2008; van der Merwe et al., 2008). The temporal histological progression of the oesophageal lesion, from early nodule to malignant neoplasm has not been reported.

The association between spirocercosis and oesophageal sarcoma was first described in 1955 (Seibold et al., 1955). This association was based on the finding of *S. lupi* worms in oesophageal nodules close to the sarcoma or the pathognomonic findings of spondylitis or aortic aneurysms in conjunction with the oesophageal tumour. The spirocercosis-associated sarcoma has been described in detail (Bailey, 1963, 1972;

Seibold et al., 1955; van der Merwe et al., 2008). Histologically the sarcoma has been classified as fibrosarcoma, osteosarcoma or anaplastic sarcoma (Ranen et al., 2007; Ranen et al., 2004). Interestingly, foci of chondroid and/or osseous metaplasia have also been observed within neoplastic and non-neoplastic nodules (Dvir et al., 2008; Ribelin and Bailey, 1958). The histological characteristics of the *S. lupi*-induced fibrosarcoma include: short or long interwoven bundles of pleomorphic cells that may vary from highly undifferentiated (anaplastic), roughly spindle-shaped cells with round or ovoid nuclei, to interlacing bundles of elongated cells resembling immature connective tissue; variable amounts of intercellular collagenous matrix, and a high mitotic index (Seibold et al., 1955). The histological characteristics of the *S. lupi*-induced osteosarcoma include: foci of closely-packed, short, spindle- or polygonal/triangular-shaped osteoblasts (with plump, ovoid nuclei) oriented to point in various directions and ostensibly filling the intertrabecular spaces; variable numbers of multinucleated cells (both osteoclasts and tumour giant cells can be identified), and variable quantities of osteoid matrix and/or woven bone, with or without foci of chondroid differentiation. In the better-differentiated osteosarcomas, conspicuous osteoid as well as spicules or trabeculae of mature mineralized bone are identified amidst solid foci of neoplastic osteoblasts (Bailey, 1963). Anaplastic sarcomas are characterized histologically by the presence of obviously neoplastic, plump, roughly spindle-shaped cells, usually in an interwoven or interlacing pattern, without the presence of clearly identifiable intercellular matrix, and numerous mitoses. 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 neoplasia in dogs.

Studying the temporal morphological changes in a lesion may help to understand the pathogenesis of the lesion as well as possibly establishing cause-and-effect relationships and stage specific prognostication and treatment. In *Helicobacter pylori*-induced gastric carcinoma in humans, another malignancy that is known to be caused by an infectious organism, the earliest defined change involves lymphocytic inflammation (gastritis). This progresses to mucosal atrophy, characterized by the introduction of fibrous stroma in the stomach mucosa. The latter change is thought to be responsible for the subsequent metaplasia, dysplasia and finally neoplastic transformation of the gastric mucosal epithelium (Correa and Houghton, 2007).

Feline vaccine-associated sarcoma is another disease condition that has some similarities to spirocercosis-induced sarcoma. The condition has been described in a large number of cats that received a killed aluminium-adjuvanted feline leukemia virus or rabies vaccination (McEntee and Page, 2001). The vaccine-induced nodule shares certain histological features with *S. lupi* nodules, including the presence of a necrotic core and peripheral inflammatory reaction. It has been assumed that vaccine-associated sarcomas arise from the overzealous inflammatory reaction within the nodule (McEntee and Page, 2001). These fibrosarcomas have been well-described staged and graded (Couto et al., 2002). Grading has also been done on a large number of cases of canine osteosarcoma for prognostication purposes (Kirpensteijn et al., 2002), as well as on oesophageal sarcomas (all of them probably *Spirocerca*-induced) (Ranen et al., 2007).

This study aims to outline the histological progression of the spirocercosis-associated oesophageal nodule from the early stage of infection to fibroblastic nodule and, finally, to sarcoma. Thus, the objective of the present study was to clarify the nature

of the inflammatory response within the *Spirocerca*-induced oesophageal nodule and to describe any morphological changes in the fibrocyte/fibroblast cell population and collagen stroma throughout the progression of the nodule. Another objective of the current study was to examine any potential similarities to other infections that progress to cancer such as *Helicobacter pylori.* We also aimed to apply the widely-accepted grading criteria for canine osteosarcoma to spirocercosis-induced neoplastic nodules and to find prognostic indicators.

5.3 Materials and Methods

Sixty two paraffin blocks containing *Spirocerca*-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. A Martius, Scarlet and Blue (MSB) stain was applied to a duplicate section in order to detect fibrin within the nodules (Jones, 2002). 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 malignancy was selected for evaluation. If a nodule was sectioned more than once, the section with the most advanced fibroplasia was selected.

