Flat-coated retriever dog tumour survey 25 years on

Author : Jane Dobson, Tess Hoather, Fernando Constantino-Casas

Categories : Companion animal, Vets

Date : July 11, 2016

Shortly after Jane Dobson came to Cambridge as a lecturer in veterinary oncology, the authors were approached by the late Sheila Godbolt and Martin Roe, who were concerned a large number of flat-coated retrievers were dying from cancer.

Initial attempts to determine the type(s) of tumour and number of dogs affected were met with anecdotal reports of dogs with “liver cancer” or “stomach cancer”, and it soon became apparent a histological approach would be necessary to define the problem, if one existed.

Tumour Survey

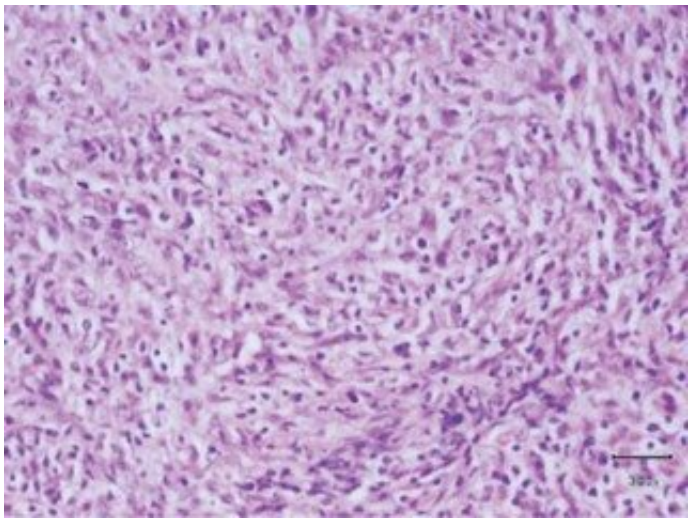

Figure 1. Haematoxylin and eosin (H and E) section of poorly differentiated histiocytic sarcoma. Neoplastic cells are pleomorphic admixed with large numbers of lymphocytes (H and E).

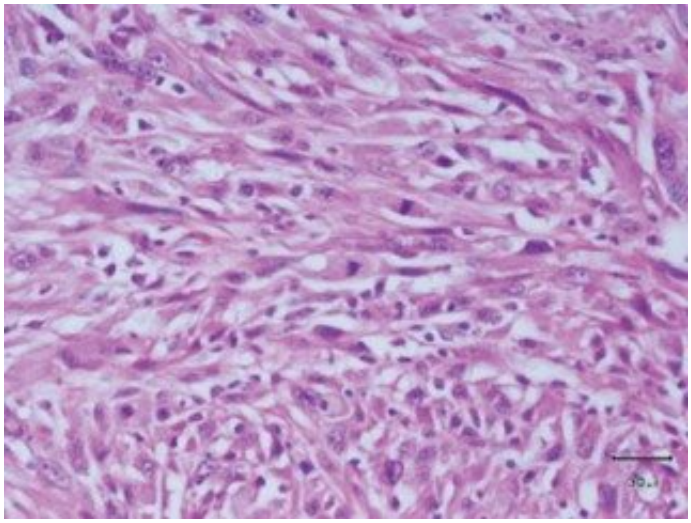

Figure 2. H and E section of spindle cell variant – histiocytic sarcoma, spindle cell subtype. Neoplastic cells are spindle to round with moderate amount of cytoplasm. Mitotic figures are present (H and E).

In March 1990, the Tumour Survey started. Vets in general practice from across the UK were invited to submit tissues suspected of being neoplastic, collected from flat-coated retrievers – either by biopsy or excision, or at postmortem examination – to the University of Cambridge Department of Veterinary Medicine.

Specimens were submitted in formalin and processed routinely to obtain haematoxylin and eosin (H and E) stained sections, which were examined and reported by colleagues in pathology. The service was free to submitting vets and owners, with the basic costs of processing covered by owners and breeders of flat-coated retrievers, who raised funds to support this work.

As word spread through the breed about the Tumour Survey, the number of submissions rose year on year and, by 1998, more than 1,000 samples were received from 782 dogs. A total of 165 samples (16%) were not neoplastic at all (cysts, skin tags and so on), while the remainder were evenly split between benign (447 lesions; 44%) and malignant (411 lesions; 40%).

