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Veterinary and Comparative Oncology is pleased to include these abstract titles from the Annual Conference and we hope you find them informative and useful. These abstracts have not been subjected to peer review or editorial revision however, and it would be prudent for the reader to exercise caution in the interpretation of the data presented.

The abstracts published in this proceedings should be treated as personal communications and should be cited as such.

Telomerase targeted cancer gene therapy in canines

M. L. Arendt, J. Morris, L. Nasir, I. M. Morgan

University of Glasgow, Faculty of Veterinary Medicine, Bearsden Road, G61 1QH, Glasgow, UK

In cancer gene therapy there are basic elements that need to be accomplished in order to produce an effective therapy. These are specific targeting of cancer cells, high expression of trans-genes and low toxicity.

We have used the telomerase promoter which is shown to be active in 85–90% of canine cancers and combined this with a two-step amplification mechanism. In this system the telomerase promoter drives the transcription of a transcriptional activator protein which in turn activates a strong minimal promoter to transcribe the trans-gene of interest. We have tested this system in cell culture using telomerase positive and negative cell lines and have shown the ability of this system to be very active in telomerase positive cells. We have also compared our system with the GAL4-VP16 system which is commonly used in gene therapy.

Results: Our system shows a high level of specificity and sensitivity and it is superior to the GAL4-VP16 system.

Conclusion: We have developed a system with a unique potential to target cancer cells and express trans-genes at a high level and believe that this system will be able to improve gene therapy mechanisms.

Prospect: We are working on developing a conditionally replicative adenovirus using CAV-1 making it capable of replicating and inducing lysis in telomerase positive cells. Simultaneously we are on using the TRAIL gene for cancer cell killing.

Most frequently encountered neoplasia in dogs in Denmark: data from the Danish Veterinary Cancer Registry

L. B. Broenden, N. Toft, T. Knudsen, J. Mikkelsen, A. T. Kristensen

Department of Small Animal Clinical Sciences, Faculty of Life Sciences, Dyrslægevej 16, 1870 Frederiksberg C, Denmark

Introduction: During the last 2.5 years a group of practitioners representing initially a few practices but lately 30 practices throughout Denmark have entered diagnosed cases in dogs to the Danish Veterinary Cancer Registry. A total of 977 cases from dogs have been reported. A legally required registry of the country's 600 000 dogs allows for weighing of the numbers of cases within a certain breed against the number of dogs of that particular breed in Denmark and estimation of relative risk.

Objective and method: The objective of the current study was to estimate prevalence of the most common neoplasia in dogs in Denmark and the relative risk of neoplasia in different breeds.

Results: Based on the registry data, at present the most common neoplasia in Danish dogs were cutaneous (394; 40%) followed by mammary (185; 19%), haemolymphatic tissues (52; 5%) and oral cavity (40; 4%). The most frequently encountered malignant cancers were mast cell tumours (76; 8%), adenocarcinoma (72; 7%), lymphoma (68; 7%) and carcinoma (63; 6%). Benign tumours most commonly entered in the registry were lipomas (123; 13%), adenomas (75; 8%), and histiocytomas (73; 7%). The relative risk of neoplasia for the breeds most commonly seen (relative risk estimates regulated for population composition) was highest in Boxers (4.38), Bernese Mountain dogs (2.50), Flat coated retrievers (1.88), and Cocker spaniels (1.75).

Conclusion: These results correspond well with data from a similar study in Norway,¹ except with regard to haemolymphatic neoplasia and the order of breeds with high relative risk is slightly different.

Reference

1. Arnesen K, Gamlem H, Glattre E, Grøndalen J, Moe L, Nordstoga K. Hundekreftregisteret i Norge 1990–1998. *Norsk Veterinærtidsskrift* 2000; **112**: 137–147.

Effect of aglepristone (Ru534) on the Ki67 proliferation index of spontaneous canine mammary tumours

V. Domingo¹, C. Recuerda¹, E. Rollón², Y. Millán³, I. Barranco³, J. Gómez-Laguna³, J. Martín de las Mulas³

¹Clinica Veterinaria Recuerda, Granada, Spain, ²Clinica Veterinaria Canymar, Cadiz, Spain, ³Depto. de A. y Anatomía Patológica Comparada, Facultad de Veterinaria, Universidad de Córdoba, Spain

Introduction: Progesterone receptor (PR) activation plays an important role in the development and growth of canine mammary tumours. The aim of this study was to investigate whether aglepristone has any effect on the proliferation rate of canine mammary tumour cells.

Methods: With the owner's consent, 12 bitches with malignant mammary tumours were injected with aglepristone (10 mg/kg, subcutaneously) on days 1, 2, 7 and 8. Both the pre-treatment biopsy (day 1) and the surgical excision specimen (day 15) were processed routinely for histopathology and immunohistochemistry (oestrogen receptor α -ER α -, PR, MIB1 antigen) studies. Blood samples were collected on days 1 and 15 to assay plasma concentrations of progesterone. The size of the tumour was recorded before the beginning of the treatment and then on days 8 and 15. Six bitches without tumours were equally treated and served as controls.

Results: All 12 tumours analyzed were classified as carcinomas, and 9 expressed both ER α and PR (75%). The Ki67 proliferation index (number of positive cells/1000 cells) was lower in the post-treatment biopsy of 6 cases with and 2 cases without hormone receptors expression. No decrease in the number of MIB1-positive cells was observed in tissues from control dogs. Plasma concentrations of progesterone increased in experimental but not in control dogs. No changes were observed in the size of the tumours, neither in the hormone receptor expression pattern, after the treatment.

Conclusions: Aglepristone diminished Ki67 cell proliferation index and progesterone levels but not on tumour size or hormone receptor expression.

Comparison of staging in dogs with appendicular osteosarcoma with radiographs and staging based on computed tomography

N. Eberle, D. Simon, A. Gerdwilker, M. Fork, I. Nolte

Small Animal Hospital, School of Veterinary Medicine Hannover, Germany

Introduction: Conventional staging of appendicular osteosarcoma in the dog is based on radiographs of the primary tumour and thorax. Examination by computed tomography (CT) is a diagnostic technique increasingly available in veterinary medicine. It was the aim of this study to compare staging performed with conventional methods to staging based on CT examination with special emphasis on the detection of pulmonary metastases of osteosarcoma as well as to analyse the data with regards to its prognostic value.

