Small Animal Medicine and Feline Chapters
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1. A MODIFIED THREE-DOSE INOCULATION PROTOCOL FOR COLONIZATION OF THE CANINE URINARY TRACT WITH THE ASYMPTOMATIC BACTERIURIYA ESCHERICHIA COLI STRAIN 83972

Mary F Thompson, Paul C Mills, Mark A Schembri, Darren J Trott

Aim: To trial a multi-dose inoculation protocol for colonisation of the canine urinary tract with the asymptomatic bacteriuria E. coli strain 83972.

Background: Asymptomatic bacteriuria is a possible alternative to antibiotics for management of recurrent urinary tract infection in dogs through the process of active bacterial interference. A multi-dose inoculation protocol is successful in achieving persistent E. coli strain 83972 bacteriuria in humans but has not been utilised in dogs.

Methods: Three doses of approximately 10^9 E. coli strain 83972 bacteria were introduced into the bladder of eight healthy female dogs over 24 hours via an indwelling urinary catheter. Three additional dogs were sham-inoculated. The presence of pyuria, depression, stranguria, pollakiuria and pigmenturia was documented for six weeks. Urinalysis and aerobic bacterial cultures were performed daily for the first four days then 2-3 times weekly for the remaining 5 weeks.

Results: Urinary leukocyte counts were increased in all dogs in the first 48 hours but to a lesser extent for the control dogs in comparison to inoculated dogs. Five of seven inoculated dogs (71%) had a positive culture on day 2. Duration of colonization ranged from one to 28 days (median 2 days). No dog appeared systemically unwell but four of eight inoculated dogs exhibited pollakiuria or stranguria. By day 8 of the trial all clinical signs had resolved.

Conclusion: Prolonged bacteriuria with E. coli strain 83972 is possible in healthy dogs with one dog remaining colonized for 28 days following the three-dose inoculation protocol. Clinical signs related to inoculation were mild and transient.

2. CHRONIC KIDNEY DISEASE AFTER I^{131} TREATMENT FOR FELINE HYPERTHYROIDISM: RISK FACTORS FOR DEVELOPMENT AND EFFECT ON SURVIVAL

A Wong, H Hisyam Teh, JA Beatty, VR Barrs

Aim: 1. To determine if pre-treatment variables can predict the development of chronic kidney disease (CKD) after I^{131} treatment for hyperthyroidism. 2. To compare survival times of cats that developed CKD post-treatment with those that did not.

Methods: Clinicopathologic data and survival times were obtained retrospectively from the medical records of 81 cats receiving I^{131} between 2006 and 2009. The impact of pre and post-treatment variables (urea, creatinine, urine specific gravity (USG), blood pressure, urine protein: creatinine ratio, body weight, I^{131} dose, serum T4) on the development of CKD was analysed by logistic regression. CKD was stratified according to the IRIS staging system. Survival times were compared using an independent sample T-test. Survival analysis was performed using Cox-regression models and Kaplan-Meir analysis.

Results: 16 (20%) cats developed CKD post-treatment, including IRIS stage 1 (n=1), stage 2 (n=14) and stage 3 (n=1). No pre-treatment variables influenced the development of CKD. 27 cats were included in the survival time analysis, of which 6 developed CKD. The difference in mean survival for cats developing CKD (563 days) or no CKD (651 days) was not statistically significant (P = 0.53).

Conclusion: No pre-treatment variables predicted development of CKD post I^{131}-treatment. There was no significant difference between the survival times in cats that developed CKD or did not. The results of this study imply no benefit from a medical treatment trial prior to I^{131} in cats that are not azotaemic and have a USG > 1.040.
3. RISK FACTORS FOR RECTAL COLONISATION WITH FLUOROQUINOLONE RESISTANT \textit{ESCHERICHIA COLI} IN DOGS ADMITTED TO A VETERINARY HOSPITAL

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\textbf{Background:} Fluoroquinolone resistance is an emerging problem in both veterinary and human medicine; however risk factors for antimicrobial resistance in companion animals have largely been extrapolated from human literature.

\textbf{Objective:} To determine potential risk factors for dogs becoming rectally colonised with fluoroquinolone resistant \textit{E. coli} during hospitalisation.

\textbf{Methods:} The medical records of 124 dogs (24 case admissions, 100 controls) were retrospectively examined to identify exposure to potential risk factors including prior hospitalisation, treatments and antimicrobial use during the 8 weeks prior to admission and faecal sample collection.

\textbf{Results:} On multivariable analysis being treated with multiple concurrent antimicrobials and having endoscopy performed increased the risk of colonisation with fluoroquinolone resistant \textit{E. coli}. Univariable analyses indicated that the risk increased following hospitalisation of \(\geq 2\) days duration, catheterisation and intravenous fluid administration for \(\geq 2\) days, treatment with multiple courses of antimicrobials and/or being referred from another veterinary hospital.

\textbf{Conclusions:} Animals that were referred, hospitalised for longer periods, had invasive diagnostic procedures performed as well as being treated with multiple courses of antimicrobials or multiple concurrent antimicrobials is associated with increased risk of rectal colonisation with fluoroquinolone resistant \textit{E. coli}. These results allow the identification of patients at higher risk of colonisation and enable appropriate infection control procedures to be implemented to reduce the risk of colonisation occurring.

4. HAEMATOLOGICAL AND BIOCHEMICAL FINDINGS IN FELINE IMMUNODEFICIENCY VIRUS (FIV) INFECTED CATS

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\textbf{Aim:} To compare haematological and biochemical data and survival times of FIV-infected versus uninfected cats. Prevalence and risk factors were also investigated.

\textbf{Methods:} Cats tested for FIV (January 2005 to October 2009) were identified from VCCC medical records. ‘FIV-infected’ was defined as unvaccinated/seropositive and ‘FIV-uninfected’ as seronegative or, for vaccinated cats, PCR-negative. Signalment, environment, haematological and biochemical values and survival times were recorded. Linear regression analyses were conducted to compare haematological and biochemical data between groups, after adjusting for potential confounders. Risk factors for FIV positivity were identified using logistic regression analyses.

\textbf{Results:} 522 cats were tested for FIV. The prevalence of FIV was 14.8%. The majority of infected cats lived in multi-cat households and had outdoor access. Mixed breed, male gender and older age were risk factors for FIV infection. Haematological and biochemical abnormalities were common in both FIV-infected and uninfected cats. Elevated plasma sodium was the only abnormality associated with FIV infection, after adjusting for age and sex. The best subset of variables to differentiate between infected and uninfected cats were sodium level, sex, monocyte count and age. Survival of FIV-infected cats was greater than uninfected cats.

\textbf{Conclusion:} The prevalence of FIV was high. Risk factors were similar to those identified in previous studies. FIV-infected cats could not be distinguished from uninfected cats based on haematological and biochemical abnormalities. FIV-infected cats lived significantly longer than uninfected cats. A negative effect of FIV on survival was not apparent in this study.
5. THE DEVELOPMENT OF AN IN VITRO ASSAY TO PREDICT THE EFFICACY OF FEL-O-VAX FIV VACCINE AGAINST DIFFERENT ISOLATES OF FIV

**Stickney AL**, Cave NJ, Dunowska M, Pernthaner T

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**Background:** Challenge studies have demonstrated protection against isolates of Feline Immunodeficiency Virus (FIV) subtypes A, B and D after vaccination with Fel-O-Vax. However, vaccination did not prevent infection with one isolate of subtype A in one study. No studies have investigated the efficacy of the vaccine against subtype C, which is concerning to veterinarians in countries such as New Zealand, where subtype C predominates.

Cell-mediated immunity (CMI) is pivotal for protection following vaccination against FIV. The humoral response is less important, and antibody titres cannot be used to predict protection. An assay of FIV-specific CMI would be useful as a screening test prior to performing in vivo challenge studies.

**Aims:** To determine if CD25 expression increases in vaccinated cats following in vitro antigen-specific stimulation, and determine its potential to test cross-reactivity against different FIV subtypes following vaccination.

**Methods:** Following vaccination, feline lymphocytes were stimulated in culture with media, FIV, con-A, and lymphocytes from unvaccinated cats were used controls. Flow cytometry was used to determine the proportion of lymphocytes expressing CD25 with, and without antigen-specific stimulation.

**Results:** Stimulating lymphocytes with FIV in vitro, results in significant up-regulation of CD25 expression in vaccinated, but not unvaccinated cats.

**Conclusion:** This assay can be used in future studies to assess cross-reactivity against different subtypes of FIV.

6. ISOLATION OF PARVOVIRUS FROM THE BONE MARROW OF EIGHT ADULT CATS USING POLYMERASE CHAIN REACTION

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**Aim:** To determine if CPV and FPV genomic sequences are present in bone marrow samples of healthy adult cats, to further investigate the possibility of persistent infection within these animals.

**Background:** The host range of CPV-2a, CPV-2b and CPV-2c differs from the original virus, CPV-2. These more recent parvoviruses have gained the ability to replicate in vitro in feline cells and also in vivo in cats. The pathogenicity of CPV in cats is variable, with some displaying severe clinical signs similar to those caused by the more common feline panleukopenia virus (FPV) and others displaying minimal to no clinical signs. Although faecal shedding generally ceases within 1 – 2 weeks post-infection, both CPV and FPV have been isolated from faecal and peripheral blood samples of healthy cats, despite the presence of virus-neutralising antibodies. Therefore, it is possible that some cats may become persistently infected with these variants.

**Methods:** Bone marrow was obtained from 32 semi-feral cats that were euthanized at an animal shelter. DNA was extracted and subjected to conventional polymerase chain reaction (PCR) to determine if CPV or FPV was present. PCR products were purified, cloned and sequenced to differentiate between CPV and FPV infections.

**Results:** Bone marrow samples from eight cats contained parvoviral DNA. Of these samples, seven were found to contain CPV and one contained FPV.

**Conclusion:** This study suggests that CPV and FPV genomic sequences are present in the bone marrow of some healthy adult cats, further supporting the possibility of persistent infection with these viruses.
7. MOLECULAR SIGNALING MECHANISMS OF EOSINOPHILIA IN ROTTWEILERS

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Background Idiopathic eosinophilic diseases are over represented in Rottweilers. Studies in people highlight the importance of interleukin(IL)-5 in mediating eosinophil maturation, differentiation, migration and polyclonal expansion. The role of cytokines leading to eosinophil expression in dogs is unknown.

Aims The current study investigates whether definable differences in cytokines associated with eosinophil production and survival are present between healthy Rottweilers with normal eosinophil counts, healthy non-Rottweilers and Rottweilers with idiopathic eosinophilia.

Methods Quantitative real-time reverse transcriptase polymerase chain reaction (QRT-PCR) assays were used to quantify messenger RNA (mRNA) encoding cytokines IL-4, IL-5, IL-10, IL-12p19, IL-12p35, IL-12p40, IL-18, interferon gamma (IFN-γ) and chemokines eotaxin-2 and eotaxin-3 in peripheral blood mononuclear cell (PMBC) samples obtained from healthy non-Rottweiler dogs with normal eosinophil counts (n=5) and Rottweilers with normal (n=6), mildly increased (n=7) and high (n=3) eosinophil counts. Quantification of serum IFN-γ was performed using a canine-specific ELISA.

Results All cytokines could be quantified with the exception of eotaxin-2 and -3. Results were normalised using three stably expressed housekeeper genes (RPL13A, SDHA and YWAZ) and a relative copy number was calculated for each sample. There were no significant differences in cytokine mRNA profiles between any of the groups. There was a tendency for IFN-γ mRNA expression to be lower in the Rottweilers with moderate to severe eosinophilia versus control dogs (p=0.062). However, this trend was not seen in the concentration of serum IFN-γ quantified by ELISA as.

In conclusion, there were no significant differences in cytokine profiles between Rottweilers with or without eosinophilia; nor a difference in breed.

8. FELINE LOW GRADE ALIMENTARY LYMPHOMA (LGAL) – HOW COMMON IS IT?


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Background: LGAL requires histology for diagnosis whereas intermediate- (IGAL) and high-grades (HGAL) of alimentary lymphoma (AL) can be diagnosed by cytology of intestinal/mesenteric lymph node aspirates. Histological studies of AL may be skewed towards an increased frequency of LGAL since cases of IGAL/HGAL diagnosed cytologically may not progress to biopsy.

Aims: To determine the relative prevalence and compare clinicopathological features of LGAL with IGAL/HGAL.

Methods: Medical records from 3 feline clinics were searched for pathologist-confirmed cases of AL diagnosed between 2004 and 2009. A chi-square maximum likelihood test or Fisher’s exact test was used to test for an association between categorical data (signalment, clinicopathological and sonographic findings) and the grade of AL.

Results: 53 cases of AL were identified, including 30 diagnosed by histology (15 LGAL, 13 HGAL, 2 IGAL) and 23 IGAL/HGAL diagnosed by cytology. LGAL cases comprised 28% of all AL. A palpable abdominal mass was more common in IGAL/HGAL (43%) than in LGAL (7%) (OR 7.6, P=0.012). Anaemia was more common in IGAL/HGAL (41%) compared with LGAL (7%) (OR 9.6, P=0.020). On abdominal sonography there was loss of intestinal wall layering in 55% of IGAL/HGAL compared to 18% of LGAL (OR 9.6, P=0.018) and there was a gastrointestinal wall mass in 41% of IGAL/HGAL and 0% of LGAL (P=0.010).

Conclusion: The relative prevalence of LGAL was lower overall when cases of AL diagnosed by cytology were included in the study population. LGAL and IGAL/HGAL are clinically distinguishable disease entities based on a number of significant clinicopathological findings.

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**Background:**
In practice, the combination of cerebrospinal fluid (CSF) analysis and magnetic resonance imaging (MRI) is often used to establish a clinical diagnosis of intracranial disease. The aim of the study was to determine if specific total nucleated cell counts (TNCCs) or cytology findings in abnormal CSF correlated with the incidence of MRI abnormalities. In addition, it aimed to determine whether set abnormal MRI characteristics could be correlated with CSF nucleated cell counts or cytology.

**Method:**
Medical records were searched for dogs that had undergone an MRI of the brain and had an abnormal CSF analysis. TNCCs were then categorised into one of three categories; mild (<25x10^6/L), moderate (25-100x10^6/L) and marked (>100x10^6/L). Cytology findings were also categorised as mononuclear – lymphocytic, mononuclear – monocytoid, neutrophilic, eosinophilic or mixed. MRI results were classified as either normal or abnormal. The MRI studies were then evaluated on the presence of the following abnormalities; focal or multifocal/diffuse disease, positive T2-weighted hyperintensity, positive FLAIR hyperintensity, mass effect and well or poorly defined lesion margins.

**Results:**
Thirty-three patients met the inclusion criteria. MRI abnormalities were found in 21/33 (64%) dogs. None of the TNCC categories were statistically different with respect to the incidence of abnormal MRI findings or specific MRI abnormalities identified. Of the cytology categories, statistical significance was identified between the mononuclear-monocytoid, and mononuclear-lymphocytic/neutrophilic groups (P=0.029).

**Conclusions:**
Mononuclear-monocytoid pleocytosis’ positively correlate with the incidence of MRI abnormalities. Other variations in TNCCs or cytology do not correlate with the incidence of MRI abnormalities or specific MRI characteristics.