The following parameters were recorded as present or not per slide examined: worms, worm eggs, worm tract(s) (with or without debris) and metaplasia (osseous, chondroid or myxomatous). The inflammatory infiltrate within or at the periphery of the nodule

was evaluated and characterized as to its intensity. Ten non-overlapping low power fields (x100) were examined and the inflammation was scored 0-3 (0 = scant or absent; 1 = inflammatory cells obviously present but markedly less than other cells; 2 = inflammatory cells roughly equal to fibrocytes; 3 = predominantly inflammatory cells). Inflammatory cells included lymphocytes/plasma cells, macrophages, eosinophils and neutrophils. The predominant inflammatory cell type was recorded per case. It was also recorded whether macrophages were present. It was noted separately if neutrophils were specifically associated with the worm, worm tract and debris or to the peripherally ulcerated rim of neoplastic nodules.

Ten non-overlapping high power fields (x400) were examined and scored (0-3) for necrosis, (0 = no necrosis; 1= small foci of necrosis or widespread single cell necrosis that required careful perusal of the section; 2 = obvious presence of necrosis, but in <50% of the field; 3 = necrosis in >50% of the field). The same scheme was used to score haemorrhage on 3 non-overlapping x40 fields. The quantity of collagen was scored 0-3 on 3 non-overlapping x40 fields (0 = no or normal amount of collagen; 1 = obvious collagen present but occupying less area than cells; 2 = roughly equal amount of collagen and cells; 3 = obviously more collagen than cells).

For the non-neoplastic nodules, 10 non-overlapping x400 fields were scored (0-3) pertaining to the quantity of fibroblasts within the nodule (0 = no or scant fibroblasts; 1= fibroblasts obviously present but definitely less than fibrocytes; 2 = roughly equal to fibrocytes; 3 = fibroblasts definitely more than fibrocytes). If the fibroblasts looked activated (characterized by excessively plump euchromatic nuclei, often with prominent single or multiple magenta nucleoli) the degree of activated fibroblasts was scored using the same scheme.

Neoplasms were identified based on poor differentiation and anaplastic changes including pleomorphism, nuclear hyperchromasia, multiple nucleoli and prominent nucleoli and or extensive mitoses. The cases were initially selected based on the archived histopathology reports from 6 trained veterinary pathologists in the Pathology Section, Department of Paraclinical Sciences, Faculty of Veterinary Science, Onderstepoort. Two experienced pathologists (SJC and MCW) then selected appropriate cases (using the light microscope) with sufficient tissues and minimal autolysis. The selected tissue sections were then further evaluated under the light microscope by 2 researchers (ED and SJC) and the final decision regarding scoring of various histological criteria was made by SJC, based on her experiential knowledge. Notably, in all ambiguous cases, MCW was again consulted and a consensus was reached. The neoplasms were firstly defined phenotypicaly as fibrosarcoma, osteosarcoma or undifferentiated sarcoma based on the criteria outlined in the introduction. Neoplasms were scored according to their degree of differentiation. Mitotic index was determined by counting the number of mitoses per 10 non-overlapping high power fields (x400). Mitotic index was further scored 0-3 as follows: 1 = 1–9 mitotic figures per ten 400x fields; 2 = 10–19 mitoses per ten 400x fields; 3 = 20 or more mitoses per ten 400x fields. Tumour matrix was scored (0-3) according to the ratio between matrix and neoplastic cells: relatively less matrix than cells (3), equal (2) or more (1). Nuclear pleomorphism (NP) was examined in 10 non-overlapping x400 fields and scored 0–4 (0 = all nuclei identical; 1 = < 25% NP; 2 = 25–50% NP; 3 = 50–75% NP; 4 = > 75% NP). When present, multinucleated giant cells (MNGC) were counted per 10 non-overlapping high power fields (x400). The MNGC were scored on a scale of 0 to 3 (0 = no MNGC; 1 = 1-20 MNGC; 2 = 21-40; 3 = >41 MNGC). The density of neoplastic cells within tumour nodules was

evaluated on a scale of 1 to 4 (1 = less than 25% neoplastic cells; 2 = 25–50% neoplastic cells; 3 = 50–75% neoplastic cells; 4 = more than 75% neoplastic cells).

Each section was also graded according to a scheme adapted, with minor alterations, from a previous publication (Couto et al., 2002), combining the necrosis score, degree of neoplastic differentiation and the mitotic score. Final scores of 3 or 4 were designated grade I; scores of 5 or 6 were designated grade II; scores of 7, 8, or 9 were designated grade III.

5.3.1 Data Analysis

For each score assigned, the average score per case was calculated from the different field's scores. The average scores for non-neoplastic and neoplastic cases were calculated and compared by student t-test. For parameters that were only evaluated as present or absent, the prevalence in each group (non-neoplastic or neoplastic) was calculated and compared by chi-square analysis.

5.3.2 Further Analysis and Grading

In the non-neoplastic cases, a combined score of fibroblasts plus activation score minus collagen was calculated. The non-neoplastic cases were divided into cases with a combined score of ≤1 (n=15) or >1 (n=27), respectively, and compared for the different parameters.