Methods: All canine patients cytologically or histologically diagnosed with appendicular osteosarcoma were included into the study. All dogs underwent radiographic examination as well as CT examination of the primary lesion and the thoracic cavity. Data recorded included epidemiologic data, conventional clinical stage and computed tomography findings. Kaplan–Meier product limited analysis was used for survival analysis. Cox forward multivariate regression analysis was used to evaluate patient variables for influence on survival time.

Results: 38 dogs were included in the analysis. Radiographically pulmonary lesions were detected in 2 cases (5%), whereas the CT imaging showed that pulmonary nodules were evident in 10 cases (26%). There was a significantly improved detection of small pulmonary nodules in the lung parenchyma with computed tomography and better prognostic assessment of clinical stage.

Conclusion: Computed tomography examinations allow improved assessment of clinical stage of canine osteosarcoma patients. Although radiography is used in diagnosis of canine osteosarcoma, additional imaging with computed tomography seems beneficial in the detection of pulmonary lesions.

Impact of Ki67 labelling index on treatment outcome of feline vaccine-associated sarcoma

C. Eckstein, C. Rohrer Bley, F. Guscetti, M. Roos, B. Kaser-Hotz

Diagnostic Imaging and Radio-Oncology, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland

Introduction: Feline vaccine-associated sarcomas are highly invasive and often rapidly growing neoplasms. High tumour proliferation has been shown to correlate with aggressive behaviour in human and canine soft tissue sarcomas.

Methods: We evaluated the growth fraction of vaccine-associated sarcomas by Ki67 immunohistochemistry in relation to outcome after therapy, stating the hypothesis that a high labelling index implicates a less favourable response to treatment.

Results: Median survival of the 46 curatively treated patients (surgery followed by definitive radiation therapy) was 43 months with almost 50% of the cats becoming long-term survivors. The median progression free interval (PFI) was 37 months. In the group of palliatively treated cats ($n = 27$) the median survival was 24 months, with a median PFI of 10 months. Median survival of 7 months (PFI 4 months) was significantly shorter in palliatively irradiated gross tumour than seen after treatment of presumed microscopic disease (survival of 30, PFI of 20 months). However, the median survival for palliatively treated cats with macroscopic disease could be significantly prolonged when chemotherapy was added ($n = 5$). Due to low patient numbers, additional benefit of chemotherapy could not be evaluated in palliatively treated cats with no visible mass. Ki67 (>10% positive) tended to negatively correlate with the PFI of curatively treated patients. Surprisingly, in palliatively treated cats a significantly longer PFI was reached, if >20% of the cells stained positive.

Conclusion: The labelling index Ki67 does not seem to provide a clear prognostic parameter for PFI or survival in feline vaccine-associated sarcoma.

Targeting of heat shock protein 32 (Hsp32) / heme oxygenase-1 (HO-1) in canine mastocytoma cells is associated with reduced growth and induction of apoptosis

E. Hadzijusufovic^{‡*}, L. Rebuzzi^{‡*}, K. V. Gleixner^{*}, A. Vales^{*}, R. Kondo^{*}, A. Gruze⁺, M. Kneidinger^{*}, M. T. Krauth^{*}, P. Samorapoompichit[¶], K. Greish[#], M. Mayerhofer^{*†}, W. F. Pickl⁺, H. Maeda[#], M. Willmann[‡], P. Valent^{*}

[‡]University of Veterinary Medicine, Clinical Department for Small Animals and Horses, Clinic for Internal Medicine and Infectious Diseases, Veterinärplatz 1, 1210 Vienna, Austria, ^{*}Department of Internal Medicine I, Division of Hematology & Hemostaseology, [†]Clinical Institute of Medical & Chemical Laboratory Diagnostics, ⁺Institute of Immunology, [¶]Center of Anatomy & Cell Biology; Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria, [#]Department of Microbiology, Kumamoto University School of Medicine, Honjo 2-2-1, Kumamoto 860-0811, Japan

Introduction: Mastocytomas are among the most frequent neoplasms in canines. Aggressive mastocytomas usually are resistant against conventional therapy and the prognosis is grave. Therefore, current research focuses on new targets in neoplastic mast cells (MC) and the development of targeted drugs. Heat shock protein 32 (Hsp32), also known as heme oxygenase-1 (HO-1), is a survival-enhancing molecule that is expressed in various neoplastic cells.

Methods: We examined the expression of Hsp32 (HO-1) in primary canine MC as well as in the canine mastocytoma cell line C2. Expression of the Hsp32 protein was examined by immunocytochemistry and Western blotting, and expression of Hsp32 mRNA by RT-PCR. To define the functional role of Hsp32, two novel Hsp32-targeting drugs, pegylated zinc-protoporphyrin (PEG-ZnPP) and styrene maleic acid-micelle-encapsulated ZnPP (SMA-ZnPP), were applied.

Results: As assessed by immunocytochemistry and Western blotting, primary neoplastic MC and C2 cells were found to express the Hsp32 protein in a constitutive manner. Moreover, we were able to show that C2 cells express Hsp32 mRNA. Exposure of C2 cells to hemin (10 μ M) resulted in an up-regulation of expression of Hsp32. Both Hsp32-targeting drugs, PEG-ZnPP and SMA-ZnPP, were found to inhibit the proliferation of C2 cells and of neoplastic MC. The growth-inhibitory effects of PEG-ZnPP and SMA-ZnPP were time- and dose-dependent (IC₅₀: 1–20 μ M), and associated with apoptosis.

Conclusions: Hsp32/HO-1 is an important survival factor and interesting new target in neoplastic canine MC. Clinical trials with Hsp32-targeted drugs are now warranted to define the *in vivo* antineoplastic efficacy of these new drugs.

Palliative management of malignant effusion with implantable vascular access ports in dogs

A. Lara¹, C. Kosarek², K. Hosoya¹, F. Alvarez¹, D. Smeak¹, A. Pozzi¹,
W. Kisseberth¹, P. Ries³, C. G. Couto¹

¹College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, ²College of Veterinary Medicine, University of Georgia, Athens, GA, USA, ³VCA Veterinary Care Animal Hospital, Albuquerque, NM, USA

Introduction: Malignant pericardial or pleural effusion (MPE) in the dog carries a poor prognosis. Survival time is determined by successful control of clinical signs by pericardiectomy, periodic thoraco-/pericardiocentesis, or response to intracavitary chemotherapy. We are currently evaluating a vascular access port (VAP) that allows long-term pleural fluid drainage with minimal discomfort for the patient, and administration of intrathoracic chemotherapy in dogs with MPE.