10. USE OF THE COAGUCHEK XS PROTHROMBIN TIME ANALYSER IN DOGS

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**Background.** Rapid point of care evaluation of prothrombin time (PT) in spontaneously bleeding patients would aid in diagnosis and appropriate therapy of common coagulopathies such as vitamin K rodenticide toxicity. In New Zealand point of care veterinary prothrombin time analyzers are not available. The Coaguchek XS is a handheld coagulation monitor, which is widely available for human testing, and could have applications for canine samples.

**Aim.** To evaluate the suitability of the Coaguchek XS device to determine PT in dogs by measuring the clinical agreement between the results obtained with the device and standard laboratory testing.

**Methods.** The prothrombin time in 25 samples from 13 dogs with suspected coagulopathy and 40 samples from 40 healthy dogs was determined concurrently using the Coaguchek XS device and standard laboratory analysis. The clinical agreement between methods was assessed using Bland-Altman analysis and error grid analysis.

**Results.** The Coaguchek XS showed a 1.56 fold positive systematic bias which when corrected for gave excellent clinical agreement between the results obtained with the device and standard laboratory testing. A measurement error occurred in 2 Coaguchek XS samples which may have been due to the combination of severe anaemia and severely prolonged PT.

**Conclusions.** Coaguchek XS can be used in dogs. Development of a device-specific reference range for dogs would improve its value.
11. SERUM GALACTOMANNAN DETECTION – EVALUATION OF A NEW DIAGNOSTIC TEST FOR UPPER RESPIRATORY TRACT ASPERGILLOSIS IN CATS

Whitney JL1, Martin P2, Beatty JA1, Barrs VR1.

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2 Veterinary Pathology Diagnostic Services, University of Sydney, NSW

**Background:** Feline upper respiratory tract aspergillosis is an emerging disease that can be difficult to treat. Diagnosis usually requires positive fungal culture and/or cytological or histological identification of fungal hyphae in affected tissues. These tests involve invasive procedures and false negative and false positive results are possible. Measurement of serum galactomannan, a polysaccharide component of fungal cell walls, is a non-invasive, alternative test used in the early diagnosis of human invasive aspergillosis.

**Aims:** To evaluate the use of serum galactomannan measurement in the diagnosis of feline upper respiratory tract aspergillosis.

**Method:** Batched serum samples were tested for serum galactomannan using a one-stage immunoenzymatic sandwich microplate ELISA (Platelia™ Aspergillosis) in four groups of cats:

1. Cats with confirmed upper respiratory tract aspergillosis (n=14)
2. Cats with other upper respiratory tract diseases (n=16)
3. Cats without non-respiratory tract diseases treated with β-lactam antibiotics (n=14)
4. Healthy cats (n=45)

**Results:** The sensitivity of the test was 28.6% and the specificity was 60%. High numbers of false positive results were identified in young cats and in cats treated with β-lactam antibiotics.

**Conclusion:** Serum galactomannan measurement has a poor sensitivity and moderate sensitivity for the diagnosis of feline upper respiratory tract aspergillosis. The specificity of the test can be improved when known causes of false positive results are removed from the analysis.

12. CURETTAGE AND DIATHERMY. A NOVEL TREATMENT FOR FELINE ACTINIC DYSPLASIA AND SQUAMOUS CELL CARCINOMA ON THE NASAL PLANUM

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3 New Zealand Veterinary Pathology, Hamilton, New Zealand

**Background:** Squamous cell carcinoma of the nasal planum is a common neoplasm in cats in New Zealand and Australia. Current common treatments in New Zealand are cryotherapy and nasal planum resection. Curettage and diathermy is commonly used in selected neoplasms in human medicine and is an easily mastered technique with 95% cure rates reported.

**Aim:** To evaluate curettage and diathermy as a treatment for early staged squamous cell carcinoma lesions of the nasal planum in cats.

**Methods:** Thirty-four cats clinically assessed to have superficial squamous cell carcinoma (staged Tis to T3) involving less than 50% of the nasal planum were treated with a 3-cycle curettage and diathermy procedure. Degree of dysplasia, response to treatment, adverse effects, owner perceptions, time to recurrence and proportion disease free at 1 year were evaluated.

**Results:** Histologically lesions ranged across degrees of squamous dysplasia from mild dysplastic change to invasive squamous cell carcinoma. A complete response to treatment was obtained in all cats. Two cats had a clinical recurrence of lesions at 161 and 192 days after treatment. The probability of remaining disease free after 12 months was 0.94 (95% CI 0.84-1.0). The median time to recurrence was not reached. The procedure was well tolerated with a good cosmetic outcome and no significant postoperative complications.

**Clinical significance:** This study suggests that curettage and diathermy is an effective treatment for feline nasal squamous cell carcinoma involving less than 50% of the nasal planum. Curettage and diathermy is an easily mastered technique, requiring minimal equipment.
POST-MORTEM STUDY OF CANINE PANCREATITIS
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The definition of pancreatitis clinically is often made based on the duration of clinical signs (acute indicates a sudden onset; whilst chronic is more insidious). Histologically, acute pancreatitis is defined as the presence of necrosis of inflammation within the body of the pancreas or surrounding peri-pancreatic tissue. Chronic pancreatitis indicates there is fibrosis or acinar atrophy that is irreversible. There is also a recently described phenomenon of chronic active pancreatitis, where there are repeated bouts of inflammation superimposed on fibrosis. One post-mortem survey identified 92% of dogs in a referral institution to have pancreatic inflammation1, whilst another study found approximately 34% of dogs surveyed in a first opinion clinic to have chronic pancreatitis2. As it seems highly unlikely that 90% of sick dogs will be clinically affected by pancreatitis, it can be surmised that histologic pancreatitis does not always translate to clinically significant pancreatitis. It has not been established what percentage of the pancreas needs to show signs of inflammation before it can be truly categorised as having clinically apparent pancreatitis. It has not been established what percentage of the pancreas needs to show signs of inflammation before it can be truly categorised as having clinically apparent pancreatitis.

There are currently no studies that reliably inform us of the specificity of pancreatic changes (variable echogenicity and pancreatic enlargement) in this disease. In addition, there are no studies that reliably compare typical abdominal ultrasound to endoscopic ultrasound, MRI or CT in dogs. It is unlikely that CT will be as helpful in canine patients as in humans, as the greatest indication of CT in people is to detect infected necrosis, which does not occur in dogs.

Post-mortem evaluation of the pancreata from dogs presenting to Murdoch University between September 2008 and May 2010 were included in this study. Dogs were included regardless of ante-mortem diagnosis. Samples were obtained from the left limb, right limb and body of the pancreas and graded using an adaptation of two previous studies1,2. Inflammation was assessed and assigned a score out of 4 for each section, and the 3 sections added together for a total possible maximum score of 12. Additionally, the presence of fibrosis was defined as mature fibrous connective tissue replacing the acinar structures or expanding the interstitium. The severity of the fibrosis was scaled 0-3 for each section as well.

38 were included for analysis, and 27 had a pancreatic inflammation score ≤ 3 (no clinical pancreatitis), 3 had a score of 4-5 (mild pancreatitis); 2 had a score 6-8 (moderate pancreatitis) and 6 had a score ≥ 9 (severe pancreatitis). 29 had a fibrosis score of ≤ 2, 18 of which had no detectable fibrosis. 7 dogs had a moderate degree of fibrosis, and 2 had severe fibrosis.

4 of the dogs had severe peri-pancreatic fat necrosis present, but less inflammation present within the pancreatic interstitium. None of these 4dogs had increased concentrations of specific canine pancreatic lipase (Spec cPL), but all had ultrasound findings consistent with pancreatitis.

An interesting finding in this study was the isolated peri-pancreatic fat necrosis. The pathological consideration of this disease is that necrosis develops as a primary event, with subsequent recruitment of inflammatory cells. As such, this group of dogs probably represents a very early phase of the disease, where they are likely to have negative biochemical changes (due to a lack of pancreatic tissue involvement), whilst they will have imaging changes. Whether this group of animals needs to be considered a separate entity clinically is debatable, as signs will occur due to systemic activation of inflammatory pathways.

To date, the current sensitivity and specificity of clinical suspicion and imaging findings to diagnose pancreatitis in dogs is not known. It may well be that good clinical acumen along with abdominal imaging may prove to be more sensitive and specific for diagnosing acute pancreatitis than any single laboratory analyte.

THYROID SCINTIGRAPHY
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Thyroid scintigraphy is a useful tool in the diagnosis of thyroid disease, providing information regarding both structure and function integral to the management of diseases of the thyroid. Thyroid scintigraphy can be particularly helpful for challenging cases where dysfunction is suspected but not supported by endocrine testing as well as for investigation of masses involving the cervical region. Although the technique is largely limited to academic institutions and specialist practices, it remains a simple, non-invasive and cost effective procedure. Radiation safety regulations require hospitalisation of the patient until the radiation has decayed and, for Victoria, this typically means that the cat or dog is hospitalised for 24 hours following administration of the technetium (99mTc) pertechnetate.

Due to radiation emission characteristics, half –life, quality of images produced and timing of the scanning procedure, the most commonly used radio-isotope is 99mTc. The isotope is administered intravenously and uptake is measured by detection of γ-radiation using a gamma camera. The cervical region and thorax are scanned in sternal, and both left and right lateral recumbency. The patient is sedated to reduce movement artifact given the length of time required to acquire the scan. The scans provide both qualitative (location) and quantitative (based on count densities) information. The 99mTc is taken up by the thyroid glands, salivary glands and gastric mucosa. It is excreted into the urine and will rapidly start to appear within the urinary bladder.

Indications for thyroid scanning are varied and include evaluation of the functional status of the thyroid glands (hypothyroidism and hyperthyroidism), establishing the location of functional thyroid tissue (unilateral, bilateral, ectopic and metastatic), calculation of radio-iodine (131I) dosage for treatment of hyperthyroid cats, assessment of thyroid tumours, evaluation of tumours of unknown origin in the cervical region and determining efficacy of treatment following surgery or 131I therapy for thyroid tumours1-12.

Diagnosis of feline hyperthyroidism is often straightforward but mild cases may present with a normal serum total thyroxine (T4) concentration, concurrent illness may reduce the T4 concentration to normal and increased free T4 concentrations can be associated with non-thyroidal illness4,5. In such cases, thyroid scintigraphy can confirm the presence of hyperthyroidism. Additionally, the scans will determine whether there is bilateral or unilateral involvement, may differentiate between benign and malignant thyroid disease and identify ectopic or metastatic tissue. False positive ectopic or metastatic tissue may be caused by accumulation of saliva in the pharyngeal region or oesophagus and skin contamination4,5.

Diagnosis of canine hypothyroidism can also prove challenging due to the variable presentation of non-specific clinical signs, inconsistent biochemical changes and influence of concurrent disease or drug therapy. Thyroid scintigraphy can distinguish between hypothyroidism and sick euthyroid syndrome7.

The majority of dogs with thyroid neoplasia are euthyroid and most investigations are initiated due to the presence of a palpable mass in the cervical region8-12. In this instance, thyroid scintigraphy will determine whether the mass is thyroid in origin. Non-thyroidal tumours may displace the thyroid glands but there will still be normal uptake by the two thyroid glands9. In thyroid neoplasia, there may be increased uptake by the tumour (more commonly) with or without suppression of the contralateral gland or there may be minimal uptake by the tumour and a normal contralateral gland9. Lack of uptake of 99mTc by the primary tumour indicates that metastases will not take up the isotope either and thoracic radiographs are therefore required in this case to determine whether metastases are present. 131I therapy is not recommended for tumours where there is minimal uptake of 99mTc11,12. Conversely, when the primary tumour demonstrates increased uptake of 99mTc, this indicates that there is a high iodine trapping ability and that treatment with 131I is likely to be effective11-12. Associated metastases are also likely to take up 99mTc. In a small proportion of dogs with primary thyroid cancer positive for 99mTc uptake, the thyroid tumour, however, will not accumulate 131I10. This may explain the apparent lack of response to 131I therapy observed in some dogs with primary thyroid cancer12. There is conflicting evidence regarding whether scintigraphy using 99mTc is more or less sensitive than thoracic radiography for detection of pulmonary metastasis arising from a primary thyroid tumour8,9. It has been suggested that lack of 99mTc uptake by metastases may be due to competitive uptake by the primary tumour. Thoracic radiographs may therefore be helpful in some cases where there is no uptake of 131I by the pulmonary parenchyma.


Small Animal Medicine and Feline Chapters
GFR AS ASSESSED USING SCINTIGRAPHY

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The glomerular filtration rate (GFR) is the volume of fluid filtered from the renal glomerular capillaries into Bowmman's capsule per unit time, thus it measures the ability of the kidneys to filter and remove wastes. Scintigraphy GFR is the least invasive and most rapid method available to measure GFR in dogs and cats, and the reliability of the method has been shown to be comparable to standard methods of GFR determination (Krawiec et al., 1986). Only scintigraphy GFR and CT GFR (O’Dell-Anderson et al., 2006) can evaluate the GFR of each kidney individually (all other methods give a global value). Animals do not have to be sedated for scintigraphy GFR whereas CT GFR requires general anaesthesia. GFR measurements have been shown to be more sensitive for detecting mild/early kidney disease compared to measurement of serum creatinine and urea (Gleadhill et al., 1995).

Indications for GFR examination include; suspected kidney disease in a nonazotemic animal, assessment of contralateral kidney function when removal of one kidney is being considered, monitoring treatment for glomerulonephritis, assessment of suitability of hyperthyroid cats for radioactive iodine treatment, as a prognostic indicator for animal with renal disease, and to evaluate for obstructive uropathies. Scintigraphy GFR has also been used to monitor renal function in cats involved in kidney transplantation procedures (Halling et al., 2003).

In a study of hyperthyroid cats (Adams et al., 1997) it was found that GFR examination (using a cut off value 2.25ml/kg/min) prior to treatment of the cats with radioactive iodine detected not only the already azotaemic cats but more importantly detected all cats that were nonazotemic prior to treatment but became azotaemic within 30 days after treatment. All the cats in the study that had a normal GFR before treatment also had a normal GFR, were nonazotemic and could concentrate their urine 30 days after the treatment. Thus, GFR scintigraphy is a very valuable test for cats that are candidates for radioactive iodine treatment for hyperthyroidism.

Technetium-99m diethylene-triaminepentaacetic acid (DTPA) is the radiopharmaceutical used for scintigraphy GFR. DTPA is not excreted or reabsorbed in the renal tubules, thus removal of the DTPA from the blood depends entirely on glomerular function. Since only a very small amount of DTPA binds to plasma protein it is possible to calculate the GFR by measuring its rate of uptake in the kidneys.

The GFR can be calculated from the scintigrams using either a regression correlation, which must be established for each lab (Gates, 1982) or by expressing GFR relative to plasma volume that is calculated using a Patlak plot of the kidney and left ventricle regions of interest (Peters et al., 1994). The advantage of the plasma volume method is that, in contrast to Gates method, hydration and body conformation should not influence the results of the GFR.

GFR scintigraphy takes about 10 minutes and is done in 4 steps. Animals are not sedated for the GFR examination, and may go home with their owners directly after the examination.