Perhaps, not surprisingly, canine cutaneous histiocytoma (CCH) was the most common of the benign submissions, accounting for 48% of benign tumours and 25% of all tumour submissions. However, the striking finding, which we reported in 2000 (Morris et al), was soft tissue sarcomas accounted for more than 55% of the malignant tumours and 25% of all tumour submissions.

Many of the sarcomas were notable for being very poorly differentiated and unclassifiable on

standard H and E sections, being variably described as poorly differentiated (actual number of tumours; n = 73), spindle cell (n = 37), round cell (n = 20), histiocytic (n = 8) or giant cell (n = 3; **Figures 1 and 2**).

Although the predominant cell type varied between tumours, various cell types were often observed within different areas of the same tumour; many tumours had an inflammatory infiltrate, later shown to consist predominantly of T lymphocytes.

Mitotic activity was common, with between three mitoses to five mitoses per high power field. The majority of these tumours had arisen deep in the soft tissue/fascia of the limbs (49 forelimb, 34 hindlimb) – particularly affecting the upper forelimb between the elbow and shoulder (**Figure 3**). Some visceral sites were also recorded. The mean age of dogs affected by these undifferentiated sarcomas was 8 years (range 0 to 13) – 78 were female and 60 male.

Over the following years, we continued to accrue samples from flat-coated retrievers through the Tumour Survey, averaging at times up to 150 to 180 samples per year. The pattern of submissions, with regards to tumour type, has not changed substantially from that reported in 2000 and, through this and other publications, the tendency for flat-coated retrievers to develop this particularly aggressive form of sarcoma has become well established.

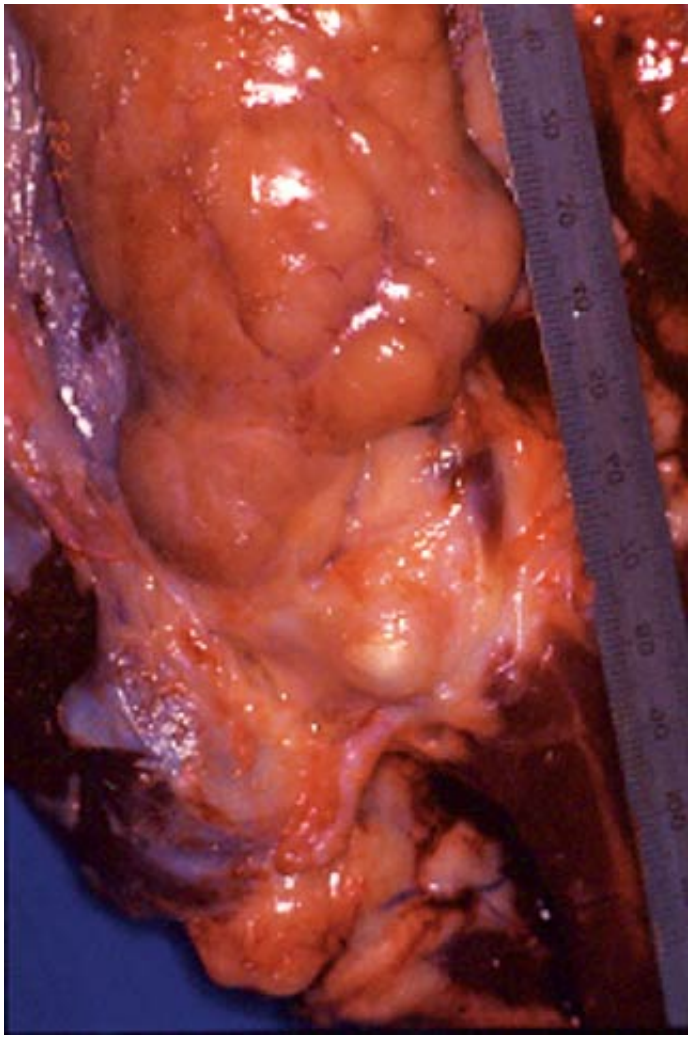

Figure 3. Gross appearance of histiocytic sarcoma within the biceps musculature.

The Tumour Survey has provided an archive of tumours from flat-coated retrievers, which has proved a valuable resource for further research directed at determining the nature of these sarcomas.

In 2002, we described the immunological and histopathologic features of 14 such tumours (Morris et al, 2002). The majority of tumours showed 100% positivity for vimentin, greater than 70% positive staining for major histocompatibility complex (MHC) class II with variable, but less than 50% staining for actin and desmin.