Methods: Eight dogs with cytologically or histologically confirmed MPE had a VAP surgically placed. A VAP silicone catheter was placed intrathoracically and a titanium port was fixed to the subcutaneous tissue of the thoracic wall to drain pleural effusion and administer chemotherapy.

Results: Survival time period ranged from 35 to 274 days (mean 65 days). There were no complications associated with port placement. One dog needed a second VAP placed in the opposite hemithorax due to an imperforated mediastinum, and another dog had the VAP replaced due to breakage of the catheter. Weekly thoracic taps were performed and the volume drained ranged between 10 and 1700 ml per visit.

Three dogs had routine taps performed at home by the owners. All patients received intrathoracic chemotherapy with 5-fluorouracil or carboplatin, Three dogs were also treated with systemic chemotherapy.

Conclusions: Implantable VAPs are a useful tool in the long-term therapeutic and palliative management of dogs with MPE. They are associated with minimal complications and may improve the patient's quality of life.

One dimensional agarose gel electrophoresis of serum proteins in dogs with lymphoma

J. G. P. Henriques, J. S. Morris, D. Eckersall

Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Bearsden, Glasgow, G61 1QH, UK

Lymphoma cells induce the release of different inflammatory proteins in patients' serum and these proteins can distinguish different types of lymphoid neoplasia. In this study we investigated the serum proteins of dogs with lymphoma using electrophoresis.

Thirty-five dogs presenting to Glasgow University Small Animal Hospital with untreated (27) or relapsed (8) lymphoma were studied. Of these, 24 had multicentric disease and most (29) were substage b; 12 B cell and 10 T cell. Serum samples collected before chemotherapy were run by agarose gel electrophoresis (Paragon®) along with 13 serum samples from control dogs. The gels were scanned and the stained bands evaluated using densitometry. The resulting electrophoretograms were divided into five regions (albumin, alpha 1, alpha 2, beta and gamma).

There was no consistent electrophoretogram shape for serum from dogs with lymphoma. Twenty dogs (57%) had increased alpha 2 and 70% of these were substage b. In most cases, the beta fraction could not be subdivided, but total beta was decreased in 10 dogs (28%). There was no increase in the gamma fraction in any dogs. Eighty percent T cell lymphomas had decreased alpha 1 and 42% B cell lymphomas had increased beta fractions. Within the alpha 2 and beta regions there were some stronger bands, which were not in controls.

Serum protein electrophoresis can help to differentiate lymphoma samples from normal dogs. We intend to continue the study and identify the proteins within the fractions more precisely using further techniques such as capillary electrophoresis or mass spectrometry.

Adjuvant gemcitabine after surgical removal of aggressive malignant mammary tumours in dogs

L. Marconato, M. R. Lorenzo, F. Abramo, A. Ratto, E. Zini

Clinica Veterinaria L'Arca, Vico Cacciottoli 46/47, 80129 Napoli, Italy

Introduction: In general, canine mammary tumours with high-risk for locoregional failure and distant metastases can not be successfully managed by surgery alone. Chemotherapy has been rarely attempted in

dogs with aggressive malignant mammary tumours. The aim of this study is to explore the efficacy and safety of adjuvant post-operative gemcitabine chemotherapy in dogs.

Methods: A prospective clinical trial in which dogs with aggressive mammary carcinoma were treated with surgical excision alone (Group 1) or with surgery and adjuvant weekly gemcitabine (Group 2) for at least four cycles was conducted. Gemcitabine was given as an intravenous infusion at the dose of 800 mg/m². Time to local recurrence, time to distant metastasis, and overall survival were calculated from date of surgical excision.

Results: Nine dogs underwent surgical excision alone, whereas 10 dogs were treated by surgery and adjuvant gemcitabine. Treated dogs received from 4 to 10 gemcitabine courses. Time to local recurrence was longer in Group 1 than in Group 2 ($p < 0.05$). Time to distant metastasis and overall survival were not different between groups. Gemcitabine treatment was well tolerated with no dogs experiencing clinically relevant hematologic or gastrointestinal toxicity.

Conclusions: Despite safe at the present dose, gemcitabine chemotherapy as an adjunct treatment to surgical excision may have limited beneficial effects in dogs with aggressive mammary carcinoma. Additional clinical trials are warranted to address whether gemcitabine alone or in combination with other chemotherapeutic drugs may improve outcome of operated aggressive canine mammary carcinoma.

Surgery alone or in combination with doxorubicin for the treatment of feline injection-site sarcomas: clinical experience on 95 cases (2000–2006)

M. Martano, E. Morello, C. Squassino, G. La Rosa, S. Zabarino, P. Buracco

Department of Animal Pathology, School of Veterinary Medicine, via L. da Vinci, 44 – 10095 Grugliasco, Italy

Introduction: Injection-site sarcomas (ISS) are tumours that develop at the site of subcutaneous or intramuscular injection in predisposed cats. They are characterized by a long latency period, low metastatic but high recurrence rates, even when aggressive therapy is adopted. The therapy of choice implies the combination of *en bloc* surgery and radiation, +/- chemotherapy. The role of chemotherapy is not clear, since it is usually employed together with other treatments, mainly in more aggressive cases. The goal of this study was to evaluate the effect of doxorubicin used both as neoadjuvant and adjuvant to radical surgery.

Methods: Ninety-five cats were divided into two groups: group A (45 cats) received 1 to 4 doxorubicin cycles, 21 days apart, with radical surgery 10 days after the second cycle; group B (50 cats) received radical surgery alone.

Results: Overall survival time ranged from 68 to 2511 days in group A and from 60 to 2364 days in group B; median was not reached. Median disease-free period was 585 days in group A (22–2511 days) but it was not reached in group B (60–2151 days). Metastasis and recurrence rates were 6.6% and 46.6% in group A, respectively, and 6% and 20% in group B.

Conclusions: Doxorubicin associated with radical surgery does not seem to significantly improve tumour control rate, even if survival is prolonged when chemotherapy is used. In this study chemotherapy was used mainly in cats with more aggressive or already recurred tumours, therefore a true comparison between groups is not completely reliable.