- The exact amount of radioactivity to be injected is measured by counting the activity in the syringe in front of the gamma camera.
- The animals are positioned in left lateral recumbency and the gamma camera is positioned dorsally to include the kidneys and the thorax. A dynamic study is obtained with a frame rate of 1 frame per 10 seconds for 5 minutes.
- The camera is rotated 90 degrees above the dog and a static lateral 60 second image is made to measure the kidney depth.
- The camera is then returned to its original position and the injection tubing, iv catheter and syringe are counted on a stand.

The most important information obtained from the GFR is the time activity curve (figure 1A and 1B). This shows the rate of uptake and excretion of the radiopharmaceutical by the kidneys, and with this information the GFR values are calculated using a computer program. The normal values we use are from measurements from a group of clinically normal dogs that were examined with scintigraphy GFR at our clinic (Kampa et al., 2003, Kampa et al., 2007). Normal dogs measured at our clinic had a total GFR greater than 2.66ml/min/kg (Gates method) or 30.8ml/min/L (Plasma method) and individual kidney values greater than 1.3ml/min/kg (Gates method) or 15.4ml/min/L (Plasma method). No normal values are available from our lab for cats, but several references consider total GFR values above 2.5ml/min/kg as normal for cats (Uribe et al., 1992, McCord et al., 2008). There has been the suggestion that cats might have a mildly lower and more variable normal GFR variable compared to dogs (Hecht et al., 2008).

Time to peak can also be measured from the time activity curve. This is the time from injection to maximum kidney activity, and normal dogs at our clinic had a range of 80-210 seconds (Kampa et al., 2002). Other studies have found wider ranges of time to peak in normal dogs and cats (Hecht et al., 2006, Hecht et al., 2008). A prolonged time to peak indicates slow renal uptake of the radiopharmaceutical, or delayed outflow of urine (can be intra-renal or post renal
causes) (figure 1C). Diagnosis of urinary tract obstruction in the renal pelvis or ureter regions can in some cases be more accurately evaluated by giving furosemide during the excretory phase of the GFR examination (Hecht et al., 2010a, Hecht et al., 2010b)

Figure 1:
A. Time activity curve of a normal dog. 1 is the vascular phase, 2 is the uptake phase, 3 is the outflow phase. Left kidney above, right kidney below.
B. Logarithmic plots of kidney uptakes (above) and left ventricle activity (below)
C. Time activity curve showing prolonged time to peak from a dog with low GFR and slow renal uptake of the radiopharmaceutical.

TRANS-SPLENIC PORTAL SCINTIGRAPHY
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Portal scintigraphy is used to investigate the presence of macroscopic vascular shunts between the portal vasculature and the systemic circulation resulting in bypass of the hepatic sinusoids, so called portosystemic shunts. When signalment, history, clinical signs and changes in blood parameters (especially high post-prandial bile acids and high ammonia) suggest a portosystemic shunt then portal scintigraphy is a rapid and reliable method of determining whether or not a shunt is present.

In 2007 we changed our portal scintigraphy technique from the traditional per-rectal portal scintigraphy to trans-splenic portal scintigraphy (TSPS) (Cole et al., 2005). The TSPS method results in a nuclear angiogram of the portal vasculature and uses a lower dose of technetium compared to the per-rectal method (Sura et al., 2007) Dogs are lightly sedated (acepromazine maleate and butorphanol, if necessary propofol is given) and cats anaesthetised (propofol) for TSPS. An injection of approximately 40-80 MBq of 99mTc-pertechnetate is suspended in 0.2ml saline and is injected using ultrasound guidance into the spleen. The images are obtained as a dynamic study using 1 frame per second over a 3 minute period. The animals may go home after they have recovered from the sedation/anaesthesia.

The information from the portal scintigraphy can be evaluated in several ways. The dynamic frames can be examined individually or these images can be played as a movie. The TSPS method gives such good definition of the pathway of the movement of the pertechnetate bolus that usually a diagnosis can be made from looking at these images. Using a summated static image from the dynamic study, regions of interest can be manually drawn around the region of the liver and the region of the heart. A time activity curve can be generated using the data from the regions of interest and show whether the activity reaches the liver or the heart first, calculate the transit time and see the relative activity in the liver region compared to the heart region (figure 1A and 1B). In normal dogs using the TSPS technique the activity arrives in the heart 5 to 10.8 seconds after the liver (Cole et al., 2005), this is called the transit time. A portosystemic shunt is diagnosed when the transit time is less than 5 seconds (often the activity reaches the heart before the liver) and the activity in the heart is persistently higher than the liver.

Figure 1 - A

![Liver and heart counts](image1a.png)

Figure 1 - B

![Liver and heart counts](image1b.png)

Figure 1: Liver and heart time activity curves for a shunt negative (A) and a shunt positive (B) TSPS study. The transit time in the normal dog is 9 seconds compared to the transit time of 2 seconds in the dog with the shunt. The dog with the shunt has persistently higher activity in the heart region compared to the liver region.

Using TSPS it is possible to differentiate single compared to multiple shunts, but it is not possible to distinguish intrahepatic shunts from extrahepatic shunts (Morandi et al. 2005). Animals with hepatic microvascular dysplasia may...
have normal portal scintigraphy studies. Four image patterns, suggesting shunt type have been recognised by the pathway of the pertechnetate from the splenic injection to the heart in TSPS images; portoazygous shunts, porto/splenocaval shunts, internal thoracic shunts (Morandi et al. 2005) and multiple acquired shunts (Morandi et al. 2010). TSPS is very good at determining whether or not a shunt is present BUT, it usually does not provide the anatomical shunt morphology information required for surgical planning of shunt ligation. Thus, a positive portal scintigraphy is usually followed up with either; ultrasound, intra-operative mesenteric portography, CT angiography or Magnetic Resonance Angiography (Time of Flight or Contrast Enhanced) examination to get the morphological information the surgeon requires.

There is limited information available regarding the use of TSPS in cats (Morandi et al. 2010) but from the few cases we have examined it has been very useful. Left gastric portocaval shunts are the most common type of shunt in cats and since the left gastric vein empties into the splenic vein TSPS should work well with these types of shunts.

An occasional problem with the TSPS technique is leakage of the pertechnetate from the intra-splenic injection into the peritoneal cavity. This poses no risk to the animal but can create some problems for image interpretation due to the increased background activity in the abdominal region and the reduced amount of activity in the bolus of activity that passes through the portal vein. This problem has occurred in a small number of our studies but we have found that repeating the intra-splenic injection several minutes later has resulted in diagnostic studies.

A technique of TSPS using $^{99m}$Tc-mebrofenin has been described for normal dogs (Morandi et al. 2007). The intra-splenic injection of mebrofenin results in a nuclear angiogram of the portal vasculature similar to that obtained with pertechnetate except that a much higher percentage of mebrofenin is retained in the liver. Thus, in normal dogs only a very small amount of activity reaches the heart in mebrofenin TSPS, and this should result in very high levels of certainty for shunt diagnosis. Additionally mebrofenin can be used to evaluate hepatic function since mebrofenin passes through the liver via the same transport mechanism as bilirubin. The hepatic function study is performed by taking static images of the abdomen every 5-15 minutes for 1-2 hours after the mebrofenin injection. Abnormal hepatic function is indicated prolonged blood pool activity, retention of the radiopharmaceutical in the liver region and prolongation of the time taken for the radiopharmaceutical to pass into the intestinal tract. The hepatic function study should be most useful in dogs that are negative for a portosystemic shunt in the angiogram phase of the examination, to further investigate the cause of their liver disease.

FILM READING SESSION
In immune-mediated haemolytic anaemia (IMHA) and immune-mediated thrombocytopenia (ITP), red blood cells and thrombocytes are destroyed as a consequence of anti-erythrocyte and anti-platelet antibody production, respectively. Antibodies bound to the surface of the respective cells cause direct lysis as a result of intravascular complement activation (in the case of IgM) or premature removal from circulation by macrophages in the spleen and liver. Immune mediated thrombocytopenia may occur alone or in association with IMHA in dogs (and in human medicine the rare condition of concurrent or sequential IMHA and ITP without known underlying cause is referred to as Evans’ syndrome). Both IMT and IMHA may be primary or secondary in nature. Secondary IMHA and ITP occur when there is an underlying disease process such as neoplasia, infectious diseases such as viral, bacterial, rickettsial, or parasitic diseases such as protozoan or metazoan infections. Furthermore, exposure to drugs, including vaccines, and other toxins may lead to immune-mediated haematological disease. In contrast, primary IMHA and ITP refers to immune-mediated destruction of red cells and platelets in the absence of an identifiable cause. Both diseases are serious, potentially life-threatening; studies of IMHA have reported mortality rates up to 70% in the first 3 weeks of treatment (Jackson et al., 1985; Burgess et al., 2000) and approximately 40% of dogs with ITP die or are euthanased during the initial episode of thrombocytopenia or because of disease recurrence (Williams et al., 1984; Jackson et al., 1985).

The clinical signs of IMHA include lethargy, inappetence, pigmentation (during intravascular haemolysis), tachycardia, pale mucous membranes and, occasionally, fever. Signs of primary haemostatic dysfunction predominate in ITP, characterised by epistaxis, lethargy, petechial and ecchymotic haemorrhages, haematochezia, ocular haemorrhages and weakness. For both IMHA and ITP certain breeds such as Cocker Spaniels, miniature, toy and standard Poodles, Old English Sheepdogs and German Shepherd dogs have been reported to have a higher incidence of disease compared with control populations, but any breed of dog including cross-bred individuals may be affected. A haematological examination is essential for the diagnosis of both conditions; in IMHA there may be gross or microscopic haemagglutulation (indicating the presence of anti-erythrocyte antibodies), spherocytosis, and increased erythrocyte fragility. A Coombs’ test is often used to support the diagnosis of IMHA but this is neither a sensitive nor specific test and does not allow distinction to be made between primary and secondary IMHA. Similarly, a variety of criteria have been applied to the diagnosis of canine ITP (Lewis & Meyers, 1996) including the severity of the thrombocytopenia, the presence of platelet fragments, normal to increased numbers of megakaryocytes in the bone marrow, detection of anti-platelet antibodies, and increased platelet counts in response to immunosuppressive drug therapy.

Protocols for the evaluation of IMHA and ITP, in particular those directed at determining the presence or absence of underlying disease, include a thorough evaluation of the patient’s history (with special reference to recent illnesses, drug therapy or vaccination, and travel history), a detailed physical examination, and further ancillary testing such as diagnostic imaging, bone marrow cytology and infectious disease profiles. The potential role of infectious and parasitic diseases in the development of IMHA and ITP cannot be overstated, yet the diagnostic tests available to practitioners for these disorders in Australia at least, are somewhat limited.

Collectively, infectious diseases are recognised as important promoters of immune-mediated disease. This is especially true of the vector-borne diseases, including leishmaniasis, anaplasmosis, babesiosis, ehrlichiosis, and other rickettsial diseases. It has been reported that vector salivary proteins injected into the dermal microenvironment during tick feeding are able to modulate the host’s immune system creating a relatively favourable environment for the survival and reproduction of infectious agents (Day 2005; Wikel et al., 2001). This manipulation of the host’s immunity tends to promote Th-2 regulated humoral responses over the protective Th-1 cell mediated responses. This has the potential to allow persistence of the infection, but more importantly from a clinical perspective, it encourages the development of inappropriate secondary immunopathology characterised by autoantibodies, complement activation and hyperglobulinaemia. IMHA and ITP are the two most common immune-mediated consequences of the production of such autoantibody, so it should not be surprising that thrombocytopenia and, to a lesser extent, haemolysis are commonly detected in association with infectious disease, especially haemoparasites.

A number of canine and feline vector-borne diseases have been reported in Australia, including babesiosis (Babesia vogeli and B. gibsoni), anaplasmosis (Anaplasma platys), haemotropic Mycoplasma infections (M haemofelis, Candidatus Mycoplasma haemominutum, Candidatus Mycoplasma turicensis, and Mycoplasma haemocanis), and most recently, Rickettsia felis. To date, canine leishmaniasis and babesiosis in cats has been identified only in recently imported individuals (unpublished observation) and the important haemotropic infectious diseases ehrlichiosis and hepatozoonosis (transmitted by R. sanguineus), have never been reported in Australia. The flea-borne pathogens Bartonella henselae and Bartonella clarridgeiae have been isolated in cats and their ectoparasites in eastern Australia.
but other species of *Bartonella* that are well recognised as causes of thrombocytopenia in dogs in other parts of the world (e.g. *B. vinsonii* subsp. *berkhoffii*) have not yet been identified in Australia.

Platelet-bound antibodies have been reported in association with *B. canis* infections (Lewis et al., 1995), *B. gibsoni* (Wilkerson et al., 2001), leishmaniasis, ehrlichiosis and *A. phagocytophilum* (Terrazzano et al., 2006; Grindem et al., 1999; Mazepa et al., 2010), usually in association with the production of other circulating immune complexes or auto-antibodies. Studies of *Babesia gibsoni* in Japan demonstrated the presence of IgG and IgM red cell-associated antibodies for erythrocyte membrane antigens (Adachi et al., 1992) and a recent European study has suggested that Coombs’ test positivity is more likely to be associated with *B. vogeli* infections than *Babesia canis* (Carli et al., 2009).

This presentation will review the immunological mechanisms by which infectious and parasitic diseases cause secondary ITP and IMHA and will discuss the diagnostic strategies available to detect these ‘stealth’ invaders that cause so much host-mediated pathology.

Immune-mediated haemolytic anaemia (IMHA) is a common cause of anaemia in dogs and cats. IMHA can be either primary (idiopathic or autoimmune) or secondary. Primary IMHA, a classic autoimmune disorder with no recognised underlying cause, is the most frequent form of IMHA in dogs. The condition typically affects young adult and middle-aged animals, and is most common in cocker spaniels, English springer spaniels, poodles, and old English sheepdogs. IMHA can also occur secondary to a wide range of infectious, inflammatory or neoplastic processes. Important causes of secondary IMHA in small animals include Feline Leukaemia Virus (FeLV) or haemobartonellosis (mycoplasmosis) in cats, and recent vaccination or neoplasia (particularly lymphosarcoma) in dogs. Various medications have also been reported to trigger IMHA. Secondary IMHA affects animals of any age or breed, and should be strongly suspected in patients with a signalment atypical for primary IMHA, such as geriatric animals. Unlike the dog, IMHA in the cat is most commonly secondary. Distinction between primary and secondary IMHA is therapeutically important because secondary IMHA will often respond poorly to treatment, or recur, unless the underlying cause is recognized and eliminated.

MECHANISMS OF RED CELL DESTRUCTION

The mechanism underlying typical cases of IMHA is antibody-mediated cytotoxic (Type II) destruction of circulating red blood cells (RBCs). Although most cases share this common mechanism, the disease is otherwise very heterogeneous: in primary IMHA, the most studied form of IMHA, both the pattern of immunoglobulin and complement involvement in RBC destruction and the site of antibody attachment to RBC membranes varies widely between patients. Although the most common immunoglobulin type involved in primary IMHA is IgG, less commonly IgM may also be implicated, along with variable involvement of complement. Antibodies have been reported to attach to various components of the RBC membrane, particularly (but not exclusively) glycoporphins.