We concluded these undifferentiated sarcomas belonged to a spectrum of tumours with varying proportions of characteristic cell types and morphological features – some of which fit the diagnostic criteria for malignant fibrous histiocytoma (a malignant pleocellular neoplasm, presumably arising from primitive mesenchymal cells, showing evidence of a fibroblastic/myofibroblastic phenotype; Hendrick et al, 1998).

Many of the tumours seemed to have a significant myofibroblast component and a moderate T cell infiltrate, but the precise lineage remained uncertain.

Since this work was published in 2002, more antibody reagents have become available to characterise cells – particularly those of the myeloid lineage, as a result of which terminology has changed. The term histiocytic sarcoma (HS) has now been adopted to encompass two ends of a spectrum of malignant tumours previously referred to as malignant fibrous histiocytoma (MFH) and malignant histiocytosis (MH). The term localised HS has been proposed to describe solitary lesions and disseminated HS, the multifocal form – previously MH. The latter is highly breed-specific – especially in the Bernese mountain dog, where it has been reported with a frequency of 25%.

MH is also prevalent in Rottweilers and other retrievers. In contrast to the multifocal, disseminated form of HS reported in these breeds, most forms of HS or HS-like tumours reported in flat-coated retrievers have been solitary tumours arising in the deep musculature or fascia of limbs or in association with joints.

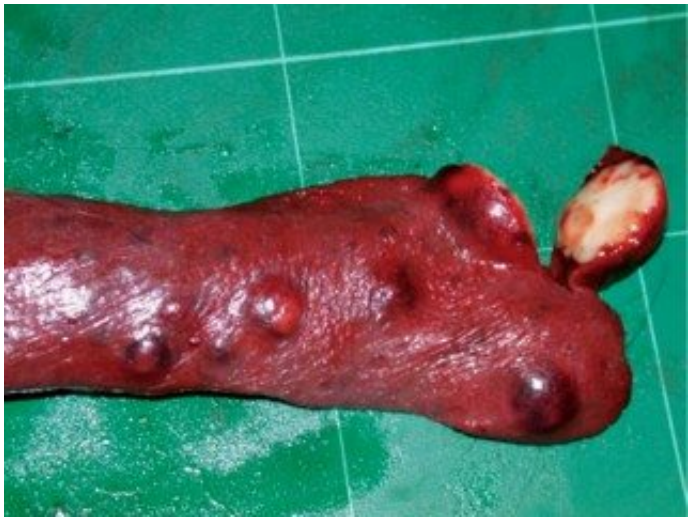

Figure 4. Gross appearance of
histiocytic sarcoma affecting the spleen.

However, we have documented an aggressive form of HS of the spleen in flat-coated retrievers and, in these dogs, the clinical presentation and findings were consistent with a haemophagocytic form of HS described by Moore and colleagues (Dobson et al, 2006).

In 2008, the Tumour Survey provided information on 180 flat-coated retrievers bearing HS-like lesions, which showed although the majority (101 lesions; 57%) were primary limb lesions, 47 dogs (26%) had visceral, mainly splenic lesions with no peripheral primary tumour (**Figure 4**).

HS' have a range of histological appearances, such that, microscopically, the diagnosis of HS can be complex. Histological findings include diffuse proliferation of neoplastic histiocytes,

multinucleated histiocytic giant cells, spindle cells, anaplastic cells and, in some cases, presence of erythrophagocytic cells. Lymphocytic infiltrates in HS have also been reported.

Immunohistochemical staining is an increasingly important technique to accurately identify the cell of origin in poorly differentiated tumours such as HS. Identification of histiocytes can be achieved with molecules involved in antigen presentation, such as MHC class II molecules and the beta-2 integrins CD11d/CD18. On the basis of immunohistochemistry, HS is MHC class II and CD18 positive, and the use of these markers has enabled HS to be differentiated from synovial sarcomas of the joint, and poorly differentiated sarcomas elsewhere in the body (Craig et al, 2002).