Reference

Vaccine-Associated Feline Sarcoma Task Force: Roundtable discussion. The current understanding and management of vaccine-associated sarcomas in cats. *J Am Vet Med Assoc* 2005; **226**: 1821–1842.

Clinical and pathological studies of feline myeloma related disorders

P. J. Mellor¹⁺, S. Haughland¹, A. Holloway¹, J. Archer¹, G. A. Polton², K. C. Smith³, S. Murphy³, P. E. McNeil⁴, D. J. Argyle⁵, M. E. Herrtage¹, M. J. Day⁶

¹Dept of Veterinary Medicine, University of Cambridge, UK, ²Davies Veterinary Specialists, Hitchin, Hertfordshire, UK, ³Animal Health Trust, Newmarket, Suffolk, UK, ⁴Finn Laboratories, Diss, Norfolk, UK, ⁵Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK, ⁶School of Clinical Veterinary Science, University of Bristol, Langford. ⁺Current address: Finn Laboratories, Diss, Norfolk, UK

Introduction: Myeloma-related disorders (MRD) are rare neoplasms in cats. Our aim was to describe clinical, clinicopathological, imaging, pathological findings and response to treatment in a large retrospective case series. *A priori* hypotheses were that feline MRD patients (1) uncommonly present with radiographic bone lesions, (2) commonly present with extramedullary involvement, (3) those with well-differentiated tumours commonly have extramedullary involvement – in contrast to reports in human patients.

Methods: Cases were sourced from all major UK referral centres and complete records obtained. Tumours were cytologically and/or histopathologically and immunohistochemically assessed, and classified as well-differentiated, intermediate or poorly-differentiated. For hypothesis testing, the differences in proportions were analysed using the two-sided *z*-test.

Results: Of 24 cases with clinical data, *n* = 7 presented with cutaneous mass(es) and of these, only two animals had concomitant systemic signs and hyperglobulinaemia. The second group (*n* = 17) had neoplasia involving abdominal organs (usually the liver and spleen), bone marrow or both. 100% of this group had systemic signs and paraproteinaemia but the majority responded well to chemotherapy with a median survival time of 12.3 months. Radiographically detectable bone lesions were significantly less common in cats (8%) than in man (80%) (*P* < 0.001). Marked extramedullary involvement at initial presentation was significantly more common in cats (67%) than in man (<5%) (*P* < 0.001). Cats with well-differentiated tumours more commonly had extramedullary involvement (80%) than in humans (20%) (*P* < 0.001).

Conclusions: Our preliminary work suggests a novel hypothesis: a primary extramedullary origin for the neoplastic transformation of a myeloma cell may be more common in feline MRD.

Systemic indolent mastocytosis in a young dog. A new presentation of mast cell disease?

L. Nielsen, A. T. Kristensen

Department of Small Animal Clinical Sciences, Faculty of Life Sciences, Dyrlægevej 16, 1870 Frederiksberg C, Denmark

A six-month-old female Jack Russell Terrier puppy was presented to the referring veterinarian due to multiple alopecic, pruritic and raised nodules in the skin of the dog. An excisional biopsy showed a well differentiated mast cell tumour. The dog was treated with a short course of corticosteroids with minimal effect.

Two years later, the dog was presented to the university clinic for examination due to a progression of the disease. Clinical examination revealed over 30 nodules scattered over the skin of the dorsum, neck, head and ears. Several nodules were red and oedematous or ulcerated and a moderate peripheral lymphadenopathy was present. Staging of the patient revealed mast cells in all examined nodules by fine needle aspiration. Furthermore mast cell infiltration was found in the spleen and several peripheral lymph nodes, while the rest of work up was negative for mast cell infiltration. Due to the age of the dog at initial presentation, the prolonged disease entity and the dog's clinical status a diagnosis of systemic indolent mastocytosis was made.

The dog was treated with cyclosporin, antihistamines and corticosteroids. The dog quickly improved showing partial remission with resolution of some nodules and reduction of more than 50% in others. Two months post diagnosis the dog is still doing well.

Systemic indolent mastocytosis is a benign disease seen in young people characterised by multiple dermal changes or maculopapular rashes and headaches. The disease is usually self-limiting with a benign course responding moderately to palliative care such as antihistamines.

Accelerated hypofractionated radiation therapy for the treatment of feline oral squamous cell carcinoma: a pilot study

V. J. Poirier, J. Buchholz, C. Rohrer Bley, B. Kaser-Hotz

Section of Diagnostic Imaging and Radiation Oncology, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

Introduction: Squamous cell carcinoma (SCC) is the most common feline oral tumor. Radiation therapy and/or surgery are the standard of care in humans and dogs. In the cat, standard radiation protocol with curative intent or palliative intent only achieved tumour control of 1.5 to 5.5 months. The purpose of this study was to evaluate time to progression and overall survival for feline oral SCC treated with accelerated hypofractionated radiation therapy (RT).

Methods: Histologically confirmed feline oral SCC treated with RT were reviewed. Two RT protocols were used: 10 daily fractions of 4.8 Gy or 14 twice-daily fractions of 3.5 Gy.

Results: Seven cats with oral SCC underwent RT. Five cats had macroscopic disease and two cats had surgery prior to the radiation. All but one cat completed the prescribed radiation protocol. Another cat died 1 week post RT of acute pancreatitis. Six cats had oesophageal tube in place and the acute effects were tolerable and self-limiting. Response could be assessed in three patients (2 CR and 1 PR) with duration of 42, 69 and 1352 days. The overall median time to progression was 105 days and the overall median survival was 143 days. Two cats are disease-free and alive at 436 days and 1352 days.

Conclusion: Accelerated hypofractionated RT was tolerated with acceptable side effects. Tumour responses were seen but short in duration. Two cats were long-term survivors.

Twenty-six cases of feline nasal lymphoma: a retrospective study

G. A. Polton, M. J. Brearley

Davies Veterinary Specialists, Higham Gobion, Herts, SG5 3HR, UK

Introduction: Nasal lymphoma is a reasonably unusual but frequently described manifestation of feline lymphoma. Clinical recommendations are drawn from a limited number of case series comprising small numbers of disparate patients managed by different means.