Antibody attachment to cell membranes triggers RBC destruction by a number of different mechanisms. With high levels of antibody attachment and, particularly, complement fixation (with involvement of the membrane attack complex), membranes may be so damaged that extracellular water leaks into the cytoplasm, causing swelling and rupture of the RBC while it is still in the circulation, so-called intravascular haemolysis.

In the absence of direct RBC lysis, antibody attachment and subsequent cell membrane damage can still lead to an accelerated rate of destruction of affected RBCs by tissue macrophages within the mononuclear phagocytic system (MPS), a process that occurs outside of the circulation (extravascular haemolysis). MPS destruction of RBCs is mediated by Fc receptors on the macrophage surface, receptors which bind the Fc component of the antibodies attached to the RBC membranes. Since the MPS is located throughout the body, extravascular haemolysis can occur in many organs, but typically is most pronounced in the liver and, particularly, the spleen.

In some patients with high levels of anti-RBC antibodies, many individual antibodies can each bind to two different RBCs, a process that causes the cells to clump together (agglutinate). Patients that exhibit significant RBC agglutination at body temperature typically have an increased rate of extravascular haemolysis, since clumping of RBC slows their passage through vessels and facilitates their removal by the MPS.

Typically, IMHA is caused by antibodies directed against circulating, mature RBC, with the marrow mounting a healthy regenerative response to the resultant anaemia. However, in some small animal patients (perhaps up to about one third), antibodies may also be directed against marrow RBC precursors at any stage in their development. Haemolytic anaemia with an inappropriately poor regenerative response will develop if antibodies are directed against cell membrane components that are present both on mature RBC and their marrow precursors. In contrast, if antibodies are directed against membrane components that are present only on marrow precursors, and not on mature RBC, non-regenerative anaemia will develop without peripheral haemolysis. Pure red cell aplasia (PRCA), in which all stages of marrow RBC precursor are dramatically reduced or absent, is the most extreme form of this process.

In primary IMHA, autoantibodies are directed against components of the patient’s own RBC membrane. Although the same process can occur with secondary IMHA, antibodies may alternatively be directed against a foreign antigen (such as a drug or virus) that is attached to the RBC membrane, against normal RBC membrane components that are antigenically similar to non-RBC antigens that are associated with the underlying disease process, or against membrane components that are normally hidden but are exposed by the underlying disease.
CLINICAL SIGNS
Signs typically associated with IMHA reflect the presence of both anaemia (lethargy, weakness, pale mucous membranes, and a haemetic heart murmur) and compensatory responses caused by tissue hypoxia and stimulation of the sympathetic nervous system (tachypnoea, tachycardia, and bounding pulses). Some patients may also show clinical signs of an ongoing immunological or inflammatory process, such as pyrexia, anorexia and, uncommonly, lymphadenopathy. Surprisingly, since the MPS within the spleen and liver is usually the main site of RBC destruction, organomegaly is only variably present in animals with IMHA. Patients with IMHA of acute onset tend to be very severely affected by their anaemia, and are often very depressed, weak or even collapsed. Hyperbilirubinaemia, bilirubinuria and tissue jaundice are often seen during acute severe episodes of IMHA. Since intravascular haemolysis is uncommon, haemoglobinemia and bilirubinuria are observed very infrequently. Patients with extravascular haemolysis due to sub-acute or chronic IMHA can compensate to some extent for their lack of erythrocytes, and may be remarkably bright despite the presence of severe anaemia. In these patients, the liver can often cope with the extra bilirubin released by RBC breakdown, and jaundice does not occur. Pulmonary thromboembolism is a well-recognised complication of IMHA, and is particularly common in those animals with acute severe anaemia that are receiving high dose glucocorticoids. Pulmonary thromboembolism should always be suspected in those anaemic patients that suddenly develop severe and persistent dyspnoea, although other causes of dyspnoea such as cardiogenic pulmonary oedema or acute bacterial pneumonia should also be considered, especially in dogs already receiving glucocorticoid and immunosuppressive therapy. Disseminated intravascular coagulation (DIC) can also complicate severe cases of IMHA.

DIAGNOSIS OF IMHA
Haematology in patients with IMHA typically reveals a moderate to severe anaemia, which is most commonly regenerative, with anisocytosis, polychromasia, a high corrected reticulocyte count and, sometimes, increased numbers of nucleated RBCs. Reticulocyte counts can however sometimes be inappropriately low, either because antibodies are also directed against RBC precursors, or because anaemia is peracute (since it takes about 3-5 days for the marrow to mount a strong regenerative response). White cell and neutrophil counts are often moderately to markedly increased, probably in response to both non-specific marrow stimulation and the inflammatory process associated with RBC breakdown. Occasionally, white cell counts can be high enough to mimic myelogenous leukaemia, a reaction sometimes called a ‘leukaemoid response’. Platelet counts are usually normal unless the animal also has immunemediated thrombocytopenia (IMT). Concurrent IMHA and IMT, a condition known as Evan’s syndrome, may affect up to approximately 10% of dogs with IMHA. Haematology can often also reveal clues that suggest a specific aetiological diagnosis:

- **Spherocytosis:** Spherocytes are small spherical erythrocytes that, when present in high numbers, strongly suggest a diagnosis of either primary or secondary IMHA. The absence of spherocytes, however, does not absolutely exclude a diagnosis of IMHA. Spherocytes are formed when tissue macrophages remove a piece of RBC membrane without cell destruction or a significant loss of cytoplasm. Spherocytes can be difficult to recognise in cats, because normal feline RBCs tend to be smaller and less discoid than canine RBCs. Experienced veterinary clinical pathologists, however, may be able to recognise the presence of spherocytes in the cat.
- **Agglutination:** Examination of blood smears may reveal microscopic autoagglutination (clumping) of RBCs. Such agglutination can form large rafts of RBC that, when a collection tube containing anticoagulated blood is closely inspected, are visible to the naked eye as multiple red speckles. Similar speckles can however be created by rouleaux formation, a phenomenon that can occur in normal animals, especially cats. Clinicians should therefore perform a saline dilution (one drop of RBCs to one drop of saline in dogs, one drop of RBCs to two drops of saline in cats) slide agglutination test to differentiate rouleaux from genuine autoagglutination. True agglutination can be seen grossly as persistent speckles despite dilution with saline, and microscopically as non-linear clumps of RBCs. A positive slide agglutination result is highly suggestive of a diagnosis of IMHA, and also suggests that the condition is likely to be acute and severe. A negative slide agglutination does not rule out IMHA, since in fact a negative result is reported to be the most common result in small animals with IMHA because most actually have non-agglutinating antibodies. Recent clinical studies of canine IMHA, however, report a much higher incidence of positive slide agglutination, perhaps reflecting a referral bias as a result of practitioners tending to refer only the more severe cases of IMHA. Automated haematology analysers sometimes register a clump of agglutinated RBCs as a single cell, often of a size too large to be recorded as a RBC at all. Resultant erroneous results may include an artefactually high MCV or, if clumped cells are not recognised as erythrocytes, lowering of the calculated haematocrit. Since the haemoglobin within all RBCs is still measured by the analyser, this leads to an erroneously high estimation of mean corpuscular haemoglobin concentration (MCHC). When agglutination is suspected to be the cause of a lower than expected haematocrit, packed cell volume (PCV), which is not affected by RBC clumping, should be monitored using microhaematocrit tube centrifugation rather than an automated analyser.
Other RBC Abnormalities: Careful examination of RBC morphology may suggest an underlying cause of either immunological or non-immunological haemolysis. Diagnostically useful RBC abnormalities include detection of parasites such as *M. haemofelis* (which may cause secondary IMHA), Heinz bodies (suggesting haemolysis secondary to oxidative damage) and schistocytosis (suggesting a microangiopathic haemolytic process such as DIC).

Serum biochemistry and urinalysis are often normal in dogs with IMHA. Potential abnormalities that may be seen in some patients include mild to moderate elevation of liver enzymes (thought to indicate hepatic hypoxia secondary to severe anaemia) and variable hyperglobulinaemia. Since serum albumin is usually normal, hypoalbuminaemia is an unexpected finding that may suggest that anaemia is in fact due to occult blood loss rather than haemolysis, or that the patient also has another illness. Mild to moderate hyperbilirubinaemia and bilirubinuria may be seen transiently in animals with acute severe anaemia. Since the liver is usually able to cope with all but the transient overwhelming bilirubin loads produced by acute severe haemolysis, severe hyperbilirubinaemia or persistence of jaundice for more than 3 to 5 days, even in the markedly anaemic animal, usually indicates the presence of concurrent hepatic disease or biliary obstruction. Haemoglobinuria and haemoglobinuria are uncommon, transient events that indicate the presence of severe intravascular haemolysis.

Immunological testing
Specific immunological testing can be used to support a tentative diagnosis of IMHA. The most widely used test is the direct antiglobulin test (DAT) or Coombs’ test, which detects antibodies and/or complement bound to RBC membranes. A standard DAT as provided by most laboratories typically uses a mix of antibodies directed against IgG, IgM (to a variable extent) and complement, and is performed at body temperature. Modifications of the routine screening DAT that may increase its diagnostic value include running the test at different temperatures and titres, and using individual antibodies against IgG, IgM, IgA and complement as well as the standard polyvalent antibody/complement mix. Positive DAT results at 4° Celsius, however, are of minimal diagnostic significance unless the patient has clinical signs consistent with cold antibody type agglutination or intravascular haemolysis.

Strictly interpreted, a positive DAT supports a diagnosis of IMHA, while a negative test suggests a non-immunological cause of haemolysis. Numerous studies, however, have shown that a DAT can often be of only mediocre diagnostic accuracy: although sensitivity and specificity undoubtedly improve with meticulous attention to test methodology, the fact remains that both false positive and false negative results do occur relatively commonly. Veterinarians should therefore be aware that since IMHA can occur in the presence of a negative DAT and, conversely, a positive test does not absolutely prove the presence of IMHA, sometimes a diagnosis must be made based on clinical judgement despite the presence of an apparently discrepant DAT result. Performing a DAT is however still recommended in all patients with suspected IMHA even if criteria such as spherocytosis or a positive slide agglutination already strongly suggest a diagnosis, since a positive DAT will add support to the diagnosis and characterise the disease further by determining the involvement of various immunoglobulin types and complement. Various other immunological tests for detecting anti-RBC antibody have been reported, including an enzyme-linked immunosorbent assay, and a direct enzyme-linked antiglobulin test but, although some of these tests may arguably be more sensitive than the DAT, they have not as yet become commonly available. Regardless of whether a DAT or an alternative test for ant-RBC antibody is used, however, clinicians should be aware that a positive result merely records the presence of antibody, and does not determine whether IMHA is primary (AIHA) or secondary.

Uncommonly, IMHA (with or without IMT) will be merely one component of systemic lupus erythematosus (SLE), a multisystemic immunological disturbance. Measurement of serum anti-nuclear antibody (ANA) is therefore indicated in those patients displaying evidence of the concurrent involvement of more than one body system, such as IMT, glomerulonephritis, polyarthritis, polymyositis or immune-mediated skin disease. In contrast, ANA is not indicated (and is usually negative) in those patients suspected to have uncomplicated IMHA.

**IDENTIFICATION OF UNDERLYING DISEASE**

Since IMHA is often secondary, particularly in cats and in dogs with an atypical signalment, confirmation of a diagnosis of IMHA is not necessarily the end of the diagnostic trail. Primary IMHA can only be diagnosed with absolute certainty once potential underlying causes have been thoroughly investigated. Unfortunately, this presents practitioners with a dilemma: although IMHA is unlikely to be treated effectively unless underlying causes have been eliminated, a complete search for such causes can be expensive, time-consuming, invasive and, in the case of primary IMHA, ultimately fruitless. Standard screening tests for underlying disease which ideally should be performed in all animals with IMHA include haematology (including careful examination of a blood smear), serum biochemistry, urinalysis, thoracic and abdominal radiography and, in cats, testing for retroviruses (particularly FeLV). Serologic and/or PCR testing for RBC parasites such as haemobartonellosis, now more correctly termed mycoplasmosis (*Mycoplasma haemofelis* in cats, *Mycoplasma haemocanis* in splenectomized dogs), *Babesia canis* (particularly in greyhounds) or *Babesia gibsoni* (particularly in pit bull terriers) is also often indicated. Since arguably rickettsial
diseases may also predispose to secondary IMHA, testing for *Ehrlichia* species may also be indicated in endemic areas. Further tests that might be considered in some patients, particularly in older animals in which underlying occult neoplasia (especially lymphoproliferative disease) is a real possibility, include abdominal ultrasonography, lymph node aspiration cytology, and bone marrow analysis.

**BONE MARROW ANALYSIS**

Bone marrow analysis (aspiration cytology and/or core biopsy histopathology) is also indicated in all patients suspected to have the non-regenerative forms of IMHA. Pure red cell aplasia is characterised by a relative or complete lack of RBC precursors within the marrow, whereas cytological or histopathological evidence of an erythroid ‘maturation arrest’ (preponderance of immature precursors, with an absence of more mature RBC precursors) suggests that, rather than being directed against very early stem cells, antibodies are directed against a later stage of marrow RBC development. Marrow cytology and/or histopathology may also reveal macrophages phagocytosing erythrocytes or RBC precursors. In such patients, when available, techniques such as immunofluorescent or immunoperoxidase staining of marrow samples may confirm the presence of antibodies directed against RBC precursors.

EMERGENCY MANAGEMENT OF IMMUNE-MEDIATED BLOOD DISORDERS
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The most common immune-mediated blood disorders in small animal patients are immune-mediated thrombocytopenia (IMT) and immune-mediated haemolytic anaemia (IMHA). Less common disorders that may have an immune-mediated component include pure red cell aplasia (PRCA), aplastic anaemia, amegakaryocytic thrombocytopenia and steroid-responsive neutropenia. Immune-mediated blood disorders can be either primary (idiopathic) or secondary. As a general rule, these disorders in dogs are most commonly primary, whereas in cats they are more likely to be secondary. Since treatment of IMHA and IMT has more similarities than differences, most therapeutic approaches apply equally well to both.

Veterinarians have been effectively treating individual patients with IMHA and IMT for many years. Standard therapy is based around transfusion as needed, coupled with immunosuppressive therapy (prednisolone or dexamethasone, with or without concurrent azathioprine, cyclophosphamide or cyclosporine) that is tapered and then discontinued. Unfortunately, however, there is a mounting body of evidence documenting that, with standard therapy, survival rates for IMT and (particularly) IMHA patients are unsatisfactory. For example, a recent retrospective study from Virginia-Maryland reported that, despite their best therapeutic efforts, the one-year survival rate for dogs with IMHA was still only 30%. Most other published studies have long-term survival rates of not much better than 50%. Deaths (naturally occurring or euthanasia) occurred either during initial hospitalisation, or at a later date due to disease recurrence or owner intolerance of long-term medication. Undoubtedly, there is a 'referral bias' that will exaggerate the severity of disease in some studies since, with recent advances in in-house diagnostics, better availability of transfusion products, and a greater understanding of immunosuppressive therapy, many general practitioners can now effectively treat the less severe blood disorders without referral. Critical patients with severe or complicated IMHA and IMT are more likely to be referred to specialist centres, and are also more likely to die despite treatment, contributing to the high mortality rates in studies that originate from referral centres. Nevertheless, despite the potential effects of this referral bias, it is still undeniable that mortality rates for the immune-mediated blood disorders are unacceptably high.