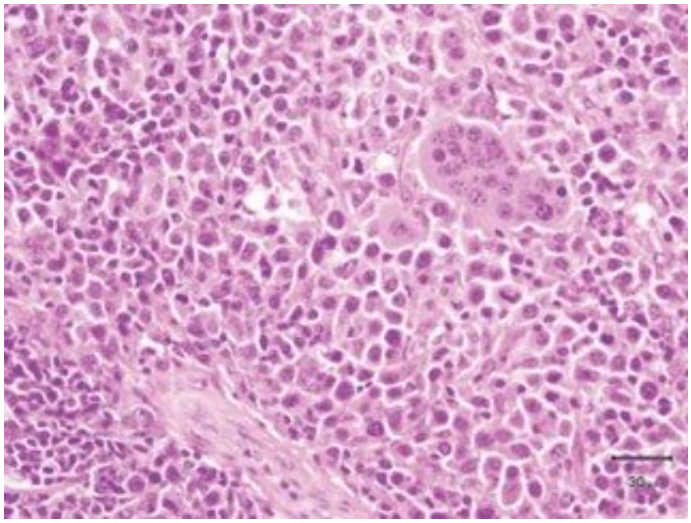

Figure 5. Haematoxylin and eosin (H and E) section of histiocytic subtype. Histiocytic sarcoma, histiocytic subtype with large round neoplastic histocytic cells with some multinucleated giant cells and scattered lymphocytes (H and E).

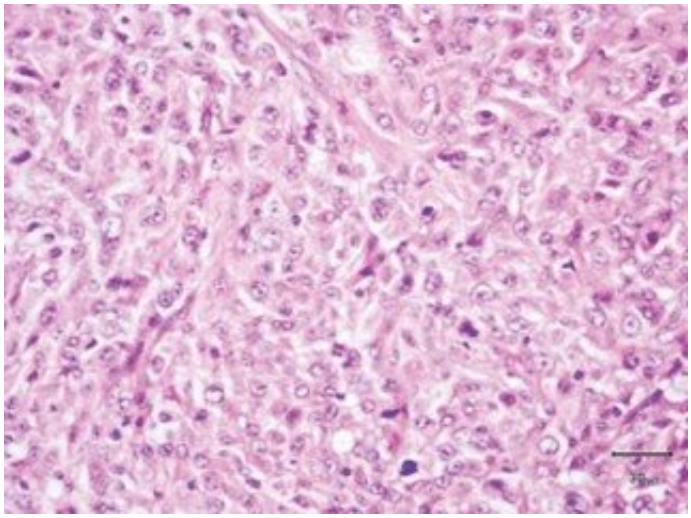

Figure 6. Haematoxylin and eosin (H and E) section of histiocytic-spindle-pleomorphic subtype. Histiocytic sarcoma, histiocytic-spindle-pleomorphic subtype with few fibroblast-like cells admixed with neoplastic histiocytic cells, with a pale eosinophilic cytoplasm (H and E).

Also in 2008, we undertook a detailed histologic and immunohistological review of 40 HS from the survey – 20 limb tumours and 20 splenic tumours – and showed two distinct phenotypic sub-types could be identified: a histiocytic subtype, most prevalent in the splenic tumours, and a histiocytic-spindle-pleomorphic subtype, mainly seen in the limb tumours (**Figures 5 and 6**).

Despite their variable morphology, all tumours expressed MHC class II and the leukocyte antigen CD18, but only those tumours in the spleen consistently expressed CD11d (**Figures 7 and 8**; Constantino-Casas et al, 2011). Since this time, we have turned to molecular techniques using microRNA (miRNA) profiling as a means to gain a better understanding of soft tissue sarcomas and histiocytic sarcomas in particular.

In a pilot study containing 18 HS' from flat-coated retrievers, the expression profile of localised and visceral HS did not lead to them forming two different groups. Instead, at least three different HS groups could be identified – two of them having tumours from both locations.

Since this was a pilot study, only the expression of 20 mature miRNAs were studied and it is possible miRNAs that distinguish the two HS tumour types were not included in the study. A group from the Netherlands has shown variation in gene expression between localised and visceral HS (Boerkamp et al, 2014).

Biological behaviour, management and prognosis

Sadly, the prognosis for dogs with histiocytic sarcoma is poor. Those dogs presenting with the disseminated form of the disease are often very sick at the time of diagnosis due to metabolic or haematological complications. The bone marrow is often affected and some forms of the disease are associated with haemophagocytosis (Dobson et al, 2006).

Although localised histiocytic sarcomas may be managed initially by surgical excision, the site and extent of the lesion often precludes surgery short of amputation. Primary tumours can be sensitive to radiation, which offers an alternative to amputation for pain relief (Dobson, 2007). However, in the flat-coated retriever, a high (70% to 90%) rate of distant metastasis exists – particularly to the viscera, including liver, spleen and kidneys (**Figure 9**).