5.4 Results

Of the 62 paraffin blocks that were evaluated, 42 were classified as non-neoplastic oesophageal nodules and 20 were classified as malignant neoplasms. In the non-

neoplastic group, evidence of worm migration was observed in almost all cases; a worm or worms were present in 69% of cases, eggs in 71% of cases and a migratory tract or tracts in 95% of cases. In the neoplastic cases, a worm or worms were present in 15% of cases, eggs in 25% of cases and a migratory tract or tracts in 55% of cases (significantly lower than was observed in the non-neoplastic nodules, $p<0.01$). In the non-neoplastic cases, inflammation was very prominent and scored 1.91±0.52, indicating that roughly half of the cell population in non-neoplastic nodules comprised inflammatory cells, compared to 0.97±0.5 in the neoplastic cases ($p<0.01$). In 40% of non-neoplastic cases the inflammatory infiltrate was predominantly lymphoplasmacytic in nature (Fig. 1), in 24% of cases, lymphocytes and neutrophils were mixed, and in 21% of cases, neutrophils predominated, compared to 25%, 5% and 70%, respectively in the neoplastic cases ($p=0.02$). Neutrophils were often distributed diffusely in a nodule, but in a few cases purulent foci were observed immediately adjacent to worm tract(s) and their associated tissue debris (Fig. 2, 3 and 4) or subadjacent to the ulcerated rim of the nodule. Macrophages were usually engorged with haemosiderin and in only two cases were macrophages organized in multifocal granulomata within a nodule. Necrosis scored 0.88±0.41 in the non-neoplastic cases and 1.47±0.5 in the neoplastic cases ($p<0.01$). Haemorrhage was relatively rare within nodules and there was no difference in the haemorrhage score between the neoplastic and non-neoplastic groups (0.68±0.71 vs.0.81±0.68). Fibrin was present in 70% of the neoplastic cases, predominantly in association with ulcerated areas, and significantly more ($p<0.01$) than in the non-neoplastic cases (29%).

The average mitotic index per nodule was 1.31±1.55 in the non-neoplastic compared to 42.85±30.79 in the neoplastic case group ($p<0.01$). The average number of

multinucleated giant cells per nodule was 0.9±1.45 in the non-neoplastic compared to 13.9±14.66 in the neoplastic group ($p<0.01$).

Fibroplasia was very prominent in the non-neoplastic cases. In this group, the fibroblast score was 2.16±0.7, the fibroblast activation score was 0.82±0.55 and collagen scored 1.91±0.7. Two typical patterns were observed within the non-neoplastic nodules regarding fibrocytes/fibroblasts and the preponderance of collagen: one group was characterized by the presence of occasional inactive fibrocytes and a relatively large amount of collagen and the other group exhibited significant fibroblastic activity and a relatively small amount of collagen. The fibroblast score plus the fibroblast activation score minus the collagen score was calculated per non-neoplastic nodule. The non-neoplastic cases were subsequently divided into cases with a combined score of ≤1 (-0.72±1.18, n=15) or >1 (2.04±0.52, n=27), respectively. These two non-neoplastic groups were significantly different ($p<0.01$) in all 3 parameters: collagen (2.66±0.52 vs. 1.51±0.36), fibroblasts (1.48±0.67 vs. 2.52±0.35) and fibroblast activity scores (0.45±0.6 vs. 1.02±0.41), with minimal overlap. However, these two groups had similar scores for inflammation, necrosis, haemorrhage and fibrin, but they were significantly different ($p<0.01$) with respect to mitotic index (0.26±0.46 vs. 1.89±1.65) and number of multinucleated giant cells (0 vs. 1.4±1.6). The group with fewer active fibroblasts and relatively more collagen was classified as the “early nodule” / stage 1 (Fig. 5) and the group with many more active fibroblasts, more marked cellular atypia, and more numerous mitoses was classified as the “pre-neoplastic nodule” / stage 2 (Fig. 6) (Table 1). In the pre-neoplastic group osseous metaplasia was present in three cases. This lesion was not observed in the early nodule, but was also observed in two osteosarcoma cases, in foci well-separated from the neoplastic osteoid matrix and spicules of new bone. In one of the 15 early

nodules, myxomatous metaplasia was seen. In the neoplastic cases 4/17 osteosarcoma cases showed chondroid/chondrous differentiation.

Of the 20 tumours, 17 were osteosarcoma (Fig. 7), and 3 were classified as fibrosarcoma (Fig. 8). No anaplastic sarcomas were identified.