Two main priorities can be readily identified from analysis of IMHA and IMT mortality data: firstly, the rate of in-hospital deaths during the initial immune-mediated crisis must be reduced and, secondly, more long-term therapy must be tailored in order to avoid relapses while minimising expense and drug-induced side effects.

INITIAL INVESTIGATION
Since effective treatment cannot proceed without a correct diagnosis, a thorough work-up is always recommended during the initial management of IMHA and IMT. Given a working diagnosis of primary immune-mediated blood disease, standard therapy during an initial crisis will typically include immunosuppressive doses of glucocorticoids with or without other immunosuppressive agents, and transfusion as needed. Even if an underlying cause for secondary IMHA or IMT has been identified and removed, immunosuppressive therapy is still usually indicated during the initial treatment phase.

EMERGENCY DRUG THERAPY
Glucocorticoid therapy is a mainstay of both the initial and the chronic treatment of IMT and IMHA. Oral prednisolone (or prednisone) dosage at the commencement of therapy should be 2 mg/kg once or twice daily. Although some clinicians prefer to commence therapy with an initial dose of either intravenous dexamethasone (0.1 to 0.2 mg/kg) or intravenous high dose methylprednisolone (11 mg/kg daily for up to 3 days), there is minimal hard evidence that starting with intravenous steroid therapy hastens recovery. Typically, regardless of route of administration or starting dose, steroids are not immediately effective.

Immunosuppressive therapy with drugs such as azathioprine, cyclophosphamide or cyclosporine is also indicated in severely affected animals. Even in severely affected patients, these drugs are usually given orally at standard starting dose rates. Cyclophosphamide, however, is sometimes also given intravenously (200 mg/m²) in dogs with acute, severe IMT or IMHA. There is little evidence that commencing with a high-dose intravenous bolus of cyclophosphamide hastens recovery. In fact, several recent retrospective studies have reported high mortality rates in IMT patients that are initially treated with cyclophosphamide, even at standard conservative oral doses. Given the limitations of a retrospective study, however, it is by no means proven that cyclophosphamide actually increases mortality rates, since factors such as case selection bias (for example, clinicians may reserve the use of cyclophosphamide for their sickest patients) may influence apparent survival rates in animals treated with cyclophosphamide. Cyclosporine is also available in a solution for intravenous use (6 mg/kg, given over 4 hours) although, like cyclophosphamide, there is minimal strong evidence that intravenous administration hastens recovery during crises.

Small Animal Medicine and Feline Chapters
Dogs with IMT may respond to a single intravenous bolus of vincristine (0.02 mg/kg). The vinca alkaloid is inexpensive and usually well tolerated, and a recent paper has reported that a single initial dose of vincristine hastens recovery of platelet numbers in some canine patients. The vinca alkaloids have both mild immunosuppressive (impairment of MPS function, and inhibition of cell-mediated and humoral immunity) and thrombocytotic (stimulation of transient megakaryocyte platelet release) properties. Intravenous vinca alkaloids induce transient platelet number increases in many IMT patients; circulating platelet life-span may be prolonged following treatment, suggesting that the increased platelet number is due to decreased destruction as well as enhanced megakaryocyte platelet release. Vinca alkaloids avidly bind to tubulin, a major component of platelet microtubules. The antibody-coated vinca-containing platelets of IMT patients are subsequently phagocytosed by tissue macrophages. Vinca alkaloids are therefore selectively delivered in cytotoxic doses to the macrophages involved in platelet destruction (so-called ‘poison platelets’).

Vincristine is the vinca alkaloid most commonly used in the dog. Intravenous vincristine markedly increases platelet numbers in some canine IMT patients, often within two to three days. Vincristine (a single intravenous dose) is therefore recommended for the emergency management of canine IMT. Intravenous vinca alkaloid boluses are cleared from the circulation too rapidly for optimal vinca-platelet binding. Although weekly vinca boluses maintain remission in some human IMT patients, most eventually become refractory. Techniques maximizing vinca-platelet binding have improved remission rates: either constant vinca infusion over four to eight hours, or transfusion with platelets pre-incubated with vinca alkaloid (‘vinca-loaded’ platelets). Although reported, similar techniques have not been thoroughly clinically evaluated in the dog. Such techniques are labour-intensive, and are not commonly used in veterinary medicine. Vincristine is extremely corrosive if extravasated. Single vincristine doses are otherwise well tolerated. Chronic vincristine therapy has been associated with reversible peripheral neuropathy in humans, and a comparable vincristine-associated neuropathy has recently been reported in the dog. Vincristine inhibits platelet function in vitro. However, clinically significant platelet dysfunction of any significant duration which can be unequivocally attributed to vincristine has not been documented in vivo.

**SUPPORTIVE/ANCILLARY THERAPY**

IMT and IMHA patients with severe blood loss or haemolytic anaemia will be suffering from generalised tissue hypoxia, and will benefit from reducing oxygen demand by instituting strict cage rest until anaemia responds to therapy. The severely compromised patient can also be supported with oxygen supplementation. Haemoglobin oxygen saturation is however already near maximal, and supplementation with oxygen therefore increases saturation only minimally. Oxygen supplementation is also laborious and expensive. Since patients with IMHA have a normal blood volume, crystalloid or colloid fluid therapy is of little benefit and may contribute to volume overload. Hypovolaemic IMT patients, in contrast, may benefit from fluid therapy. An additional benefit of strict cage rest in IMT patients is that rest reduces the chances of traumatic vascular injury, which in turn reduces the chances of life-threatening bleeding in severely thrombocytopenic animals.

Since patients with IMHA are prone to pulmonary thromboembolism and DIC, particularly those with severe anaemia and/or a positive slide agglutination, and those requiring transfusion, some clinicians recommend using prophylactic heparin during the hospitalisation of severely affected animals. A safe low dose of heparin that does not cause spontaneous bleeding, and does not require careful monitoring of coagulation parameters, is 75 to 100 U/kg three to four times daily subcutaneously. Much higher doses of heparin (starting at 200-250 U/kg SC four times daily), titrated upwards in order to prolong partial thromboplastin times by at least 1.5 times baseline values, may however be more effective at preventing thromboembolism. Measurement of plasma heparin levels, with subsequent dosage adjustments to attain a therapeutic range, may prove to be another means of maximizing the benefit of heparin therapy. Plasma heparin assays have recently become available, via inhibition of factor Xa assays. The standard form of heparin that is currently used in veterinary medicine is unfractionated heparin. However, the use of low molecular weight forms of heparin such as dalteparin or enoxaparin (which, in people, have a more predictable bioavailability than unfractionated heparin) may allow safer and more effective anticoagulation: we are currently using enoxaparin at a dose of 0.8 mg/kg SC q6hrs, based on Xa inhibition assays. Although reported, similar techniques have not been thoroughly clinically evaluated in the dog. Such techniques are labour-intensive, and are not commonly used in veterinary medicine. Vincristine is extremely corrosive if extravasated. Single vincristine doses are otherwise well tolerated. Chronic vincristine therapy has been associated with reversible peripheral neuropathy in humans, and a comparable vincristine-associated neuropathy has recently been reported in the dog. Vincristine inhibits platelet function in vitro. However, clinically significant platelet dysfunction of any significant duration which can be unequivocally attributed to vincristine has not been documented in vivo.

**TRANSFUSION**

Cage rest and standard glucocorticoid and immunosuppressive drug therapy are successful in most small animal patients with non-life-threatening IMHA and IMT. However, initial response to therapy can sometimes be sluggish (a week or more), particularly in those animals with poor marrow responsiveness due to either peracute anaemia or immune-mediated damage to bone marrow RBC or platelet precursors. In the meantime, transfusion may be needed to support those patients with life-threatening acute and severe anaemia (PCV less than about 15%, or signs of severe compromise,
such as collapse, nystagmus or stupor). Transfused red blood cells often have a very short life span (days or even hours) in patients with IMHA, and transfusions may actually increase the rate of haemolysis (‘add fuel to the fire’). For this reason, transfusions should be avoided when possible in stable patients with IMHA. However, in those IMHA patients that are severely compromised, blood transfusions are life-saving, and should not be withheld. Transfused platelets in IMT patients typically have an extraordinarily short circulating survival time and, in fact, platelet numbers have often not even detectably risen immediately after a platelet transfusion. Transfusion to replace lost platelets is therefore rarely of value in IMT patients, although there is no real contraindication to trying a single test dose of a platelet product. Transfusion of RBC products in order to support hypovolaemic or anaemic IMT patients, on the other hand, can often be life-saving, even if the transfusion had no impact on platelet numbers.

In normovolaemic animals, such as most patients with IMHA, whole blood may be safely transfused at a rate of up to approximately 20 ml/kg/hour, at a maximum daily volume of 20 ml/kg. Multiple transfusions as often as every day or two may be needed in very severely affected animals. Since IMHA patients are typically normovolaemic, volume overload after transfusion can become a significant risk in animals that have already recently received high volumes of blood or other fluids. In these patients, blood transfusions should be given slowly (maximum rate of 4 ml/kg/hour). When available, packed red blood cells are preferable to whole blood. Since cross-matches are often positive in patients with IMHA (because the animal has antibodies against its own RBC, and can even ‘cross-match’ positive against its own blood, as well as donor blood), compatible or universal donors should be used if blood typing is available.

Over the past decade or so, bovine purified polymerised haemoglobin was used as an effective means of providing temporary (several days) oxygen-carrying support for the severely anaemic IMHA patient. Bovine polymerised haemoglobin was a very convenient blood product for use in general practice, in that it was associated with almost no risk of transfusion reaction, could be safely used without blood typing or cross-matching, and could be stored for up to two years at room temperature. Although the product was developed and marketed for use in dogs at doses of 10-30 ml/kg, bovine polymerised haemoglobin was also reported to provide effective temporary support to anaemic cats at a dose of 10 ml/kg given over several hours. Since the product was a colloid, it had the potential to cause volume overload if given too fast. One retrospective study reported a very high mortality rate in canine IMHA patients that received bovine polymerised haemoglobin, although these results may potentially have been affected by a pre-treatment case selection bias (that is, the sickest patients got the polymerised haemoglobin). Bovine polymerised haemoglobin was expensive and, unfortunately, has become for all practical purposes unavailable, at least for the foreseeable future.

In IMT patients with severe blood loss anaemia or hypovolaemia, fresh or stored packed red cell or whole blood products can be life saving. In severely hypovolaemic IMT patients with ongoing bleeding, blood can be given to effect at rates that can greatly exceed 20 ml/kg/day if needed. Although products such as platelet concentrate, platelet-rich plasma and fresh whole blood can be given in order to provide platelets, the transient survival time of most transfused platelets typically renders such treatments ineffective. The main focus of transfusion therapy in IMT patients therefore should be to provide red cell and volume support in the bleeding patient.

ADVANCED EMERGENCY THERAPY

Unfortunately, some animals with IMHA and IMT, despite appropriate standard therapy and multiple transfusions, succumb to severe anaemia or blood loss during the first weeks of treatment. Additional treatment options which may be used in a crisis include gammaglobulin, plasmapheresis and splenectomy.

High intravenous doses of human immunoglobulin (HIVIG), as a 6 to 12 hour infusion at doses ranging from 0.5 to 1.5 g/kg, occasionally cause rapid and sometimes sustained remission of immune-mediated disorders, including IMHA, PRCA and IMT. Human intravenous immunoglobulin is a pooled preparation of IgG obtained from the plasma of multiple healthy blood donors. Although HIVIG were initially produced for treatment of immunodeficiencies, they have also been shown to be beneficial in the treatment of human immune-mediated diseases such as IMT and IMHA. The main proposed mechanism of action of HIVIG is that the ‘antibody soup’ bathing the MPS binds to and overwhelms available macrophage Fc receptor sites, leaving no receptors left to bind antibody-coated cells. Alternatively, there may be some antibodies in the HIVIG soup that actually bind to and inactivate circulating anti-platelet or anti-RBC antibodies. The use of HIVIG in dogs is associated with few side effects, although there is some concern that treated animals have a higher incidence of pulmonary thromboembolism. Certainly, a high rate of pulmonary thromboembolism in HIVIG-treated patients was reported in the human literature, raising concerns about using HIVIG in canine IMHA patients. Pulmonary thromboembolism is less of a concern in IMT patients and, recently, HIVIG has been shown to safely and effectively shorten the duration of thrombocytopenia in dogs with IMT. Human gammaglobulin has not attained common usage in veterinary medicine, probably because of high cost and occasional limited availability.

Small Animal Medicine and Feline Chapters
Plasmapheresis and splenectomy, although reported to be useful in isolated cases, have also not entered into common use, and are usually considered treatments of last resort. Plasmapheresis, when available, is a very effective method of rapidly removing unbound anti-RBC or anti-platelet antibodies from the circulation, although antibodies that are already bound to cell membranes will persist and may cause ongoing disease.

Splenectomy is potentially a particularly effective treatment for IMT and IMHA because many different splenic elements contribute to the mechanisms reducing circulating blood cell numbers: anti-RBC or anti-platelet antibody production (splenic lymphocytes), antibody-coated platelet or RBC destruction (splenic MPS), and platelet or RBC sequestration (splenic vasculature). Splenectomy is the treatment of choice for most humans with chronic IMT or IMHA, with higher remission rates than medical therapy. In human IMT patients, platelet numbers often rise within several hours of splenectomy, with maximal increases within one to two weeks. Most human IMT and IMHA patients (60% to 80%) subsequently maintain adequate platelet or RBC counts without further medical therapy. Splenectomized patients that do require further treatment frequently demonstrate an improved response to medical therapy. Splenectomy is therefore recommended early in the course of chronic human IMT or IMHA. Splenectomy has not been thoroughly clinically evaluated in a large group of small animal IMT or IMHA patients, although several recent case series reporting early splenectomy in small groups of canine IMHA patients certainly showed some promising preliminary results. Other than these recent studies, published post-splenectomy remission rates for canine IMT and IMHA (each study limited to small patient groups) vary from poor to excellent. Since response rates appear to be unpredictable, early splenectomy currently cannot be strongly recommended for canine IMT or IMHA, particularly as medical therapy is often far better tolerated than it is in people. Splenectomy is indicated in canine patients refractory to glucocorticoids and immunosuppressive agents, particularly if associated drug side effects are unacceptable. Life-threatening post-splenectomy complications in people (overwhelming infection, SIC) are rare in the dog. The most commonly reported small animal post-splenectomy complication, erythrocyte parasitaemia (Haemobartonella [Mycoplasma], Babesia), usually responds well to medical therapy. Persistent IMT or IMHA post-splenectomy indicates ongoing platelet or erythrocyte destruction by the non-splenic MPS (usually hepatic macrophages). Uncommonly, post-splenectomy platelet or RBC destruction can also occur within an 'accessory spleen' (a detached splenic remnant with residual MPS function). Some authors recommend exploratory laparotomy in humans with persistent IMT or IMHA: removal of an accessory spleen may induce complete remission.