The authors have also documented unusual patterns of metastasis with diffuse infiltration of the leptomeninges, leading to an acute neurological deterioration (Marcinowska et al, 2014a). Some indication exists that the anticancer drug lomustine (CCNU) may play an adjuvant role in the management of localised HS. In a small study of 16 dogs treated with aggressive local therapy and adjuvant lomustine chemotherapy, median survival for all dogs of 568 days was reported. However, 2 dogs had local recurrence and 8 dogs developed metastatic disease, and, in these cases, the median time to relapse was 201 days (Skorupski et al, 2009).

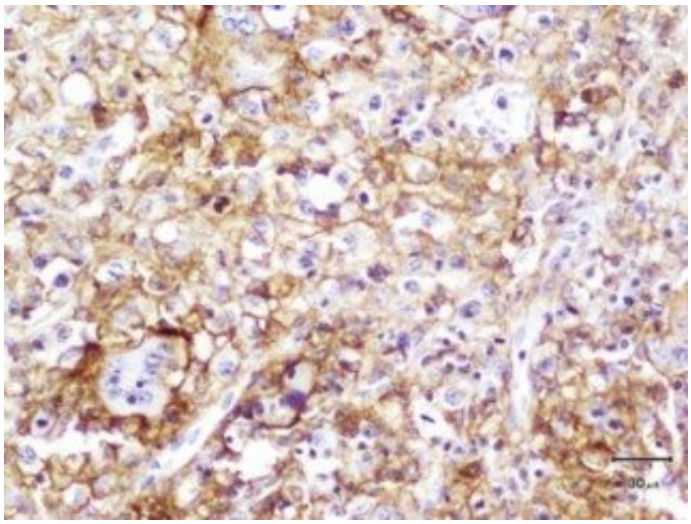

Figure 7. Immunostaining showing positive CD18. Histiocytic sarcoma with large numbers of cells with cytoplasmic immunopositivity for CD18.

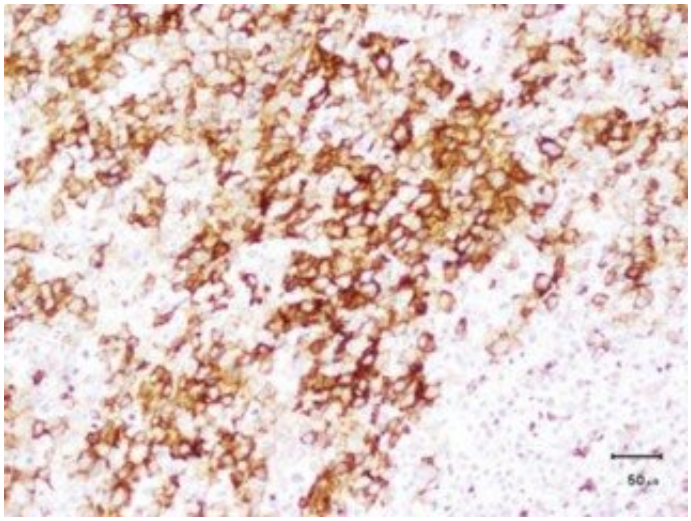

Figure 8. Immunostaining showing positive CD11d. Histiocytic sarcoma with strong diffuse cytoplasmic immunostaining for CD11d.

The response rate to lomustine in 56 dogs with gross disease was reported to be 46% (overall response), but with a median survival of 106 days (Skorupski et al, 2007). A combination chemotherapy protocol, with the addition of doxorubicin to lomustine (plus/minus cyclophosphamide), has shown efficacy in some dogs with histiocytic sarcoma (Cannon et al, 2015) and liposomal clodronate has also been reported to have some efficacy in treatment (Hafeman et al, 2010).

Many dogs with disseminated histiocytic sarcoma are euthanised at the time of diagnosis due to the widespread nature of the lesions and associated morbidity. Disseminated HS is generally considered to be poorly responsive to therapy. It remains to be seen whether more targeted therapies might play a role in the management of histiocytic disease in the future – first we need to identify potential targets.