The neoplastic nodules exhibited a high mitotic index score (2.6±1.01) and cell density (3.45±0.83), moderate nuclear pleomorphism (1.64±1.09), extracellular matrix (1.65±0.82) and necrosis (1.46±0.5) and relatively few tumour giant cells (i.e. osteoclasts were not counted) (scored 1.1±0.79) (Table 2). Eighteen cases (90%) showed 13.9±14.66 multinucleated cells (1-58) per 10 high power (400x) fields. This is also reflected by the prevalence of the individual grades within the neoplastic group (Table 2). Using a combined grading system, 11 cases were graded 2 (55%) and 9 were graded 3 (45%).

5.5 Discussion

The present study describes and analyses the progression of canine spirocercosis, from inflammatory oesophageal nodule, to pre-neoplastic fibroblastic nodule and, finally, to sarcoma. This concept has largely been adapted from a similar scheme which was used to map the progression of *Helicobacter*-induced gastritis to gastric adenocarcinoma in humans. In the latter work, such a classification system created the basis for studies on pathogenesis, prognosis and stage-specific therapy (Correa and Houghton, 2007). We propose that non-neoplastic *S. lupi* nodules can be divided into 2 stages: An early inflammatory stage, where the nodule is characterized histologically by lympho-plasmacytic inflammation, fibrocytes and abundant collagen, and a pre-neoplastic stage, where the nodule is characterized by the presence

of activated fibroblasts and reduced collagen, as well as lympho-plasmacytic inflammation. The latter group is regarded as preneoplastic (and therefore more mature than the first group) because: mitoses were more numerous, and the greater proportion of fibroblasts showed some degree of atypia (including multinucleated cells that were often polygonal in shape, and, in some cases, even plump and fusiform with a fairly linear arrangement of plump ovoid central or paracentral overlapping nuclei; these cells were usually intimately associated with plump fibroblasts and intervening wavy collagen fibres). The combined score of fibroblasts plus fibroblast activation score minus collagen indeed resulted in two such distinctive groups. However, within each of the 3 parameters there was minor overlap between the 2 groups, meaning that there are some early cases with reduced collagen and/or increased numbers of immature activated fibroblasts and vice versa. These cases no doubt represent the intermediate phases, indicating that the proposed stages are just a simplified reflection of a continuous process with characteristic temporal and spatial features, rather than a strictly stage-wise process. A larger study would probably have revealed more intermediate phases between the first 2 stages and the final neoplastic stage. Inflammation in both stages of the non-neoplastic nodule was characterized by a predominance of lymphocytes and plasma cells. However, in the earliest stage of the non-neoplastic nodule, there were 3 cases in which inflammation was characterized by a predominance of eosinophils. These particular cases may represent still earlier infection, or they may be representative of nodules containing dead parasites. If such a stage exists, it is particularly difficult to detect as the nodules are likely to be very small and the disease at this point is subclinical.

In *Helicobacter*-induced adenocarcinoma the following stages have been described: Inflammation, dysplasia, metaplasia and, finally, neoplasia. Similar to the

Helicobacter cases, the current study indicates that *Spirocerca*-induced oesophageal nodules are characterized by an early inflammatory stage followed by fibroplasia, increasing fibroblastic activity with cellular atypia, occasional focal metaplasia and, finally, neoplasia. However, contrary to the *Helicobacter* cases, in the present study, the entire oesophageal lesion was never predominantly metaplastic. Three cases in the pre-neoplastic group had foci of osseous metaplasia and might represent a metaplastic phase somewhere between non-neoplastic and obviously neoplastic cases. Alternatively, the metaplastic change may be an incidental change not related to the progression of the lesion to neoplasia.

Helicobacter pylori is by no means the only pathogen considered to be carginogenic in humans. Several other organisms have been implicated as causing neoplasia in humans by virtue of the chronic inflammatory reaction associated with them. These include Epstein-Barr virus, human papillomaviruses, hepatitis B and hepatitis C viruses, human immunodeficiency virus type 1, *Clonorchis sinensis*, *Opisthorchis viverrini* and *Schistosoma hematobium* (Herrera et al., 2005; Schottenfeld and Beebe-Dimmer, 2006). All of these infectious agents and macroparasites are known to induce chronic inflammation, followed, sometimes, by neoplastic change (Moss and Blaser, 2005). However, in comparison to the abovementioned pathogens, neoplastic transformation appears to be far more prevalent (about 20%) in spirocercosis (Dvir et al., 2001). More importantly, where oesophageal sarcoma (excluding leiomyosarcoma) is almost invariably associated with *S. lupi* in the dog, in other oncogenic pathogen-associated neoplasia, most cases cannot be associated with the pathogen and the pathogenesis remains unknown (Herrera et al., 2005). No doubt, spirocercosis-associated sarcoma provides a very promising model system for the

study of pathogen-induced neoplasia. As such, it certainly warrants further in-depth investigation.