PRACTICAL BLOOD TRANSFUSION IN THE DOG & CAT, AND ALTERNATIVE BLOOD PRODUCTS
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WHAT BLOOD PRODUCTS HAVE WE GOT?
- Animal blood is an animal remedy and as such must be licensed if it is to be sold. To my knowledge, there are two licensed commercial sources of canine blood products in Australia. Packed red blood cells and fresh-frozen plasma are available from the University of Melbourne’s Canine blood bank. Caniplas™ (fresh frozen plasma) is available from some drug wholesalers. Plasma is sometimes further divided into cryoprecipitate (containing von Willebrand’s factor, fibrinogen and factors VIII, VIIIc, XIII) and cryosupernatant (non-labile clotting factors e.g. II, VII, IX, X and plasma proteins) by blood banks.
- Unfortunately, there is no commercial source of cat blood available in Australia at this time.
- Fresh whole blood can be sourced from clinic donors for in-house use. You can also make your own packed red cells and fresh plasma for in-house use if you have the need and the equipment.

WHAT BLOOD PRODUCTS OR ALTERNATIVES MIGHT WE GET?
- **Albumin solutions: human or canine:**
  Canine serum albumin and human serum albumin (HSA) products are available overseas. Human serum albumin is available for use in human medicine in Australia, though veterinary practices are unable to access this product for use in dogs.
  Albumin is used as a colloid for resuscitation and in hypoalbuminaemic patients. Immune mediated reactions, both immediate and delayed, have been reported in dogs treated with HSA. Human trials have failed to show improved outcome in patients treated with HSA for fluid resuscitation or hypoalbuminaemia.

- **Platelets**
  Severe thrombocytopenia or more rarely, thrombocytopathia can result in spontaneous bleeding. The most commonly seen cause of this in dogs is immune mediated thrombocytopenia. Platelet transfusions are not generally indicated in these patients as the transfused platelets survive only a matter of minutes or hours in the recipient. This may be long enough to provide short-term haemostasis in life-threatening situations such as intracranial haemorrhage.
  Fresh whole blood is currently the only source of platelets available in Australia.
  Fresh platelet concentrates cryopreserved and lyophilised (freeze dried) canine platelets are available in the US. The platelets have decreased activity and a shorter lifespan than freshly harvested platelets.

- **Haemoglobin based oxygen carriers**
  HBOC’s, such as Oxyglobin® have been developed. This product has never been for sale in Australia, and has been out of production. Recently, the media reported that an HBOC was sourced directly from the US manufacturer and used at the Alfred Hospital in Melbourne to save the life of a woman who refused blood transfusions for religious reasons.
  Oxyglobin® had several advantages: it provided effective temporary oxygen carrying ability without the need for cross matching or sourcing blood products; it had a long shelf life; it could be used across different species.
  Its use is not without issues however. It causes marked discolouration of plasma meaning many diagnostic blood tests cannot be used for several days. It is a potent small particle colloid and can easily result in volume overload, especially in normovolaemic anaemic cats.

WHEN IS A TRANSFUSION INDICATED?
The main indications for transfusion in small animal practice are anaemia and disorders of coagulation. Other indications include specific plasma component deficiencies and hypoproteinaemia.

**When do we give a transfusion of red blood cells or fresh whole blood?**
Clinically, we care about anaemia or a low packed cell volume (PCV) because it represents a decrease in the oxygen carrying capacity (CaO₂) of blood and therefore in decreased delivery of oxygen (DO₂) to the tissues.
In health, there is an excess of oxygen delivered to the tissues in the arterial blood. The cells extract what they need to leaving up to 75% of the oxygen to be returned to the circulation in venous blood. The proportion of delivered oxygen that is used in the tissues is referred to as the oxygen extraction ratio. Normal is about 25%. The tissues are easily able to increase this to a maximum of 50% e.g. during exercise or when the amount of oxygen being delivered is decreased. If oxygen use increases or delivery declines further, the tissues must start to metabolise anaerobically and accrue an oxygen debt.
Figure 1: Effect of haematocrit on viscosity and oxygen delivery

The PCV or haematocrit not only determines DO$_2$, it is also the main determinant of blood viscosity. Raising haematocrit simultaneously increases oxygen content and viscosity. Decreasing PCV and therefore viscosity will increase blood flow as long as cardiac output is maintained and there is no vasoconstriction. Decreasing viscosity has the additional advantage of decreasing cardiac afterload and augmenting stroke output.

The upshot of all this is, that because of compensatory changes in CO and oxygen extraction, progressive anaemia will not impair tissue oxygenation until the haematocrit reaches a dangerously low level – probably less than 15% in non-critically ill dogs and people, lower in cats.

Transfusion medicine is a very inexact art. To quote Marino, “transfusing red blood cells to correct anaemia is one of the most fickle and arbitrary interventions in critical care medicine”. In most cases, patients are transfused to a number: a haematocrit or haemoglobin value that has been chosen as the lowest “safe” number. The number is not usually chosen for that particular patient, or even for that disease process, but by the hospital or the attending clinician. And that is if there is even a consistent number: few human ICUs have standardised transfusion practices. Transfusions are often given to people without a documented evidence of need or benefit in the patient. I suspect that this is not quite such a problem in veterinary medicine as we have to source the blood and then justify the cost of the transfusion to the owners.

Transfusions are not without risk, have been shown to independently worsen outcome and, of special significance in veterinary medicine, contribute significantly to the cost of treatment.

Several questions need to be answered when we consider transfusing anaemic patients:

1. Is a low haematocrit harmful to the patient?
2. What is the lowest tolerable haemoglobin concentration in this patient?
3. Will blood component transfusion benefit the patient?
4. What harm will potentially be done to the patient by a transfusion?

A reasonable indication for the transfusion of red blood cells is to augment the oxygen carrying capacity of the blood, but:

- There is a lack of data defining the haemoglobin concentration in humans and in animals that hinders adequate oxygen delivery and initiates tissue hypoxia.
- Mild to moderate anaemia does not compromise oxygen delivery as long as intravascular volume is maintained. Studies show that the oxygen extraction ratio (VO$_2$/DO$_2$) progressively increases as a compensatory response to haemodilution. Isovolaemic haemodilution to a haemoglobin concentration of 5 g/dL in resting humans does not produce evidence of inadequate systemic oxygen delivery or adverse clinical effects.
- A Canadian trial (Transfusion Requirements In Critical Care, the TRICC study) demonstrated that a restrictive transfusion strategy (transfusion trigger: haemoglobin concentration of 7 to 9 g/dL) was at least equivalent if not superior to a liberal transfusion strategy (transfusion trigger: haemoglobin concentration of 10 to 12 g/dL) in critically ill patients. The average number of transfusions was reduced by 54% when the lower threshold was used, and 33% of the patients in the restrictive group did not require transfusion. The 30-day mortality rate was similar in both groups. In younger patients with lower illness severity scores, the mortality rate was significantly lower for the restrictive transfusion group than for the liberal group.
- PCV is in itself variable: plasma volume has a huge influence on haematocrit. In humans, the PCV increases by 4.1% in healthy volunteers when they go from a supine to a standing position and the plasma volume decreases by 420 ml. This is equivalent to a unit of blood!

The optimal haemoglobin concentration in critical illness is unknown. Patients with cardiac disease appear to require a higher haemoglobin concentration i.e. 10 to 12 g/dL, than patients with normal cardiac function. Critically ill patients have a changing plasma volume due to fluid shifts, IVF therapy and haemodynamic instability. The haematocrit is thus very variable and an unreliable indicator of oxygen delivery and the requirement for a transfusion.
So, when is a transfusion needed?
The transfusion trigger may be difficult to determine, especially in acute blood loss situations because PCV and TP do not accurately reflect the extent of blood loss until intravascular volume is normalised. Also, hypoperfusion contributes to the hypoxia. Transfusion therefore should be based on the status of the patient not just the PCV.

**Information we can use to decide whether a blood or red cell transfusion is indicated includes:**

- **Goal PCV:**
  - Flawed, unreliable but the “number” we use and rely on.
  - Less than 15% likely to be impacting on DO₂
  - Ideally > 20% in a dog with cardiac insufficiency?
  - Preferably > 25% prior to surgery in the dog, > 18% in the cat

- **Cardiac output:**
  - Increased viscosity when the PCV is much over 30% can decrease CO and cancel the benefit of the increased haematocrit.
  - Heart rate is often normal at rest in anaemic animals. Checking HR after a gentle walk outside can give us information about the adequacy of DO₂ with exercise. A slight increase in HR is appropriate after movement, especially if the animal is a bit painful. If the HR is markedly increased after a walk, inadequate DO₂ is one differential.

- **Systemic oxygenation:**
  - Lactate: inadequate DO₂ will result in anaerobic metabolism and increased lactate.
  - Base deficit: an indicator of anaerobic metabolism.
  - Oxygen utilisation, VO₂: clinically can be approximated by the arterial-venous oxygenation difference i.e. SaO₂ (from the pulse oximeter) – SvO₂ (from venous blood gases). Normal is about 25%, maximal OER is about 50%. If the OER is approaching 50%, it is markedly increased and VO₂ is about to become “supply dependant”.

In general, dogs with a PCV less than 20%, a PCV less than 25% due to acute loss or ongoing haemorrhage require blood products. In cats, normal PCV is a lot lower so they are often clinically stable with a PCV of 15% or less. Animals with acute loss of 20% or more of their blood volume will benefit from either whole blood or packed red blood cell transfusion + crystalloid fluid administration.

**When do we give a transfusion of plasma?**
Plasma is indicated to treat a coagulopathy and in some cases of hypoproteinaemia. Fresh whole blood is also a good source of clotting factors and contains protein. Stored whole blood (up to 4 weeks) will still contain some clotting factors and contains protein.

- Animals with an ACT or APTT greater than 150% of normal (i.e. longer than 180 seconds in most cases) are at risk of spontaneous haemorrhage.
  - Dogs that present with a coagulopathy, usually due to anticoagulant rodenticide intoxication, require one unit of whole blood (400 – 500 ml) or plasma (approximately 200 – 250 ml) per 10 – 20 kg. I will usually administer 1 unit per 20 kg then recheck clotting times.
  - Plasma transfusions will increase serum albumin by approximately 5 g/dL for every 20ml/kg of plasma given. That means that a 10 kg dog will have an increase in serum albumin of 5 g/dL per unit of plasma; a 30 kg dog an increase of only 1 – 2 g/dL.

**PRACTICAL TIPS: HOW DO WE GIVE TRANSFUSIONS?**
Points to remember:

- Have a concrete reason for the transfusion. Blood products are not “tonics” or “cure alls” nor should they be used prophylactically.
- Premedication with antihistamine? To reduce the incidence of acute hypersensitivity reactions? No clinical trials have yet been published to prove that this decreases transfusion reactions.
- I do not use steroids in my patients unless they are indicated for another reason. Steroids have not been shown to decrease the incidence of reactions and even one shot of dexamethasone in a hypovolaemic dog can cause profound GIT ulceration.
- Once a blood pack has had the administration set put into it, the transfusion should be completed within 4 hours to minimise the risk of bacterial growth in the blood product.
- Adhere to strict asepsis: blood is used as a microbiological culture medium!
- Use a blood administration set with a filter.
- Do not administer blood products with calcium containing fluids, hypo- or hypertonic solutions.
- Plasma has not been shown in human clinical trials or in animals to improve the outcome of pancreatitis or parvovirus patients unless they are coagulopathic or hypoalbuminaemic.
Monitoring during a transfusion
Start the transfusion at a low rate i.e. 1 ml/kg /hour. Monitor heart rate, temperature, and respiration rate every 5 minutes initially. Ideally the animal should also be on continuous ECG monitoring to detect any arrhythmias which may be the first sign of a transfusion reaction. If there are no problems in the first 15 minutes, the rate of product administration may be slowly increased up to a maximum rate of 10 ml/kg/hour. Continue monitoring closely for the first 1/2 an hour (cage side monitoring), then check every 15 - 30 minutes while remaining in the room with the animal at all times.
In an emergency situation, blood may be given more rapidly, though the faster products are administered, the greater the chance of a reaction.

Is Crossmatching Necessary?
Crossmatching assesses the effect that recipient serum antibodies will have on the donated red blood cells (major crossmatch) and the effect that donor serum will have on the recipient red blood cells (minor cross match).
Dogs have 8 different blood groups, dog erythrocyte antigens (DEA). Not all of them are important when it comes to transfusion reactions. DEA 1.1 is the most important cause of transfusion reactions, with 1.2 second and 7 also playing a role. Dogs have a low incidence of naturally occurring antibodies to red cells and are unlikely to react strongly to a first transfusion. So, if a dog has not had a transfusion before, and has not had puppies, a cross match is probably not strictly necessary unless the dog is likely to require repeated blood transfusions e.g. immune mediated haemolytic anaemia. Any transfusion given more than 4 days after the first one should be cross matched.

Cats do have naturally occurring alloantibodies and so should always be blood typed or cross matched prior to a transfusion. Cat AB blood typing cards are available commercially. You can purchase these cards for use in your practice, or send blood to a lab for typing.

There are 3 blood groups in cats:
- A (75 - 95% of cats): If a type A cat receives type B blood, the red cells will be destroyed within 2 days.
- B: If a type B cat receives type A blood, an acute, often fatal transfusion reaction occurs. The red cells will survive a maximum of 1 hour.
- AB (<0.5% of cats): Type AB cats have no alloantibodies. They should ideally receive type AB blood otherwise they should get type A blood. If they are given type B blood, the donor blood will react strongly against the A of the recipient and a “graft vs. host” reaction will occur.

There is also the newly discovered Mik antigen. A Mik –ve cat can have naturally occurring anti-Mik alloantibodies which can result in an acute haemolytic transfusion reaction after an AB-matched blood transfusion if the donor cat is Mik +ve.

Rapid Vet-H major Crossmatch kits for in-house cross matching in cats and dogs without the need for cell washing, test tubes, incubations, microscopes etc. have been available in the USA for several years but I have not seen them in Australia yet.

Dose of blood to give:
Packed red blood cells and fresh frozen plasma are administered at 10 – 15 ml/kg, whole blood at 20 – 25 ml/kg. Blood products are usually administered slowly, over 2 – 3 hours, to reduce the chance of reactions and volume overload. In an emergency situation it may be necessary to administer them as a rapid IV bolus i.e. over 10 – 15 minutes.

- Cats: 50 – 60 ml is usually all we have. Remember that whole blood is a fluid and that in normovolaemic but anaemic cats, fluid overload is a very real concern.
  Volume to give = Bwt (kg) x 66 x (goal PCV – recipient PCV)/donor PCV
- Dogs: Rule of thumb: 10 ml/kg PRBCs or 20 ml/kg whole blood will increase PCV by about 10%
  In a 20 kg dog with a PCV of 10%, give 15 x 20 = 300 ml PRBCs to raise the PCV to 25%.
  Volume to give = Bwt (kg) x 85 x (goal PCV – recipient PCV)/donor PCV
  In a 20 kg dog with a PCV of 10%, give 20 x 85 x (25-10)/70 = 364 ml PRBCs to raise the PCV to 25%.

Cat blood transfusions
Donor:
- Young, healthy, nice...
- 4.5 kg lean body weight
- PCV > 30%, ideally > 35%, before blood collected
- FeLV, FIV, M haemofelis negative
Anaesthetic:
- Minimal CV depression and vasodilation
IV fluids to replace volume of blood taken? Blood volume in a cat is around 66 ml/kg. Up to 20% of blood volume (60 ml in a 4.5 kg cat, 52ml in a 4 kg cat) can be donated provided IV fluids are administered.