Cohort study

The Tumour Survey provided valuable information on the type of tumour affecting the breed, but did not really provide an indication of the prevalence of the problem within the breed. In 1994, the authors recruited 174 healthy dogs aged 2 to 7 years old to a Health Study and these were followed by an annual health census until death (Dobson et al, 2009).

A total of 72 dogs (42%) died from confirmed neoplasia – 20 (11.6%) died of unconfirmed tumours and 61 (35%) died from non-neoplastic conditions. The cause of death was unknown for 19 dogs and 2 dogs were lost to follow-up. Soft tissue sarcoma (especially histiocytic sarcoma) was the predominant cancer type, affecting 32 dogs (44%) of neoplasms); 6 dogs died with malignant melanoma and 3 with lymphoma. Median age at death was 9 years for dogs with tumours and 12 for non-neoplastic fatalities.

The results confirmed soft tissue sarcoma – particularly histiocytic sarcoma – was a major cause of mortality in the breed and age of onset for sarcoma showed a major peak at 8 years and a minor one at 11 years, with an average age of onset at 8.13 years.

Examination of the pedigrees of affected dogs in the Health Study, plus a further 170 affected dogs identified through the ongoing Tumour Survey, showed they all shared six ancestors (three males and three females) four to nine generations in the past, suggesting a close relationship between all affected flat-coated retrievers. Examination of affectation status within sibships strongly suggested the disease was familial (**Figure 10**; Aguirre-Hernandez et al, 2005).

The molecular genetics of histiocytic sarcoma has been investigated by molecular cytogenetic profiling (Hedan et al, 2011) and genome-wide association studies (Shearin et al, 2012). Using genome-wide array comparative genomic hybridisation, copy number aberrations (CNAs) were assessed in 146 histiocytic sarcomas – 101 from Bernese mountain dogs (68 from the US and 33 from France) and 45 from flat-coated retrievers (all from the US; Hedan et al, 2011).

Numerous CNAs were found – both gains and losses – throughout the genome, almost all of which were shared between the two breeds, suggesting they are more associated with the cancer phenotype than with breed. A subset suggested involvement of known cancer-associated genes including deletions of the tumour suppressor genes cyclin-dependent kinase inhibitor (CDKN) 2A/B, retinoblastoma protein and phosphatase and tensin homolog.

Interestingly, dysregulation of CDKN2 has also been associated with susceptibility to histiocytic sarcoma in Bernese mountain dogs by genome-wide association study (GWAS; Shearin et al, 2012).

In some dog breeds and other species, increased disease risk – including increased risk of particular cancers – is associated with the presence or absence of particular MHC alleles. The authors have also investigated whether any associations exist between a diagnosis of histiocytic sarcoma and MHC II allotype in 40 affected flat-coated retrievers, compared to 40 control flat-coated retrievers.

In this population, the MHC class II diversity was very restricted, but no significant difference existed between affected and controls, making it unlikely MHC alleles have any causative relationship with the high prevalence of histiocytic sarcoma in the breed.

Looking ahead

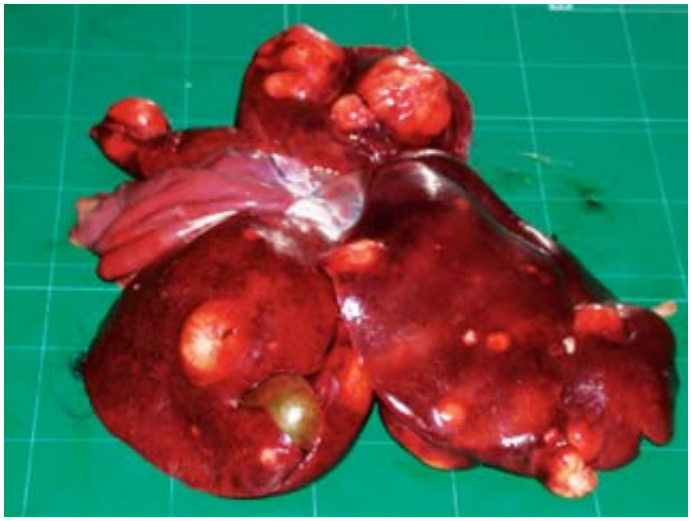

Figure 9. Gross appearance of metastatic nodules of histiocytic sarcoma in the liver of an affected dog.

Since it began, the authors have received 2,975 submissions to the Tumour Survey. Many of these submissions had more than one sample for processing, making the total number of samples more than 3,000. These, along with the information collected, have been invaluable in understanding the high incidence of histiocytic sarcoma within the breed and will provide a valuable archive of tissue for future studies. Specific details of these tumours will be published separately.