The severity of the inflammatory infiltrate, especially in the early oesophageal nodule, has not been described before. The finding of pockets of neutrophils within nodules has been described, but not the high prevalence and severity of the lympho-plasmacytic infiltrate. Interestingly, in a previous study, 8% of spirocercosis cases showed lymphocytosis in their complete blood count (Mylonakis et al., 2006). Lympho-plasmacytic inflammation has been described in association with *Helicobacter*-induced gastritis, where T helper lymphocyte type 1 (Th1) cells and their associated cytokines are thought to play a significant role in carcinogenesis (Wilson and Crabtree, 2007). Lymphoplasmacytic inflammation has also been described in vaccine-associated sarcomas in cats (Couto et al., 2002). Another hypothesis linking lymphocytes and tumour progression is based on the finding of T lymphocytes responsible for suppression of the anti-tumour immune response, namely T-regulatory cells (Beyer and Schultze, 2006). This reaction has been described in a murine model of fibrosarcomas (Beyer and Schultze, 2006), and it is generally characterized by large numbers of lymphocytes in or around the tumours. The role of the lymphocytic infiltrate in the progression of the spirocercal nodule and subsequent neoplastic transformation may warrant further investigation. The first step would be to characterize the lymphocytes involved in the spirocercosis-associated immune response.

Purulent inflammation seems to be particularly associated with ulceration and necrosis in the neoplastic cases, and with the worms, worm tracts with debris, and worm eggs in the early cases. In the neoplastic cases, fibrin was also more abundant.

However, both fibrin and neutrophils are considered to be indicators of acute inflammation, and in spirocercosis-associated nodules they should be considered predominantly in association with foci of necrosis and acute ulceration.

A competing, but not mutually exclusive, hypothesis for the progression of the spirocercal nodule to malignant neoplasia is that the parasite itself, rather than the inflammatory response it produces, is directly responsible for tumourogenesis. This could conceivably be mediated by the synthesis and release of chemical mediators or analogues of host cell-cycle factors such as fibroblast growth factor by the intra-nodular worms. This hypothesis has not, so far, been tested but we hope to remedy this in the near future.

The spirocercosis-induced sarcoma is an aggressive tumour, much more aggressive than feline vaccine-associated sarcomas (Couto et al., 2002), and even more aggressive than non-spirocercosis-associated canine osteosarcoma (Kirpensteijn et al., 2002). A comparison of the prevalence of each graded variable (Table 2) between spirocercosis-associated canine oesophageal osteosarcoma and previously reported non-spirocercosis-associated canine osteosarcoma (Kirpensteijn et al., 2002), revealed that large amounts of necrosis and tumour matrix, as well as increased tumour cell density and increased numbers of MNGC (specifically tumour giant cells), are more commonly associated with spirocercosis-associated sarcoma. However, nuclear pleomorphism seemed to be less pronounced in spirocercosis-associated osteosarcoma in the present study, compared to previous reports of non-spirocercosis-associated canine osteosarcoma. Tumours of different grades within the types of spirocercosis-associated sarcomas may also represent different stages in cancer progression. Further investigation of a greater number and range of spirocercosis-induced oesophageal

nodules from barely-discernable early nodules through to end-stage sarcoma may improve our understanding of the progression and pathogenesis of the oesophageal lesion. It is hoped that the current study has laid the foundations for such an endeavour.

5.6 Tables

Table 1

Histological parameters in the early and preneoplastic non-neoplastic groups

	Early nodule (n=15)	Preneoplastic nodule (n=27)	P value
Collagen (score 0-3)	2.66±0.52	1.51±0.36	<0.01
Fibroblast (score 0-3)	1.48±0.67	2.52±0.35	<0.01
Activated fibroblast (score 0-3)	0.45±0.6	1.02±0.41	<0.01
Combined score: fibroblast + fibroblast activation - collagen	-0.72±1.18	2.04±0.52	<0.01
Mitotic index score (average number of 10 field)	0.26±0.46	1.89±1.65	<0.01
Multinucleated giant cells (average number of 10 fields)	0±0	1.4±1.6	<0.01
Inflammation score	1.92±1.12	1.89±0.75	0.44
Necrosis score	1.07±0.73	1.14±0.82	0.73

Table 2

Frequencies of histological scores for neoplastic variables in the 20 cases with spirocercosis-induced sarcoma

Variable	0	1	2	3	4
Mitotic index score		10%	20%	70%	
Nuclear Pleomorphism	15%	30%	35%	10%	10%
Necrosis		45%	50%	5%	
Matrix		15%	40%	45%	
Cell density			20%	15%	65%
Multinucleated cells	20%	55%	20%	5%	

