Poster presentations

hyperadrenocorticism and 3 dogs with other diseases not known to cause significant proteinuria. A comparison of TTR and clusterin levels between the SCI and control groups was performed by Western blotting. Based on Fisher's exact test, the presence of TTR and clusterin was found to be significantly associated with SCI ($p = 0.0033$ for TTR monomer, $p = 0.0046$ for TTR dimer and $p = 0.0123$ for clusterin). The TTR dimer had 100% sensitivity for SCI, but only 58.3% specificity. The TTR monomer had 83% sensitivity and 83% specificity, while clusterin had 83.3% sensitivity and 75% specificity. It was, however, not possible to distinguish between SCI cases and hyperadrenocorticism cases. Albumin depletion of sam-

ples was performed to facilitate the detection of low-abundance proteins and to standardise the levels of low-abundance proteins that were loaded onto the gel and analysed by western blotting. However, although the albumin content was reduced and TTR was still detectable by Western blotting in 3 SCI samples, there was generally a concomitant reduction of low-abundance proteins, which is not ideal. In conclusion, TTR and clusterin are promising biomarkers of spinal cord injury. However, as urinary concentrations of these proteins can also increase in dogs with compromised kidney function, further validation studies are required to establish if there are threshold levels of these proteins that are specific for SCI.

Palatability in dogs of Nutri-Plus Gel[®], a highly energetic complementary feed indicated to cover the additional energy needs of dogs and cats

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Athletic dogs have high energy requirements. A sufficient energy intake is necessary to maintain ideal body weight and muscle condition scores, as well as a good performance levels. A common mistake is to increase the volume of the regular diet, which may lead to digestive problems that can interfere with the activity of the dog, such as diarrhoea, flatulence or frequent defecation. There are few well-balanced complete diets on the market that are adapted to athletic dogs. Adding a highly energetic complementary feed to the usual complete diet during periods of increased activity can be an interesting alternative.

Nutri-Plus Gel[®] is a complementary feed that contains high-energy ingredients, animal proteins, vitamins and trace elements. It is typically recommended to cover energy needs during intense physical efforts (e.g. hunting dogs) or

as recovery nutrition after intense exercise, when only small volumes can be accepted by the animal. Spontaneous intake is considered to be an essential criterion for a complementary feed that is supposed to be given on a regular basis by the dog owner. The objective of this study was to assess the palatability of Nutri-Plus Gel[®] in dogs.

Two sub-studies (A and B) were conducted in an independent research centre highly experienced in performing palatability trials. 36 and 34 healthy adult dogs of various breeds, both males and females, were included in trials A and B, respectively. Two criteria were used to assess palatability: prehension and total consumption. Prehension was defined as the act of taking the product spontaneously into the mouth, independently of whether it was then consumed. Total consumption was defined as the act of swallowing more than 95% of the quantity of product offered. Each dog received a teaspoon (5.5 grams) of the product per 5kg of body weight.

Prehension was noted in 89% (32/36) and 94% (32/34) of dogs, in trials A and B respectively. Total consumption was recorded in most of the animals in which prehension had been observed (84%, 27/32 and 72%, 23/32 in trials A and B, respectively).

The excellent prehension rate and the high level of consumption observed in these studies indicate that Nutri-Plus Gel[®] is highly palatable for dogs, most often leading to a spontaneous intake of the product. These results are consistent with observations previously made on the field.

Nasal melanoma in a 14 year old cross-breed dog

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A 14-year-old, neutered male crossbred border collie was presented for investigation of a 5 month history of sneezing and muco-haemorrhagic right-sided nasal discharge.

Physical examination revealed reduced airflow through the right nares but was otherwise unremarkable. Haematology was unremarkable and biochemistry revealed only a mild elevation in amylase at 1367 U/L (<1245 U/L). A CT scan revealed a soft tissue mass effect in the dog's right nasal cavity, with significant turbinate lysis and focal destruction of the right nasomaxillary suture. No lesions compatible with

metastatic disease were identified on CT of the dog's head, neck, thorax and abdomen. Rhinoscopy confirmed the presence of a nasal mass, and biopsies and cytology samples were procured. No evidence of metastatic disease was found by fine needle aspirate cytology of both mandibular lymph nodes.