After 25 years of accruing data and samples, the authors believe the Tumour Survey has served its purpose and run its course. The number of samples submitted has declined year on year for the past five years, and Tess Hoather, the stalwart of running the survey, has retired. The Tumour Survery closed at the end of 2015 and the authors will no longer offer the free histopathology service for the breed. However, their interest in flat-coated retrievers and other breeds affected with histiocytic sarcoma will not end.

In 2013, in conjunction with the Flatcoated Retriever Society's breed health subcommittee, the authors set up a website database to monitor the "health" of the breed by recording the cause of death when dogs die. We believe this is an innovative and effective way to monitor the health of the breed that may shed light on new or emerging problems and show trends in health issues. In the event of a flat-coated retriever death, the authors very much hope readers will encourage owners to help the breed by visiting www.flatcoated-retriever-society.org and clicking on "Health", then "Cause of Death Register" from the drop-down menu to complete the short questionnaire.

Future studies

The microenvironment of the tumour, along with the immune system, has an important role in both the development and progression of cancer. On the one hand, the immune system can eradicate emerging malignant cells, but on the other, by influencing the tumour microenvironment, it can promote the growth, invasion and metastasis of malignant cells.

In recent years, the tumour microenvironment and presence of infiltrating immune cells (T cells and macrophages) has become of increasing importance to our understanding of the relationship between cancer and the immune system. Of particular interest is the recognition of regulatory T cells within tumours that appear to downregulate the immune system and are associated with a poorer outcome in many human tumours.

These cells may offer a target for future cancer management strategies, so their role in cancer progression is important to understand. We have demonstrated many of the T cells infiltrating histiocytic sarcomas are indeed regulatory T cells (Marcinowska et al, 2014b).

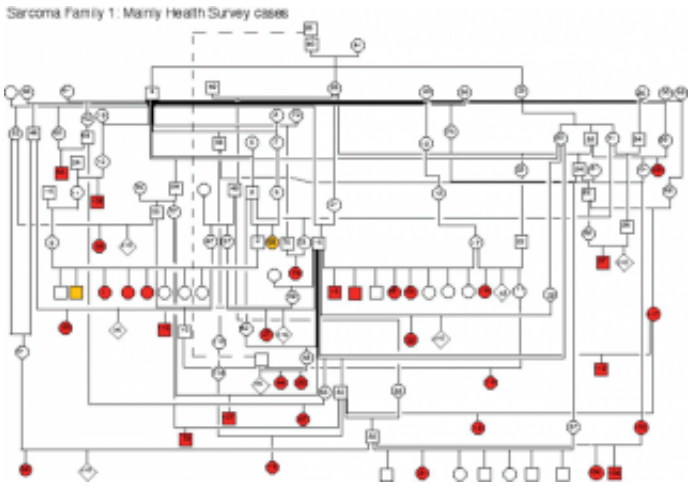

Figure 10. Pedigree of one family, courtesy of David Sargan. Squares are males, circles are females. Red denotes histologically confirmed histiocytic sarcoma and yellow denotes cancer of unknown type. In the higher generations, there may have been cases with tumours that we were not aware of.

Although the precise cellular origin of histiocytic sarcoma is unknown, the immunophenotype is suggestive of a myeloid dendritic antigen presenting cell lineage. It is interesting this prominent T cell infiltrate should be present in a tumour comprising cells that modulate the immune response, dendritic cells.

It is known dendritic cells play a pivotal role in determining immune tolerance versus immunity; therefore, a key step in better understanding the relationship between the tumour, its microenvironment and the immune system is essential to understanding how the tumour influences immune function.

The finding that a significant proportion of tumour-infiltrating T cells expressed forkhead box P3, suggesting them to be regulatory T cells, raises interesting questions of cause and affect, which the authors aim to address in future studies. The authors' team at the Queen's Veterinary School Hospital (QVSH) Cancer Therapy Unit maintains a strong interest in the breed and in HS in general. Sarah Mason has a particular interest in the medical and multimodal therapy of HS and Aleksandra Marcinowska continues her research into HS tumour microenvironment.