5.7 Figures

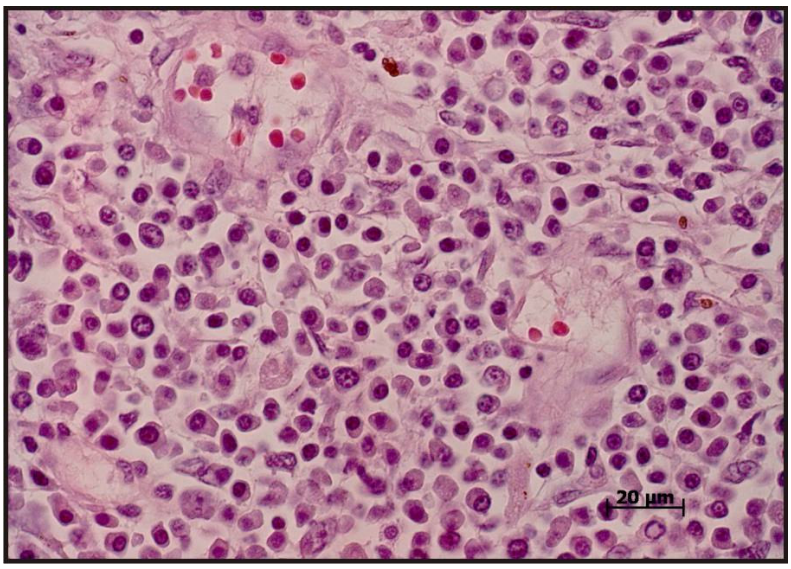

Figure 1: Florid lymphoplasmacytic cell infiltrate within a non-neoplastic oesophageal nodule (pre-neoplastic / stage 2), H&E.

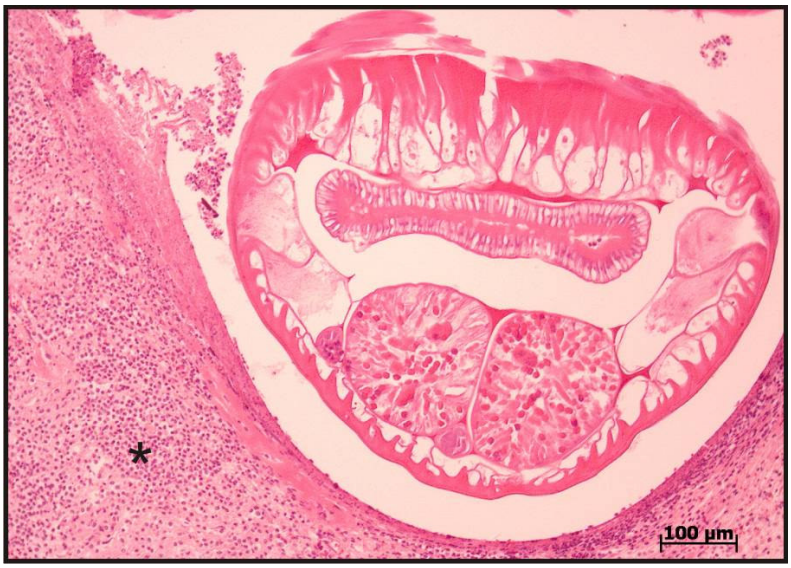

Figure 2: *Spirocerca lupi* larva surrounded by a rim of necrotic cell debris and degenerate neutrophils (asterisk) within a non-neoplastic oesophageal nodule (pre-neoplastic / stage 2). H&E.

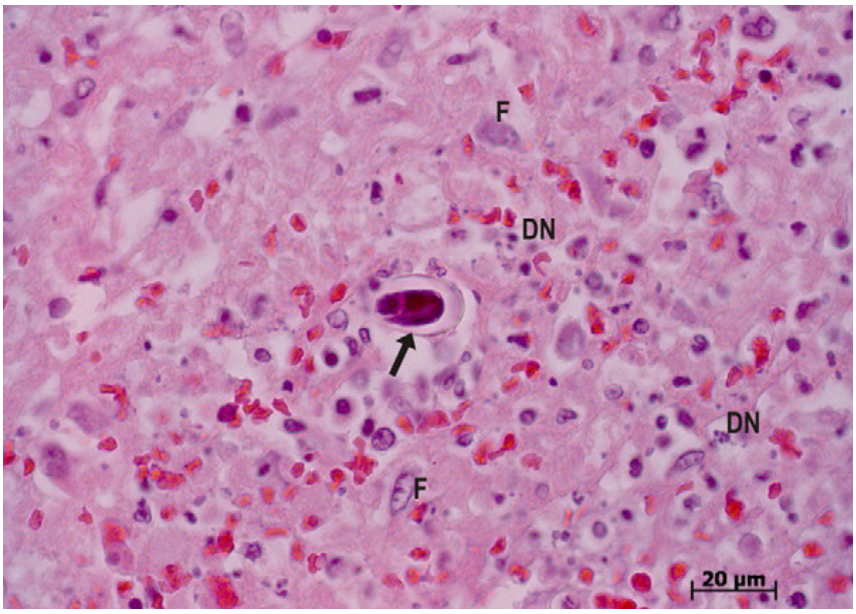

Figure 3: *Spirocerca lupi* egg (arrow) surrounded by degenerate neutrophils (DN), occasional fibroblasts (F) and haemorrhage within a non-neoplastic oesophageal nodule (pre-neoplastic / stage 2). H&E.