Monitored closely

My technique:

- Ideally three people:
  1. Monitors the donor
  2. Gains IV access and holds the needle steady
  3. Draws back on syringe, rolling syringe as blood is collected to mix

- Materials for blood collection: 60 ml syringe + extension set primed with 2ml of 1000 U/ml heparin + 18g needle.

- Materials for blood administration: 100ml or 250ml bag of saline spiked with and emptied via a blood administration set

- Method:
  1. Collect blood into pre-prepared syringe, remove the extension set.
  2. Inject blood into the prepared saline bag using a clean 16g needle
  3. Administer the blood either via a pump or gravity through the blood admin set that you drained the saline bag with.


Normal coagulation is designed to reduce blood loss in response to injury, and to limit clotting to the site of that injury. In the normal course of events, platelets aggregate (primary haemostasis), coagulation factors arrive and are activated and ultimately fibrin forms to stabilise the clot (secondary haemostasis). Fibrinolysis begins almost immediately. It is a normal part of the coagulation cascade and is designed to dissolve the clot and restore blood flow.

Abnormal coagulation results in a thrombus that is not advantageous to the animal i.e. a clot that results in partial or complete obstruction of flow through a vessel and ischaemia of an organ or tissue, or a clot that fragments and causes thromboembolism at distant sites.

DEFINITIONS

Thrombus: an intravenous clot

Embolus: a blood vessel obstruction, not usually caused by a thrombus however when a portion of a blood clot breaks off and travels to another location it may obstruct a blood vessel.

Thromboembolus: a portion of a blood clot that has broken off and travels to another location and obstructs a blood vessel.

Thrombolytic: a substance, either administered or endogenous, that breaks down clots.

Thromboprophylactic: a drug that is intended to prevent or reduce the formation of clots.

SO WHAT CAUSES PATHOLOGICAL THROMBOSIS?

Virchow gives us the classic framework for thinking about coagulation. For thrombosis to occur, a patient must have the following three predisposing factors: decreased blood flow, damaged endothelium and hypercoagulability.

Remember that hypercoagulability may exist due to an increase in pro-thrombotic mediators, a decrease in anti-thrombotic mediators or an alteration in normal fibrinolytic activity.

A cascade of events occurs once one factor in the triad is altered, resulting in the other factors coming into play: damage to endothelial cells exposes subendothelial collagen, results in expression of selectins and von Willebrand factor and the release of pro-coagulant microparticles into circulation. This increases the local and systemic coagulability.

Endothelial damage will also result in inflammation and swelling of other endothelial cells in the immediate area which will result in decreased blood flow.

Many conditions in small animal medicine are recognised to alter coagulation:

<table>
<thead>
<tr>
<th>Decreased blood flow</th>
<th>Increased coagulation</th>
<th>Endothelial damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feline HCM and left atrial enlargement</td>
<td>Any condition associated with the development of DIC, SIRS, Sepsis</td>
<td>Catheter placement</td>
</tr>
<tr>
<td>Inactivity/recumbency</td>
<td>Surgery</td>
<td>Cardiac jet lesions</td>
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<tr>
<td></td>
<td>Protein Losing Nephropathy</td>
<td>Trauma (including surgery)</td>
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<td>Protein Losing Enteropathy</td>
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<td>Hyperadrenocorticism</td>
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<td>Major trauma</td>
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Table 1: Conditions associated with increased coagulability in small animal medicine.

The typical ICU patient has several risk factors for thrombosis; some present on arrival, others developing during treatment. In human ICU’s, most patients that stay for longer than a few days are candidates for thromboprophylaxis. Despite this, up to 25% of human ICU patients will still have evidence of deep vein thrombosis.

The diagnosis of clinically significant hypercoagulability is problematic in veterinary medicine. Clinical tests of coagulation such as PT and APTT are designed to detect prolonged clotting. Low plasma levels of anti-thrombin (AT) or increased levels of fibrin degradation products, d-dimers and fibrinogen provide laboratory evidence of hypercoagulability. Thromboelastography (TEG) is a technique that will detect increased rate or clot formation in a blood sample, but it cannot yet be clinically utilised.

Detection of a thrombus can also be difficult: Doppler flow ultrasound, angiography, contrast CT or MRI can all be used but these diagnostics are not yet freely available in clinical practice. In addition, most of these tests require a general anaesthetic which may not be appropriate in the critically ill animals. It is thus likely that many, probably the majority, of cases of thrombosis in small animals go undiagnosed.
TREATMENT OF THROMBOSIS

There are two approaches: prevention or thromboprophylaxis in cases that are recognised to be at increased risk of thrombosis and treatment or thrombolysis in diagnosed cases. The clinical signs and consequences of thromboembolism will depend on the anatomical location and size of the thrombus.

Arterial thrombi tend to be composed primarily of platelets and form in areas of rapid blood flow. Anti-platelet drugs are typically used for arterial thrombus prophylaxis. Arterial thrombosis tends to present acutely, and early resolution will decrease complications associated with the clot (tissue necrosis, neuropathy) and with clot lysis (reperfusion injury).

Venous thrombi tend to be composed of fibrin and red blood cells and usually form in areas of venous stasis, or in patients AT deficiency. Anticoagulants are used to prevent venous thrombi, with warfarin therapy commonly used in people predisposed to deep vein thrombosis. Venous thrombosis is usually a less acute presentation than arterial thrombosis. Treatment may involve treatment of the underlying hypercoagulable condition, supportive care and anticoagulants to prevent further thrombosis while the bodies fibrinolytic system breaks up the clot.

**Thromboprophylaxis:**

Thromboprophylactic drugs are broken down into two groups: those that act on platelets and anti-coagulants.

- **Aspirin:** the archetypal platelet antagonist.
  
  Aspirin irreversibly inhibits COX activity in platelets, predominantly (but not exclusively) effecting COX 1 activity. COX 1 mediates the conversion of PGGH to TXA2; inhibiting the production of TXA2 markedly attenuates the aggregation of platelets in response to arachidonic acid metabolites i.e. inflammatory stimuli. There appears to be less COX 1 mediated attenuation of the platelet response to thrombin.
  
  The effects of aspirin in inhibiting platelets are dose-dependent and subject to wide individual variation. Some patients have been recognised as having an aspirin-resistant genotype. At high doses, it appears that aspirin (and other COX inhibitors) may not be a thromboprophylactic and could promote platelet aggregation and thrombosis. In the endothelium, both COX 1 and COX 2 mediate the production of prostacyclin (PGI2), the most potent endogenous inhibitor of platelet aggregation. High dose aspirin (81 mg/cat Q 48 – 72 hours) will transiently inhibit COX 1 and COX 2 activity in endothelial cells. Endothelial cells contain nuclei so are able to synthesise new sources of COX enzymes. Within a few hours of high dose aspirin therapy, prostacyclin synthesis will resume, but there has been a potentially pro-aggregation time frame when thrombin has still been able to stimulate platelet aggregation but there has been decreased prostacyclin available to inhibit this aggregation.

  - **What evidence is there for the use of aspirin thromboprophylaxis in small animals?** The use of aspirin in cats for prevention of recurrence of arterial thromboembolism (ATE), both high and low (5mg/cat Q 72 hours) doses are of questionable efficacy. The low dose may be associated with fewer side effects and possibly decreased recurrence.
  
  Dose recommendations for aspirin thromboprophylaxis: 1 mg/kg Q 24 hours in dogs; 5 mg/cat Q 72 hours.

- **Other anti-platelet drugs:** Glycoprotein IIb/IIIa antagonists e.g. Abciximab. The GPIIb/IIIa receptor is responsible for platelet aggregation. Clopidogrel (Plavix®) a thienopyridine derivative. These drugs alter platelet function via irreversible inhibition of the adenosine diphosphate (ADP) receptor which results in inhibition of platelet activation and binding with fibrinogen. Specifically, ADP receptor blockade inhibits the GPIIb/IIIa receptor. What evidence is there for the use of anti-platelet drugs in small animals? Initial results of altered platelet aggregation after treatment with abciximab in a feline arterial injury model are promising. Trials are needed to see if these results translate to clinical cases. A study evaluating clopidogrel dosed at 18.75-75 mg /cat Q 24 hours showed an impact on platelet function but trials on prevention of ATE have not yet been published. Dose recommendations for clopidogrel: 1-3 or 2-4 mg/kg Q 24 hours in dogs; 1-3 mg/kg or 18.75-75 mg /cat Q 24 hours.

- **Anticoagulants:**
  
  - **Heparin** is a thrombin inhibitor. Unfractionated heparin (UH) binds with AT and this complex inhibits thrombin (factor IIa) and Xa. It may also effect factors IX, XI, and XII and cause decreased platelet function. Recommendations are to treat until the APTT is prolonged by 1.2 – 2.5 times, at which point the animal may be at risk of complications due to bleeding. Small alterations in dose can have marked effects on the APTT. Most cats treated with UH receive either “low dose” heparin (50 – 100 U/kg) or “high dose” (200 – 3000 U/kg) Q 6 – 8 hours. Cats have wide individual variation in heparin pharmacokinetics with some requiring up to 475 U/kg to maintain plasma concentrations in the therapeutic range.
  
  - **Low molecular weight heparin (LMWH)** preferentially inhibits only factor Xa. It has a longer half life than unfractionated heparin in humans, but requires dosing Q 6 hours in dogs and more frequently in cats to maintain adequate levels of anti-Xa activity. Whether the targeted level of anti-Xa activity used in monitoring LMWH therapy is therapeutically relevant has not yet been determined.

  - **What evidence is there for the use of heparin thromboprophylaxis in small animals?** Studies in cats with ATE have used widely varying doses of both unfractionated and LMWH and shown wide
individual variations in dose response. As heparin requires frequent parenteral dosing to maintain therapeutic levels, it is generally not suitable for long term therapy.

**Thrombolytic therapy:**
Most veterinary studies on thrombolytic therapy or clot removal have been performed in cats with ATE. Treatments are associated with many complications including bleeding, pulmonary thromboembolism (when treating a venous thrombus) electrolytes abnormalities after reperfusion, and death during surgical procedures. At this time, conservative treatment, i.e. supportive care and therapy for the underlying cardiac disease, results in a slightly (though not statistically) improved outcome compared to interventional treatments.

- **Thrombolytic drugs**
  Drugs including urokinase, streptokinase and tissue plasminogen activator have been used in animals to increase the rate of clot breakdown. These drugs work by activating plasminogen to produce plasmin and thereby dissolving fibrin clots. Thrombolytic drugs are most effective in recently formed thrombi as older thrombi have extensive fibrin polymerisation which makes them more resistant to thrombolysis.
  Studies in cats with these drugs have reported poor outcomes with survival rates from 0 - 45%. A recent prospective study investigating the use of tissue plasminogen activator in cats with ATE was terminated early due to an unacceptable level of adverse outcomes. Catheter directed thrombolysis targets the clot directly with the thrombolytic agent. This aims to decrease side effects associated with systemic coagulopathy. No prospective trials using this technique have been carried out in veterinary patients.

- **Balloon dilation**
  Balloon dilation combined with catheter directed thrombolysis to successfully treat 2 dogs with pace-maker induced thrombosis has been described.

- **Vascular stenting**
  Stents are placed in people with occlusion of vessels secondary to atherosclerotic plaques or in vessels with aneurysms. Stents cannot be removed once placed. Up to one third of human patients will still have vessel occlusion at the site of the stent. Stents are used alongside catheter directed thrombolysis for the treatment of thrombosis. Case reports on the use of stents in dogs for the treatment of aortic thrombosis and to help with coil embolisation in the caudal vena cava for the treatment of porto-systemic shunting and in a cat for the treatment of vena cava thrombosis have been published.

- **Percutaneous mechanical thrombectomy**
  Rheolytic thrombectomy devices use a double lumen catheter which is advanced to the level of the thrombus. Pressurised saline is used to physically lyse the thrombus and create a vacuum in the region immediately adjacent to the catheter. The second lumen of the catheter is used to remove of the thrombus fragments. A prospective study in 6 cats using this method reported an overall survival rate of 50% which is similar to conventional medical therapy, but also a 33% procedural fatality rate.

- **Embolic trapping devices**
  Various mesh devices have been used in people “downstream” of venous thrombi to prevent clot fragments from travelling to the lungs. The trapped fragments then undergo normal fibrinolysis. I have not found any reports of the use of these devices in animals, though they could potentially be used in cases of caval thrombosis secondary to adrenal tumours.

- **Surgery**
  Surgical clot removal may be indicated in some animals e.g. those with organ infarction. Again, complications due to reperfusion and re-embolisation may occur. Surgery in clinically unstable animals such as cats with ATE secondary to cardiomyopathy is highly risky.

Thrombosis, thromboprophylaxis and thrombolytic therapies are all areas of active research and study in veterinary medicine. Currently, there is no satisfactory way to monitor clinical hypercoagulability in animals. Diagnosis of thrombosis can be technically challenging, and treatment even more so. While I think that there will be significant advances in this field in the next few years, it is notable that the problems associated with hypercoagulation and thrombosis continue to plague human criticalists.

**References:**
Specific indications for sonographic evaluation of the spleen include the palpation of generalised splenomegaly or an abdominal mass, abdominal trauma, and haemoperitoneum. The spleen may also be evaluated to stage patients for metastatic disease (for example mast cell tumour metastases), or in cases of immune mediated disease for example immune mediated thrombocytopenia which may be secondary to neoplasia. It is important to recognise, however, that sonographic abnormalities are usually non-specific. Interpretation of splenic ultrasound findings must be made in conjunction with other clinical information - history and signalment, the clinical examination, the findings of blood tests, and the results of sampling of the spleen by fine needle aspiration or cytology.

ENSURE YOU EVALUATE THE ENTIRE SPLEEN

Ultrasound provides you with a thin cross-sectional image, and the ultrasound beam must be ‘swept’ through the entire organ to ensure a thorough examination. The spleen should be evaluated in both transverse and longitudinal planes. The head of the spleen is fixed by the gastrosplenic ligament, and resides in the left craniodorsal abdomen. It may be visualised from a ventral abdominal approach, however gas or ingesta within the gastrointestinal tract may prevent evaluation. Alternatively the splenic head is seen by a left dorsal intercostal approach through the 11th to 12th intercostal space. The body and tail of the spleen extend along the left and ventral abdominal wall. The splenic vein should be traced to the portal vein, and the splenic hilus evaluated for the presence of splenic lymph nodes. Consideration should be given to the presence of ‘accessory spleens’, small islands of splenic tissue implanted on the mesentery, which may be mistaken for lymph nodes (Rossi et al. 2010).

Splenic size is assessed subjectively. Splenomegaly may be iatrogenic due to sedatives or anaesthesia, particularly caused by acepromazine and barbiturates (O’Brien et al. 2004). The spleen has a characteristic triangular shape in transverse plane and a ‘strap’ like shape in longitudinal plane. The head of the spleen curves axially. The normal splenic capsule is smooth, visible as a hyperechoic line when the ultrasound beam strikes the capsule perpendicularly. The normal spleen has a fine granular echotexture, and is hyperechoic relative to the left renal cortex and the liver. The intra-parenchymal splenic veins are visible as a converging ‘y’ shape near the splenic hilus, where they exit the spleen to join the splenic vein proper. A common incidental finding are multifocal hyperechoic nodules most commonly near the splenic hilus around the splenic veins, or peripherally. They may cast a distal acoustic shadow. These are ‘myelolipomas’, collections of fatty tissue of no pathologic significance (Schwarz et al. 2001).