Methods: A single centre retrospective study was performed evaluating a cohort of feline patients diagnosed with nasal lymphoma from 2000–2006 inclusive. Treatments included radiotherapy alone, 4 weeks of COP followed by radiotherapy, COP alone and other. Outcome comparisons were made on an intention to treat basis.

Results: Eight cats were Siamese. Gross specimens were retrieved for histopathological evaluation by forced nasal flush and grab biopsy in 12 and 10 cases, respectively. Two cases also had disease affecting non-nasal sites: the gingiva and regional lymph node. Complete clinical remission was achieved in 23/26 cases. Median remission duration for the whole cohort was 98 days. Median survival time was 337 days, (range 0–1642). There was no statistically significant difference in survival outcome with the different management strategies though it is notable that all patients living more than 12 months after diagnosis received radiotherapy. Three patients developed terminal radiotherapy complications. Death occurred due to undiagnosed renal disease and renal lymphoma each in one case. CNS lymphoma was not described.

Clinical significance: This study has demonstrated marked variations in response to the treatments prescribed. The incidence of renal or CNS manifestations following relapse was insignificant. A multicentre study to further examine the natural history of this disease is justified.

Radiation enhances vascular endothelial growth factor expression in the canine mastocytoma cell line C2

I. Sekis^{1,2}, M. Willmann¹, L. Rebuzzi¹, W. Gerner³, T. Kaeser³,
J. G. Thalhammer³, A. Saalmueller³, M. Kleiter¹

¹Clinic of Internal Medicine and Infectious diseases, ²Clinic of Anesthesia and Perioperative Intensive Care, ³Clinical Immunology, University of Veterinary Medicine, Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Introduction: Vascular endothelial growth factor (VEGF) plays an important role as proangiogenic and autocrine growth factor in several malignancies. Radiotherapy is a standard treatment in dogs with incompletely excisable mast cell tumors. Irradiation up-regulates VEGF secretion in several neoplasms resulting in radioresistance and relapse. The objective of this study was to evaluate the influence of irradiation on VEGF and VEGF-receptors (Flt-1, Flk-1/KDR) expression in a canine mastocytoma cell line.

Methods: Experiments were carried out in the canine mastocytoma cell line C2. Cells were irradiated with single doses of 2, 4, 6 and 8 Gy from a linear accelerator source. Cell Survival data were obtained by MTT and ³H-thymidine proliferation assays. VEGF levels were measured in cell lysates and supernatants by ELISA prior and 24, 36 and 72 hours post radiation. VEGF receptor expression was evaluated by flow cytometry.

Results: C2 cell survival rates post irradiation decreased in a dose dependent manner. Non-irradiated cells produced VEGF protein constitutively. Irradiation induced a significant dose and time dependent increase in VEGF synthesis, demonstrating ongoing up-regulation. Non-irradiated C2 cells expressed Flk-1/KDR in the cytoplasm but not on their surface. The influence of irradiation on receptor expression is currently under investigation.

Conclusions: Irradiation enhanced VEGF expression in the tested C2 cell line. This increased angiogenic potential could be one way of protection of tumour cells against radiation damage. Whether this up-regulation plays a role in radioresistance and if tumour control could be improved by combining radiotherapy and VEGF(R) inhibitors requires further investigation.

Outcome of neoadjuvant and adjuvant chemotherapy with doxorubicin versus surgery alone in the treatment of feline associated sarcomas: a retrospective study of 37 cases

D. Stefanello¹, M. Cantatore¹, M. Digiancamillo¹, V. Grieco², E. Riccardi²,
O. Travetti, V. Fiorbianco¹, V. Bronzo², S. Romussi¹

¹Dipartimento di Scienze Cliniche Veterinarie, Università di Milano, Italy, ²Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università di Milano, Italy

Introduction: Feline Vaccine-Associated Sarcomas (FVAS) are mesenchymal neoplasms whose treatment is still unrewarding. Wide surgery with megavoltage radiotherapy is considered the gold standard treatment in FVAS. Radiotherapy units are not available for veterinary patients in Italy, moreover the

usefulness of chemotherapy as an adjuvant tool is far to be proven. The aim of this retrospective study is to compare clinical outcome of cats treated with wide surgery alone or in combination with two different doxorubicin-based chemotherapy protocols.

Methods: The medical records of cats with histologically confirmed diagnosis of FVAS were reviewed. Feline patients were enrolled in three different treatment groups. Group A: doxorubicin (1mg/kg every 3 weeks) twice before and twice after wide surgery; group B: four doxorubicin cycles after wide surgery (1 mg/kg every 3 weeks); group C: wide surgery alone. In each group the frequency of local recurrence (X test $p < 0.05$) and the disease free interval (DFI) for not censored data (Kaplan–Meier and log-rank test $p < 0.05$) has been evaluated.

Results: The median follow-up was 501 days. The recurrence rate was calculated in 50% in group A, 20% in group B and 29% group C. The median DFI was 476 days in group A and was not reached in group B and C but ranged from 40 to 476 days. No statistically significant differences were not found between the groups.

Conclusion: Adjuvant and neoadjuvant chemotherapy did not seem to influence the DFI. Further studies are needed to definitively delineate the therapeutic role of doxorubicin in FVAS.

Adhesion factor expression in canine mast cell tumour associated fibroblasts

F. Taylor, J. Dobson, M. Sheppard, T. Scase

Department of Veterinary Medicine, Queen's Veterinary School Hospital, University of Cambridge, Madingley Road, Cambridge. CB3 0ES, UK

Introduction: Factors involved in mast cell (MC) adhesion include stem cell factor (SCF) and SynCAM (also called SgIGSF). Observations of our culture system suggest that neoplastic MCs (NMCs) only appear to proliferate when tightly adhered to a monolayer of associated fibroblasts (FBs). This experiment investigates the expression of adhesion molecules in FBs from primary MC tumours (MCTs) compared with FBs from normal canine skin.

Materials and methods: Tumour tissue from clinical cases of MCT was cultured to obtain mixed colonies of NMCs and FBs. NMCs appeared to apoptose at approximately 2 weeks, and the remaining fibroblasts were cultured onto microscope coverslips at a fixed density. FBs from dogs without MCT were cultured using the same method. Cells were fixed and incubated overnight using SynCAM antibody (SynCAMAb) or SCF antibody (SCFAB). Images were obtained following secondary fluorescent antibody labelling using an immunofluorescence microscope.