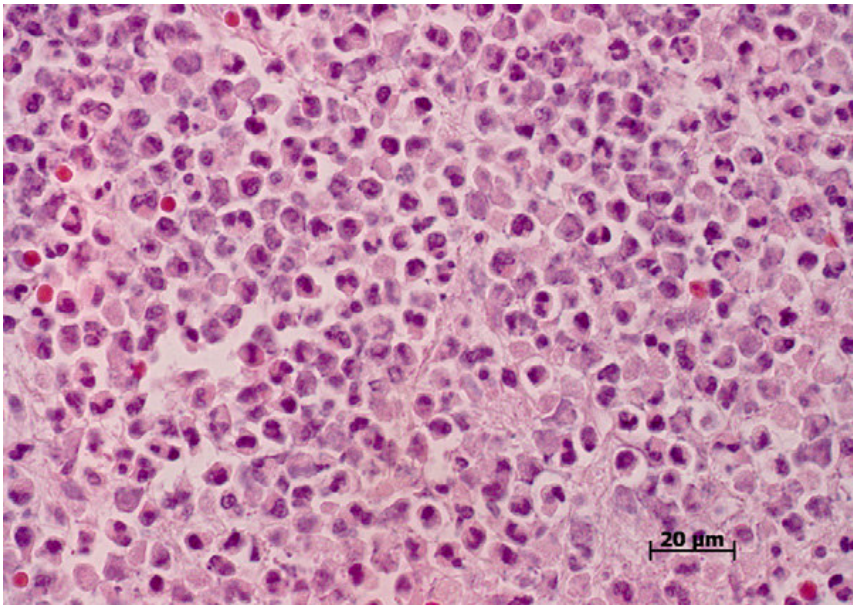

Figure 4: Purulent exudate (associated with *Spirocerca lupi* worm and tract) within a non-neoplastic oesophageal nodule (pre-neoplastic / stage 2). H&E.

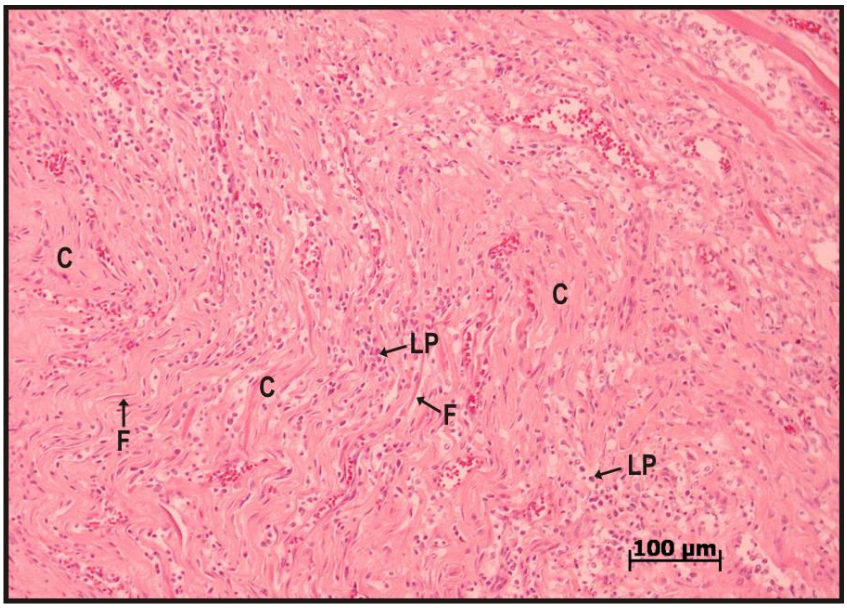

Figure 5: Collagen (C), fibrocytes (F) and intervening lymphoplasmacytic cell infiltrate (LP) within a non-neoplastic oesophageal nodule (early / stage 1). H&E.