We would, therefore, like to hear of flat-coated retrievers affected with histiocytic sarcoma. The QVSH cancer therapy team is always happy to see patients diagnosed with HS and advise on treatment options that can be adapted for individual patients. It is also pleased to advise on any aspect of diagnosis and case management, and would welcome the opportunity to receive tissue collected for diagnostic purposes from such cases.

Acknowledgements

The authors would like to thank the trustees of the flat-coated retriever Tumour Survey and all those who have raised funds to support this work, Madeline Fordham and all the staff in the histopathology laboratory who have processed the samples. David Bostock and the many pathologists and residents who have reported for the Tumour Survey over the years, and David Sargan and Jesus Aguirre-Hernandez for contribution to molecular and genetic-based studies.

References

- Aguirre-Hernandez J et al (2005). Inheritance of histiocytic sarcomas in flat-coated retrievers, *BSAVA Congress Sci Proc*, BSAVA, Gloucester: 634.
- Boerkamp KM et al (2014). The two main forma of histiocytic sarcoma in the predisposed Flatcoated retiever dog display variation in gene expression, *PLoS One* **9**(6): e98258.
- Cannon C et al (2015). Evaluation of a combination chemotherapy protocol including lomustine and doxorubicin in canine histiocytic sarcoma, *J Small Anim Pract* **56**(7): 425-429.
- Constantino-Casas F et al (2011). The clinical presentation and histopathologic-immunohistochemical classification of histiocytic sarcomas in the Flat Coated Retriever, *Vet Pathol* **48**(3): 764-771.
- Craig LE et al (2002). The diagnosis and prognosis of synovial tumors in dogs: 35 cases, *Vet Pathol* **39**(1): 66-73.
- Dobson JM (2007). Radiation response of appendicular histiocytic sarcoma in flat-coated retrievers, *European Society of Veterinary Oncology (ESVONC) spring meeting*, ESVONC, Terneuzen.
- Dobson JM et al (2006). Histiocytic sarcoma of the spleen in flat-coated retrievers presenting with regenerative anaemia and hypoproteinaemia, *Vet Rec* **158**(24): 825-829.
- Dobson JM et al (2009). Mortality in a cohort of flat-coated retrievers in the UK, *Veterinary and Comparative Oncology* **7**(2): 115-121.

- Hafeman S et al (2010). Evaluation of liposomal clodronate for treatment of malignant histiocytosis in dogs, *Cancer Immunol Immunother* **59**(3): 441-452.
- Hedan B et al (2011). Molecular cytogenetic characterization of canine histiocytic sarcoma: a spontaneous model for human histiocytic cancer identifies deletion of tumor suppressor genes and highlights influence of genetic backgrounds on tumor behavior, *BMC Cancer* **11**: 201.
- Hendrick MJ et al (1998). *Histological Classification of Mesenchymal Tumors of Skin and Soft Tissues of Domestic Animals* (2nd ser, vol 2), Armed Forces Institute of Pathology, Washington DC: 22.
- Marcinowska A et al (2014a). Histiocytic sarcoma in a flat-coated retriever with central and peripheral nervous system sarcomatosis, *Vet Rec Case Rep* **2**: e000029.
- Marcinowska A et al (2014b). Assessment of infiltrating lymphocytes in histiocytic sarcoma of flat coated retriever: a comparison of different locations, *European Society of Veterinary Oncology (ESVONC) Congress*, ESVONC, Terneuzen.
- Morris JS et al (2000). A histopathological survey of neoplasms in flat-coated retrievers: 1990 to 1998, *Vet Rec* **147**(11): 291-295.
- Morris JS et al (2002). Immunohistochemical and histopathologic features of 14 malignant fibrous histiocytomas from Flat-Coated Retrievers, *Vet Pathol* **39**(4): 473-479.
- Mellanby RJ et al (2004). Immune-mediated haemolytic anaemia associated with a sarcoma in a flat-coated retriever, *J Small Anim Pract* **45**(1): 21-24.
- Shearin AL et al (2012). The MTAP-CDKN2A locus confers susceptibility to a naturally occurring canine cancer, *Cancer Epidemiol Biomarkers Prev* **21**(7): 1,019-1,027.
- Skorupski KA et al (2007). CCNU for the treatment of dogs with histiocytic sarcoma, *J Vet Intern Med* **21**(1): 121-126.
- Skorupski KA et al (2009). Long-term survival in dogs with localized histiocytic sarcoma treated with CCNU as an adjuvant to local therapy, *Vet Comp Oncol* **7**(2): 139-144.