Results: FBs from MCTs cultured for 3 weeks exhibited much higher expression of SynCAM than FBs from normal tissue. FBs from MCTs that had been in culture for 6 weeks showed intermediate levels of immunostaining. There was no difference in SCF expression between FBs from MCT and FBs from normal tissue.

Conclusions: The results of this study suggest that adhesion molecule expression (SynCAM) by mast cell tumour associated fibroblasts may be important in the development and maintenance of MCTs in the dog. The expression of this protein may be induced by the presence of NMCs, and may play a role in promotion of MCTs.

Efficacy and toxicity of a new formulation of Paclitaxel (Paclical® Vet) in a phase I + II study for the treatment of malignant tumours in dogs

P. Rivera¹, J. Haggstrom¹, H. Nyman², N. Wikstrom¹, H. von Euler¹

¹Department of Clinical Sciences, Division of Small Animal Clinical Sciences and ²The University Animal Hospital, Division of Diagnostic Imaging; Swedish University of Agricultural Sciences (SLU), SE-750 07 Uppsala, Sweden

Introduction: Paclitaxel (Taxol®) in humans and dogs requires extensive premedication and slow infusion (3–24 hours) due to side effects caused by the solvent Cremophor EL®. The purpose of this ongoing phase I + II study is to determine the maximum tolerated dose, toxicity and efficacy of a new Cremophor EL®-free formulation of paclitaxel (Paclical® Vet) in dogs with refractory or recurrent solid tumours and high stage lymphomas.

Methods: Paclical® Vet (Oasmia Pharmaceuticals, Uppsala, Sweden), dissolved in Ringer-Acetate, was given as an 15–30 min IV infusion of 175 mg/m² with subsequent dose reduction if toxicity was observed (range 175–100 mg/m²). Treatment was repeated every 21 days for at least 3 cycles or until disease progression. No premedication, besides sedation, was routinely administered. A pharmacokinetic study was performed in 24 dogs.

Results: Twenty-eight dogs received paclitaxel. Dose-limiting neutropenia at day 4 was observed at 175 mg/m², whereas the mean dose 150 mg/m² was generally tolerated. Other side effects included alopecia, transient inappetence, vomiting/diarrhoea. One case of mild hypersensitivity was detected in 90 doses administered. No drug-related cardiotoxicity was detected. Overall response (complete# and partial#) was 67%. Mastocytomas (7) and squamous cell carcinomas (4) responded 100%. Pharmacokinetics revealed a fast tissue distribution of paclitaxel, with an α -T_{1/2} of 10 minutes. The median clearance was 16.6 L/h/m², which is similar to humans.

Conclusions: This is the first successful trial of a paclitaxel formulation in dogs. Although survival analysis and response duration is not yet determined, the tumour response and controllable side-effects call for a multicentre trial in selected diagnoses.

A pilot study to assess the effects of adjunctive treatment with propentofylline in dogs with nasal tumours treated with radiotherapy

J. M. Dobson, R. N. Gear

Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES

Introduction: Tumours of the nasal cavity and paranasal sinuses are relatively common in the dog. Radiotherapy is currently regarded as the treatment of choice for this disease but in most cases treatment

is palliative; survival times range from 8–14 months, most dogs succumb to tumour recurrence as opposed to metastasis. The presence of regions of hypoxia in solid tumours is considered to be an important cause of failure of tumour control by radiation. Improving tumour oxygenation may therefore increase tumour radiosensitivity without increasing normal tissue toxicity. The purpose of this pilot study was to assess whether propentofylline, an agent which potentially may increase tumour perfusion and hence oxygenation, could improve radiation response in canine nasal tumours.

Methods: Forty dogs with intranasal tumours were treated with a hypofractionated radiation regime using 4×9 Gy fractions at 7 day intervals to a total dose of 36 Gy. Twenty dogs also received Propentofylline (5 mg/kg per os twice daily) during and after radiotherapy.

Results: The mean survival time for all dogs in this study was 383 days (range 64–1244 days) with a median of 227 days (95% CI: 146, 307). The dogs treated with propentofylline survived longer than the controls (median survival of 268 days versus 216 days respectively) but the difference in survival times was not statistically significant.

Clinical significance: The results of this small pilot study suggest that Propentofylline may warrant further investigation as a potential radiosensitiser.

Strong conservation and single sequence peculiarities of canine apoptosis-associated molecules as compared to their human counterparts

B. Schade, A. Rickenbacher, S. M. Keller, J. Wimmershoff, F. Guscetti

Institute of Veterinary Pathology, Vetsuisse Faculty of the University of Zurich, Winterthurerstr. 268, CH-8057 Zurich, Switzerland

Background: In contrast to rodents, dogs present with a range of spontaneous neoplasms biologically and histologically similar to human cancers. Strong conservation of relevant pathways is an important determinant for the validity of such models.

Methods: We have cloned and sequenced the coding sequences of a representative set of apoptosis-associated molecules in canines (including Bcl-2 family members, Inhibitor of Apoptosis Proteins, their mitochondrial inhibitors, Caspases and p53) and we have assessed by computational analysis their degree of conservation with the human and murine orthologues.

Results: Basing on the analysis of 18 cDNAs, 11 of which are novel for the dog, we found a high degree of similarity between human and canine molecules, domains and motifs related to the intrinsic apoptotic pathway. However, sequence differences with potential functional impact were observed to varying extents in both dogs and mice. In dogs, these changes include functionally relevant residues of p53, the predicted Inhibitor of Apoptosis Protein binding motif of the mitochondrial protein Omi/HtrA2, and some caspase-cleavage sites within apoptosis-related molecules. Canine XIAP yields a caspase-cleavage site previously considered unique to humans.

Conclusions: Our comprehensive, systematic analysis of canine apoptosis-related molecules unveiled both strong similarities with their human counterparts and single potentially significant interspecies

variations. The former suggest that in most cases the dog might provide an appropriate frame for modelling therapies targeting the intrinsic apoptotic pathway. The latter might have significant functional effects in both normal and tumour cells that will require further studies.