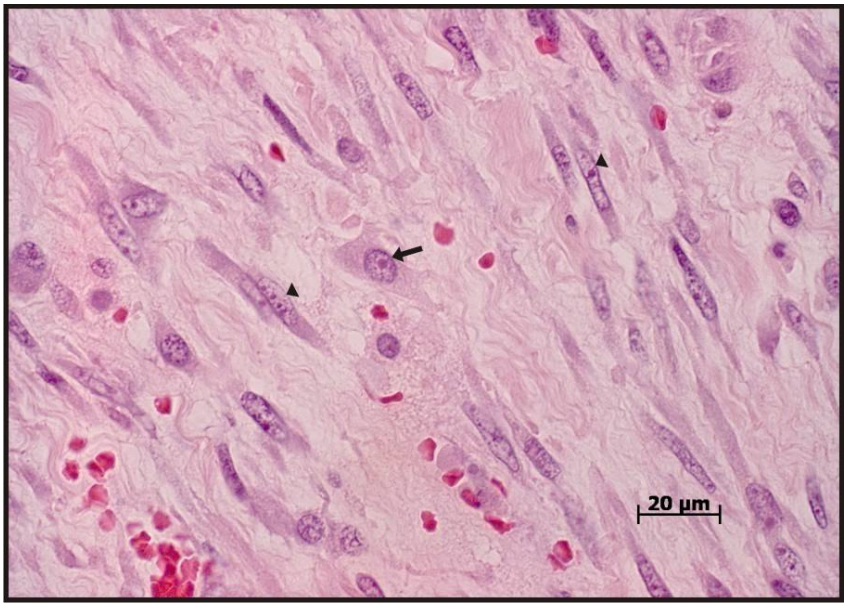

Figure 6: Fibroplasia within a non-neoplastic oesophageal nodule (pre-neoplastic / stage 2). Plump, spindle-shaped (arrowhead) to polygonal (arrow) fibroblasts with a large nucleus to cytoplasmic ratio, basophilic cytoplasm and prominent magenta nucleoli. H&E.

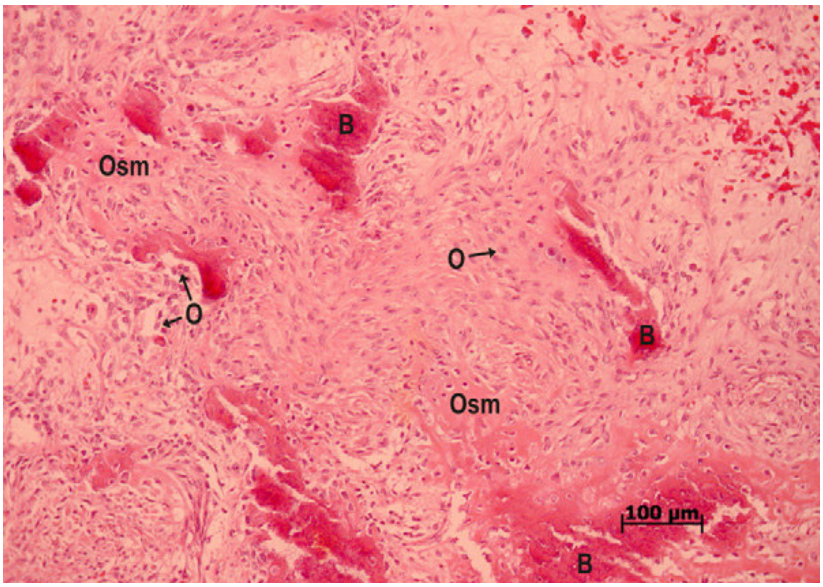

Figure 7: Well-differentiated oesophageal osteosarcoma . Neoplastic pyriform osteoblasts (O) in association with osteoid matrix (Osm) and fragments of mineralized bone (B). H&E.

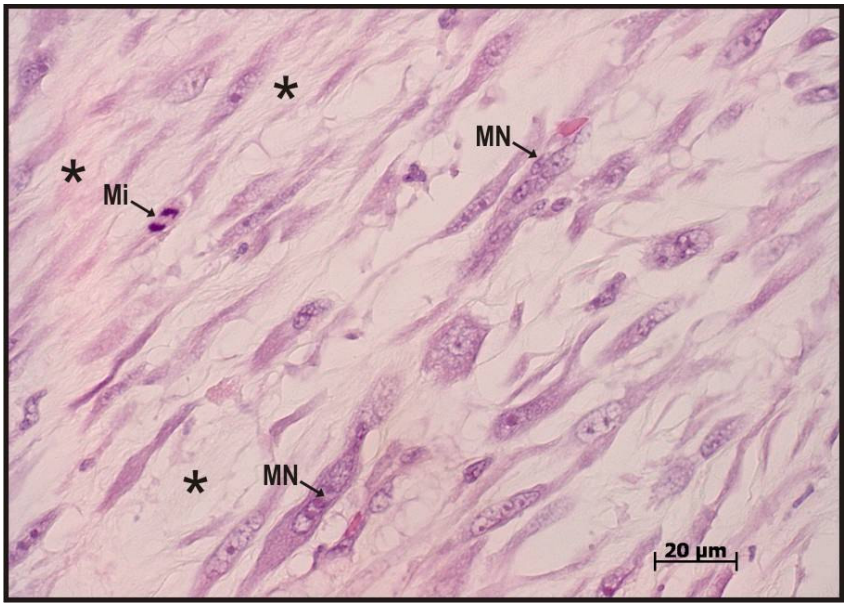

Figure 8: Poorly-differentiated oesophageal fibrosarcoma. Neoplastic spindle-shaped cells showing nuclear atypia, multinucleated cells (MN) and mitoses (Mi), amidst intervening fibrillar collagenous matrix (asterisk). H&E.