Cytology of the nasal mass demonstrated a population of pleomorphic polyhedral to spindloid cells among sheets of columnar ciliated epithelium. Nuclei were centrally placed and round with stippled chromatin and one or multiple prominent nucleoli; occasional macronuclei or multinucleate cells were observed. A number of cells contained green pigment, and in conjunction with the cellular pleomorphism were considered suggestive of melanoma. Histopathology described a neoplasm consisting of pleomorphic round to polygonal cells containing varying numbers of nuclei (1-9), abundant eosinophilic cytoplasm with light-brown intracellular granules, and less than 1 mitosis in 10 x400 fields, on a

background of inflammation. The histogenesis of the tumour was not clear although the differentials of melanoma, olfactory neuroblastoma, neuroendocrine tumour and undifferentiated carcinoma were considered most likely. Fontana Masson stain was performed to detect melanin granules and was weakly positive. Immunohistochemistry demonstrated strong staining of the neoplastic cells for PNL-2 and Melan-A. Eight percent of the cells stained positively for cytokeratin

and 20% for vimentin. These results were considered diagnostic of a melanoma.

The dog received a hypofractionated radiotherapy protocol (four, weekly fractions of 8 Gray) and achieved a complete clinical response which persists at the time of writing (4 months). To the author's knowledge, this is the first report of the diagnosis and successful treatment of a melanoma in the nasal cavity of a dog.

Approaching tumour heterogeneity using laser capture microdissection coupled with gene expression on formalin-fixed paraffin canine mammary tumours

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Canine mammary tumours (CMT), as its human counterpart, are characterized by cellular heterogeneity. The concept of tumour heterogeneity is increasingly being recognised since it will be important for understanding the molecular pathogenesis of these tumours. The use of laser capture microdissection (LCMD) in formalin-fixed, paraffin-embedded (FFPE) tissues is a recent methodology in pathology that permits the rapid and reliable extraction of RNA from different pure cell populations. The combination of LCMD and gene analysis has the potential to reveal novel pathogenic pathways

and biomarkers by which normal cells progress to invasive malignant cells. However, in order to get high-quality RNA from FFPE, optimization of protocols has to be previously performed. The goals of this study were first, to validate the LCMD technique in CMT by using two different slide types (glass membrane and frame membrane); second, to validate two commercial kits for RNA extraction; and third, to identify selected tumour biomarkers of interest in obtained samples. Thirty-nine FFPE samples from benign and malignant CMT were selected to identify normal epithelial and myoepithelial cells, malignant epithelial and myoepithelial cells and stroma cells by Arcturus[®] LCMD. RNA extraction was done by the RecoverAll Total Nucleic Acid Isolation Optimized for FFPE samples (Ambion) and by RNAqueous Micro-kit (Ambion). Gene expression by using classic polymerase chain reaction (PCR) was performed in order to study the expression of progesterone receptor (PR) and the antiangiogenic markers VEGFA and VEGFR2. HPTR1 and RPL32 were used as housekeeping genes. Isolation of the different cell populations was successfully performed by using PEN-Membrane Glass slides. Whereas RecoverAll Total Nucleic Acid Isolation Optimized for FFPE samples yielded poor RNA concentration and quality by NanoDrop, RNAqueous Micro-kit produced a significant improvement in the RNA concentration and quality and allowed the success amplification for both housekeeping and tumour biomarkers genes. In conclusion, LCMD coupled with RNA extraction by the RNAqueous Micro-kit and classic PCR is a reliable methodology for the molecular characterization of these heterogeneous cell populations in FFPE samples of CMT. However, further studies are required to optimize this methodology in order to get a better understanding of tumour heterogeneity.

Retrospective evaluation of metronomic cyclophosphamide in epithelial and mesenchymal malignant tumours

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Continuous low dose chemotherapy administration has been reported to have anti-angiogenic effects with efficacy against multiple tumour types. This study aimed to retrospectively assess the use of metronomic cyclophosphamide for histopathologically confirmed solid malignant canine tumours.

Patient records from 2009 to 2014 were reviewed at a multi-disciplinary referral hospital. Dogs without histological

confirmation of diagnosis were excluded. Thirty-seven cases of epithelial or mesenchymal malignancy in dogs treated with 10mg cyclophosphamide daily/every other day were identified. This included 14 high grade soft tissue sarcomas, 5 soft tissue sarcomas of undefined grade, 4 intermediate grade soft tissue sarcomas, 3 splenic haemangiosarcomas, 3 incompletely excised low grade soft tissue sarcomas, 2 thyroid carcinomas, and 1 each of adenocarcinoma, bladder haemangiosarcoma, splenic fibrosarcoma, apocrine gland carcinoma, metastatic prostate carcinoma, and metastatic carcinoma of unknown origin. Two were lost to follow up. Twenty-two had at least one attempt at surgical excision and three underwent radiotherapy prior to initiation of chemotherapy.

Three of the dogs (8.6%) developed haematuria, but no other adverse effects were reported. The haematuria resolved in all three dogs after cessation of cyclophosphamide.

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