Immunohistochemical expression of TopBP1, Ki67, and p53 in relation to histological type of canine mammary tumours

J. S. Morris, C. Nixon, O. J. A. King, L. Nasir, I. M. Morgan, A. W. Philbey

Division of Pathological Sciences, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Bearsden, Glasgow, G61 1QH, Scotland, UK

TopBP1 (DNA Topoisomerase II-binding protein 1) is a protein with 35% amino acid sequence homology in the carboxyl terminal and several structural domains in common with the BRCA1 gene product. Using immunohistochemistry, we examined the expression of TopBP1 in 132 canine mammary samples (46 benign, including 17 mixed mammary tumours, 17 complex and 12 simple adenomas and 86 malignant, including 41 complex and 45 simple carcinomas) in relation to histological type and grade, proliferation index (Ki67) and p53 expression. There was positive staining for TopBP1 in all samples. In general, the percentage of samples with high staining increased with malignancy. More malignant than benign tumours had over 80% ($P < 0.02$), 60% ($P < 0.047$) or 40% staining ($P < 0.055$). Benign mixed mammary tumours stained more than other benign tumours at very high levels of staining (over 80%). Most staining was nuclear but additional cytoplasmic staining was noted as the degree of malignancy increased and more carcinomas than benign tumours had cytoplasmic staining ($P < 0.03$). More benign mixed mammary tumours than other benign tumours had cytoplasmic staining (< 0.024). The number of neoplasms with more than 20% of cells immunopositive for Ki67 ($P = 0.00$) and p53 ($p < 0.02$) showed the expected trend of increasing with biological grade with benign mixed mammary tumours showing the lowest expression. In conclusion, TopBP1 is expressed in canine mammary tumours with more positive samples and more cytoplasmic expression associated with increasing malignancy. Benign mixed mammary tumours express more cytoplasmic TopBP1 than might be predicted from their benign biological behaviour.

Maintenance combination chemotherapy in feline lymphoma: results of treatment in 23 cats

D. Simon, N. Eberle, L. Laake-Singer, I. Nolte

Department of Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover, Hannover, Germany

Background: Different chemotherapy regimes have been described for the treatment of feline lymphoma. The aim of the present study was to evaluate the efficacy and toxicity of an asparaginase- and doxorubicin-based chemotherapy in cats with lymphoma.

Methods: Cats with histologically or cytologically confirmed diagnosis of lymphoma were eligible for the study. The chemotherapy protocol consisted of a cyclic combination of L-asparaginase, vincristine, cyclophosphamide, doxorubicin, methotrexate, and prednisolone with a planned total treatment time of 122 weeks. Kaplan–Meier product limit analysis was used for remission and survival analysis.

Results: 23 cats were included into the study. Median age and weight were 9.5 years and 5.5 kg, respectively. Complete remission (CR) rate was 74% ($n = 17$). Four cats (17%) attained partial remission (PR). Median duration of first CR was 264 days (range 45–2485 days). 6-month-, 1-year and 2 to 5-year remission rates were 75%, 50%, and 34%, respectively. Duration of PR ranged between 23 and 63 days. Median survival in cats with CR was 296 days (range 50–2520 days). 6-month, 1-year, 2-year and 3 to 5-year-survival rates in cats with CR were 77%, 47%, 34%, and 27% respectively. Median treatment time in cats with CR amounted to 128 days (18 weeks).

Conclusions: In this population of cats, treatment led to satisfactory remission rates, remission and survival durations. In spite of an intended continuous treatment, median treatment time amounted to approximately 4 months, giving rise to the aspect that, as has been shown in dogs, short-term, discontinuous chemotherapy may be similarly efficacious as long-term maintenance treatment in feline lymphoma.

Evaluation of angiogenesis in normal lymph nodes and lymphomas of dogs

B. Wolfesberger¹, Z. Tonar⁵, K. Witter³, A. Guija de Arespacohaga²,
M. Skalicky⁴, I. Walter³, J. G. Thalhammer¹, G. Egger³

¹Clinic of Internal Medicine and Infectious Diseases, ²Central Laboratory, ³Institute of Histology and Embryology, ⁴Institute of Pathophysiology, University of Veterinary Medicine, Vienna, Austria, ⁵Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University in Prague, Czech Republic

Introduction: Microvessel density is a frequently used parameter of angiogenesis, which is tightly regulated by pro- and anti-angiogenic factors. Angiogenesis is defined by the growth of blood vessels originating from existing vasculature and it seems to play a key role in progression of tumours. We compared the microvessel density of lymph node biopsies diagnosed with lymphoma and normal lymph nodes of dogs. Furthermore we wanted to know if there is any correlation between microvessel density and vascular endothelial growth factor expression in canine lymphoma.

Methods: Combined immunohistochemistry (von Willebrand factor) and lectin histochemistry was used to highlight microvessels in 40 untreated canine lymphomas and 14 normal lymph nodes. For the evaluation of microvessel density, the number of profiles of blood vessels per unit area was counted. In each specimen 50 image fields (a total area of 5.68 mm²) were sampled in a systematic random way.

Results: We found a significant difference between the microvessel densities (MVD) of normal compared to all neoplastic lymph nodes (177 ± 35 versus 241 ± 72). Classifying lymphoma samples according to the Working Formulation showed no significant differences in MVD between low-grade, intermediate-grade or high-grade malignancies. There was no correlation between microvessel density and vascular endothelial growth factor expression in lymphoma cells.

Conclusions: Observing increased angiogenesis in tumour samples compared to normal lymph node tissue of dogs, additional anti-angiogenic therapy as lymphoma treatment could be taken into consideration, but which of the many pro-angiogenic factors will be the optimal target, has still to be elucidated.

Identifying cancer stem cells in canine osteosarcoma

H. Wilson¹, R. Chun¹, K. M. Young¹, K. Friedrichs¹, M. Huelsmeyer¹,
D. J. Argyle²

¹Departments of Medical Sciences and Pathobiological Sciences, University of Wisconsin, Madison, WI, USA,
²Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

Osteosarcoma is the most common primary bone tumour in dogs and has an aggressive clinical course with less than 20% of dogs surviving 2 years. The main reason for death in these patients is metastatic disease that demonstrates resistance to common chemotherapeutic protocols. There is increasing evidence that cancer is a stem cell disease, in that the bulk tumour population is derived from a very small population of cancer stem cells (CSCs) that are highly resistant to chemotherapeutic or radiation therapy.

In this study we sought to identify sub-populations of sarcoma cancer cells that have a 'stem cell' phenotype. D-17 (canine osteosarcoma) and MG63 (human osteosarcoma) cells were cultured in non-adherent culture conditions using serum-starved media, allowing for the outgrowth of primitive cells in colonies termed sarcospheres. Sarcospheres were characterized using a combination of RT-PCR and immunostaining for primitive markers (Nanog, OCT3/4, STAT-3).

We found that we could consistently produce sarcospheres from D17 cells and that marker analysis confirms these to be primitive stem-like cells. The identification of sub-populations of cancer cells has immense implications for the development of new therapies.

Further, the similarities between human and canine natural disease adds strength to the potential of the dog as a model for human disease.

Multi-modality therapy of a dog with an advanced undifferentiated nasal carcinoma, and of the side effects induced by the tumour and radiation: a case report

J. P. de Vos¹, J. Butinar², E. Picavet³, W. Klaassen⁴, S. Klueter⁵, D. Krastel⁵,
G. Hildebrandt⁶

¹De Ottenhorst, clinic for companion animal medicine, van Diemenstraat 83, 4535 AR Terneuzen, The Netherlands, ²Animal Hospital Postojna, Postojna, Slovenia, ³Dental practice Picavet, Dental care DeSteenbrug, Sint Jansteen, The Netherlands, ⁴Klaassen Laboratories for Dental Mechanics, Terneuzen, The Netherlands, ⁵Department of Small Animal Medicine, University of Leipzig, Germany, ⁶Department of Radiotherapy and Radio-oncology, University of Leipzig, Germany

Material and methods: A 6-year old female mongrel dog was presented in July 2004 with a histopathologic confirmed undifferentiated nasal carcinoma. The mass protruded through a left maxillary oral-nasal fistula. On CT-images there was extension of the tumour over both nasal cavities with septum destruction, but no involvement of the cribriform plate and frontal sinuses. Clinical exam and thoracic X-rays showed no evidence of metastases. Treatment with piroxicam (0.3 mg/kg sid) and carboplatin (300 mg/m² per 3-weeks iv) resulted in a partial remission of 2 months. Succeeding intranasal high-dose-rate brachytherapy (¹⁹²Iridium) was performed using an afterloading system (Microselectron HDR; Nucletron) and MRI-based dose distribution calculation by the planning system PLATO (version 14.2.4.; Nucletron).¹ This hypofractionated twice weekly protocol, consisting of eight 4 Gy fractions, resulted in a complete remission. Subsequently to the maxillary fistula, multiple oral-nasal fistulae developed within 1 year post-radiation over a large, bone lacking, palatine area. Surgical attempts to close the largest maxillary fistula were unsuccessful and finally all the defects were covered with a custom-made removable Vitallium frame prosthesis with an attached methylmetacrylate obturator. One year after radiation, a large bone and skin defect developed on the dorsal part of the nose, which was reconstructed with a transposition flap from the forehead, pivoting more than 90°. Except a small bilateral dehiscence of 2–3 mm, the end result is functionally and cosmetically acceptable.

Results: Thirty-one months after start of therapy the tumour is still in complete remission, the additional problems are controlled, and the dog's quality of life is excellent.

Reference

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Indications and considerations for hemi-pelvectomy in the dog

J. P. Bray

Davies Veterinary Specialists, Higham Gobion, Herts, SG5 3HR, UK

Introduction: Hemi-pelvectomy has been described as an aggressive surgical procedure. There are very few case series in the veterinary literature that reviews hemi-pelvectomy in the management of disease affecting the proximal hind limb and pelvic bones. This retrospective paper describes the outcome of 26 dogs and two cats that were considered candidates for hemi-pelvectomy.

Methods: The medical records of patients that were identified as candidates for hemi-pelvectomy during the period 2004–2007 were reviewed. Disease conditions and anatomical considerations that may have affected the feasibility of the resection, reconstruction and/or potential long term survival were identified.

Results: Twenty-eight patients (26 dogs and two cats) were considered candidates for hemi-pelvectomy during the study period, but surgery was only performed in 16 of these. A neoplastic condition affecting the pelvis, femur or soft tissues of the thigh was the reason for surgery in all but two cases. The reasons for not proceeding with surgery included a high-grade malignancy, extension of the mass across the midline,

or assessment by a more conservatively-minded surgeon. In the surgical group, a high potential for curative outcome was the most common indicator for proceeding with surgery. Coaxial imaging was considered essential to assess the viability of the proposed resection. Nine of the surgical patients remain alive at the end of the study period, with a mean survival time of 535 days.

Conclusions: Recovery and rehabilitation following hemipelvectomy is rapid and post-operative function is similar to that observed with routine hindlimb amputation. Pre-operative planning, including incisional biopsy, clinical staging and co-axial imaging, are essential to ensure a successful outcome is achieved.

Transanal pull-through rectal amputation for the treatment of colorectal carcinoma in 10 dogs

C. Squassino, E. Morello, S. Iussich, F. Sammartano, M. Martano, S. Zabarino, R. Caccamo, P. Buracco

Department of Animal Pathology, School of Veterinary Medicine, University of Turin, Italy

The objective was the evaluation of postoperative complications and oncological outcome after transanal rectal pull-through amputation for treatment of canine colorectal adenocarcinoma and carcinoma *in situ*.

Ten owned dogs with single colorectal adenocarcinoma or carcinoma *in situ* were treated by a full thickness rectal amputation through either a simple transanal (six dogs) or a combined abdominal/transanal (four dogs) pull-through technique.

Seven adenocarcinomas and three *in situ* carcinomas were removed with 3–6 cm of macroscopic healthy margin. Postoperative complications included tenesmus and rectal bleeding that spontaneously resolved in all cases, and rectal stricture in three cases that resolved without additional surgery. Persistent fecal incontinence was evident in only one dog. The mean disease-free and mean survival periods were 30.4 and 30.7 months, respectively, with recurrent disease in 25% of cases. The two recurrences had a disease-free interval of 4.5 and 20 months, respectively.

In conclusion *en bloc* surgical excision of canine colorectal adenocarcinoma may afford long survival when associated with a more favourable TNM and a low histological stage. Complications of the transanal approach are usually mild and self-limiting, while in case of the combined abdominal/transanal approach they can be more frequent (50%) and severe. Both transanal rectal pull-through amputation and the combined abdominal/transanal approach are useful for *en bloc* rectal amputation of rectal/colonic neoplasia. The abdominal/transanal approach should be reserved for tumors cranial to the cranial third of the rectum.