FOCAL OR MULTIFOCAL CHANGES

Splenic nodules are a common and non-specific finding. An important differential is nodular hyperplasia, a frequent finding in older dogs. Hyperplastic nodules are usually isoechoic and only recognised when they distort the splenic capsule. In some cases they may alter splenic echotexture and echogenicity, thus cytologic or histopathologic evaluation of lesions is crucial in forming a diagnosis. Differentials for splenic nodules include: myelolipomas, nodular hyperplasia, extramedullary haematopoesis, early haematomas, infection, abscess, splenic necrosis, neoplasia (primary or metastatic).

Splenic abnormalities are often recognised in patients with primary immune-mediated haemolytic anaemia. In one study, 7 of 27 dogs (26%) had splenic abnormalities, including splenomegaly with patchy hypoechoic nodules, and heteroechoic nodules. These changes were attributed to extramedullary haematopoesis, lymphoid hyperplasia, and acute infarcts. (Cruz-Arambulo, 2007).

Splenic necrosis or infarction may be wedge-shaped with the broad base toward the periphery. As the infarcts age, they change from poorly margined hypoechoic or complex lesions, sometimes with a ‘lacy’ appearance, maturing to hyperechoic regions that may distort the splenic margin. Evaluation for the absence of blood flow using Colour or Power Doppler may be useful to distinguish from other lesions, although an absence of signal may reflect slow flow and incorrect Doppler Gain and Pulse Repetition Frequency settings.

Splenic abscesses are uncommon and highly variable in appearance. They usually have irregular poorly defined margins and are hypoechoic. Some lesions are complex with variable cystic and solid components. Areas of intense hyperechogenicity, casting ‘dirty’ distal acoustic shadow, indicates the presence of gas-forming microorganisms. Adjacent mesenteric fat may be inflamed with a hyperechoic, hyperattenuating appearance, and focal effusion.

Splenic haematomas have variable appearance; small, early haematomas are hyperechoic, while larger haematomas are anechoic to hypoechoic collections of unclotted blood. In the later stages as the clot organises, the echogenic content is surrounded by anechoic fluid. On serial ultrasound evaluation, haematomas reduce in size as they resolve. Splenic haematomas may be large in size, and may be confused with malignancies such as haemangiosarcoma.
Splenic neoplasia may manifest with solitary or multifocal masses. The type of splenic neoplasm cannot be determined from the ultrasound appearance. The sonographic appearance of splenic haemangiosarcoma (Wrigley et al.1989) and lymphoma (Wrigley et al.1988) have been described, but definitive diagnosis requires cytologic or histopathologic evaluation. Beware the patient with hypoechoic splenic and hepatic lesions; whilst neoplasia is possible, consideration must be given to concurrent splenic and hepatic nodular hyperplasia. The presence of a solitary ‘target’ lesion - hyperechoic centre with a hypoechoic rim - has a positive predictive value for malignancy of 74%. Multiple target lesions in the spleen and liver increases the positive predictive value to 81%. (Cuccovillo et al. 2002). If neoplasia is suspected, thoracic radiographs should be taken to evaluate for pulmonary metastases. Echocardiography may also be considered to check for right auricular masses.

In recent years, contrast-enhanced ultrasound has been evaluated for the differentiation of malignant from benign splenic lesions (Rossi et al. 2008, Ohlerth et al. 2008, Nakamura et al., 2010). Splenic malignancies are hypoechoic relative to adjacent normal parenchyma in the early and late vascular phases compared to benign lesions. In the late vascular phase, hypoechoic pattern was associated with malignancy, with a sensitivity of 81% and specificity of 85% (Nakamura et al., 2010)

DIFFUSE SPLENIC CHANGES

In most cases, ultrasound is not helpful to distinguish between causes of diffuse splenic disease due to the overlap of sonographic appearances. Pathologic differentials for diffuse splenic disease include: infection, immune mediated disease, lymphoma, leukaemia, neoplastic infiltrates, splenic congestion, splenic torsion, chronic haemolytic anaemia, and parasitic infection (e.g. with Mycoplasma haemofelis, Babesia, or Ehrlichia).

Splenic congestion usually presents as an isoechoic to slightly hypoechoic spleen. It occurs with disturbances to splenic or portal circulation, such as splenic vein thrombosis, splenic torsion, portal vein compression e.g. by severe hepatic disease. Splenic torsion, splenic vein thrombosis, and acute splenitis may all lead to splenic infarction and necrosis, with marked splenomegaly with a hypoechoic and ‘lacy’ appearing parenchyma (Saunders et al. 1998). A hilar perivenous hyperechoic triangle may be seen with splenic torsion (Mai, 2006). Careful evaluation of the spleen and portal veins with B-mode and Colour Doppler is recommended. Colour Doppler of the splenic parenchyma is also useful to see if blood flow is present within the spleen. With diffuse neoplastic disorders such as lymphoma or mast cell infiltration, the parenchyma may appear more coarse and heterogeneous. Fine needle aspiration and cytology is recommended in such cases.

SAMPLING OF THE SPLEEN

If splenic abnormalities are found on ultrasound, sampling is recommended unless the diagnosis is obvious from other clinical information. Ultrasound guidance allows direct sampling of focal lesions. Sampling is most commonly performed by fine needle aspiration, which is minimally invasive, relatively inexpensive, and may be performed under sedation. A non-aspiration technique is preferred as there is less blood contamination and produces higher quality cytologic samples (LeBlanc et al. 2009). In one study cytologic diagnosis corresponded with the histopathologic diagnosis in 61.3% of cases, while in 22.6% of cases, histopathology was required to distinguish between reactive and neoplastic conditions. Multiple similar appearing lesions were associated with malignancy, while solitary lesions were more often benign. (Ballegeer et al. 2007).

CONCLUSION

While the sonographic appearance of diffuse or focal splenic disease may suggest one diagnosis is more or less likely, the marked overlap in sonographic appearance of lesions means that fine needle aspiration or biopsy is required for diagnosis. Correlation of sonographic findings with other clinical information is important in the interpretation of findings. Contrast enhanced ultrasound appears to be a promising technique for differentiating between malignant and benign focal splenic lesions.

The cornerstone of management of both IMHA and IMT is immunosuppressive therapy, particularly with glucocorticoids. The major mechanisms of immunosuppressive therapy are to decrease antibody synthesis, decrease the binding affinity between antibodies and blood cells, and decrease destruction of antibody-coated cells by the MPS. However, since even if new antibody synthesis is arrested immediately, pre-existing immunoglobulins may survive in the circulation for weeks, reduction of antibody synthesis is usually not an important part of initial treatment.

**INITIAL IMMUNOSUPPRESSIVE THERAPY IN DOGS**

Initial immunosuppressive therapy for stable dogs with IMHA or IMT usually consists of oral prednisolone or prednisone, starting at of 2 to 4 mg/kg/day, given once daily or split twice daily. The most important short-term effect of glucocorticoids is inhibition of the MPS (‘medical splenectomy’). Treatment response is rarely immediate: when glucocorticoids alone are effective, a rise in red cell or platelet numbers is usually seen between 3 and 7 days after commencing therapy, but can be delayed for as long as one month. Glucocorticoids are not an entirely benign drug class, and side effects may jeopardise both patient health and owner compliance. One of the most common causes of death in IMHA or IMT patients is euthanasia due to unacceptable drug side-effects and, in my opinion, the drug class that confers the most unacceptable side-effects is the glucocorticoids. Alternate-day or tapered glucocorticoid therapy minimizes both predictable side effects (PU/PD, polyphagia, and hyperventilation) and eventual iatrogenic hyperadrenocorticism (alopecia, weight gain, muscle wasting, weakness and predisposition to infection, hypertension and pancreatitis). Adjunctive therapy (immunosuppressive agents or splenectomy) may enable further glucocorticoid dose reduction. Some clinicians give medications that may minimize steroid-associated GI ulceration: sucralfate, misoprostol, omeprazole and H2 blockers are often recommended. Although there is minimal evidence that this has previously been reported. Azathioprine is usually well tolerated, and the potential side effects of pancreatitis and hepatotoxicity are rare. Azathioprine can infrequently cause an idiosyncratic severe myelosuppression (usually within a few weeks of commencing therapy), which is usually reversible if white cell counts are closely monitored and...
leukopenia is promptly detected. Cyclosporine is a powerful selective T-cell immunosuppressive agent that is usually well tolerated and that is not myelosuppressive. Cyclosporine would arguably be the drug of choice for dogs with IMHA or IMT if it were not for the high cost associated with therapy. The high cost of cyclosporine is compounded by the added expense of regular monitoring of therapeutic blood levels. Cyclosporine has an oral bioavailability that varies widely between formulations and oral therapy must therefore be adjusted to attain an acceptable trough (collected at 12 hours if the drug is given twice daily) whole blood drug level (500 ng/ml is the standardly recommended trough level, although some clinicians anecdotally report success at reduced trough levels of 250 ng/ml or even lower). Different laboratories run cyclosporine assays using different methodologies which have varying abilities to accurately measure the concentration of pharmacologically active cyclosporine in the sample. For this reason, it is advisable whenever possible to use a blood therapeutic range that is generated for the specific assay used by the laboratory. The starting dose of oral cyclosporine in an olive oil base (Sandimmune) is 10 to 25 mg/kg daily (split into two doses), while the preferred microemulsified form (Neoral) is better absorbed, and therefore requires lower oral starting doses (5 to 10 mg/kg daily, split into two doses). The cost of microemulsified cyclosporine may be reduced a little by using generic Neoral bioequivalents or by using the canine-approved product Atopica (which is essentially a Neoral bioequivalent product, but comes in capsule sizes that are more convenient for dosing cats and small dogs). Cyclosporine doses can often be halved (thereby reducing expense) by the concurrent administration of ketoconazole, which impedes hepatic cyclosporine metabolism. Cyclosporine can cause GI disturbances, gingival hyperplasia and uncommon hepatotoxicity.

Even when not commenced during the initial therapy of IMHA or IMT, cyclophosphamide, cyclosporine or azathioprine often need to be added within a few weeks of starting treatment with corticosteroids. Indications for adding concurrent immunosuppressive agents include a failure to respond to glucocorticoids alone, or intolerable steroid side effects such as polydipsia, polyuria, polyphagia and tachypnoea. Addition of these extra immunosuppressive agents often enables a more rapid reduction in corticosteroid doses.

INITIAL IMMUNOSUPPRESSIVE THERAPY IN CATS

Cats do not tolerate immunosuppressive agents as well as dogs and it is often difficult to formulate standard dosage forms down to a size that is safe. Fortunately, cats are remarkably tolerant of long-term high doses of glucocorticoids and most cats with IMHA or IMT will respond to oral steroids alone (starting dose of prednisolone of 4 mg/kg/day). For this reason, oral azathioprine (at a markedly reduced dose of 0.3 mg/kg/day), cyclophosphamide (same dose as dogs), or cyclosporine (4-15 mg/kg/day [Sandimmune] or 1-5 mg/kg/day [Neoral], split twice daily, for a whole blood trough level of 250-500 ng/ml) are very rarely needed. Tablet sizes that are too big to allow appropriate cat dosages can present a real problem with azathioprine and cyclophosphamide. Azathioprine must usually be compounded into a suspension, a process that is best done by an experienced compounding pharmacist since, otherwise, unequal mixing throughout the bottle and settling of the active ingredient can lead to inadvertent underdoses and overdoses. Cyclophosphamide tablets should not be split because, firstly, this increases owner exposure to this mutagenic and carcinogenic drug and, secondly, the active ingredient is often unequally dispersed throughout the tablet. Attaining a starting regimen of 50 mg/m² every other day is therefore often not possible. One trick that can be used to ensure that, at least, an appropriate weekly dose of cyclophosphamide is attained in cats (and small dogs) is to calculate the total weekly dose of drug required (50 mg/m² every other day translates into 175 mg/m² weekly), and then to dose this at an interval that allows entire tablets to be used.

MONITORING RESPONSE TO THERAPY

Haematocrit and/or platelet counts should be monitored daily until an appreciable response to immunosuppressive therapy is observed, and the PCV rises above approximately 15% in cats and 20% in dogs, or the platelet count rises above 100,000/µl. From this point on, the haematocrit and/or platelet count should be monitored at least weekly until anaemia or thrombocytopenia resolve completely. Given an adequate initial treatment response, therapy should be gradually tapered to an acceptable maintenance dose that can be given safely long-term without significant side effects. In dogs, prednisolone and azathioprine can often both be tapered to 0.5 to 1 mg/kg every second day, a maintenance dose that is usually well tolerated. Similar maintenance doses of prednisolone may be achievable in cats although, since steroid side effects are typically minimal, higher doses are also usually acceptable. Since cyclophosphamide therapy can eventually cause sterile cystitis (which can be irreversible), there is no safe maintenance dose of this particular drug. Since both cyclophosphamide and azathioprine can be unpredictably myelosuppressive, white cell counts should be regularly monitored throughout therapy. Furthermore, since azathioprine can (rarely) cause an idiosyncratic hepatotoxicity, liver enzymes should also be monitored regularly for elevations far beyond those expected with glucocorticoid therapy alone.

One simple approach to tapering therapy in patients with IMHA or IMT is to recheck haematocrit and/or platelet numbers approximately every 2 weeks and then, if the disease is still in remission, to reduce the dose of one drug by 50% after each visit. In animals receiving several immunosuppressive agents, the drug which is causing the most side effects is tapered first. In most cases, this means that prednisolone is tapered first and, once steroid doses are low enough to cause no side effects, then the other drug is similarly tapered. Once a well-tolerated maintenance dose of all
drugs is attained, therapy should in my opinion ideally be continued for at least a further three months, with monthly monitoring of haematocrit and/or platelet numbers. Presuming that an underlying cause of IMHA or IMT has not been identified and eliminated, tapering therapy too rapidly increases the risk of a life-threatening relapse. Chronic oral azathioprine therapy itself can, in dogs, cause a persistent mild non-regenerative anaemia (PCV greater than 25-30%) that is sub-clinical, and the presence of a mild anaemia in dogs on this drug should therefore not be misinterpreted as evidence that treatment is inadequate. Once anaemia or thrombocytopenia have been in remission for 3 to 6 months, therapy can be cautiously withdrawn. Haematocrit and/or platelet numbers should be monitored regularly over the months following cessation of therapy.

**CHRONIC REFRACTORY IMMUNE-MEDIATED BLOOD DISORDERS**

An initial adequate response to standard immunosuppressive therapy does not necessarily guarantee that patients with IMHA or IMT will be simple to manage in the longer term. Unfortunately, in some patients, anaemia or thrombocytopenia relapse as therapy is tapered. Persistence with higher than maintenance doses of glucocorticoids and other immunosuppressive agents is however, not an acceptable alternative due to drug side effects. In these patients, additional medications that may then permit an acceptable dose reduction of the standard drugs include danazol, chlorambucil, leflunomide and mycophenolate mofetil.

Danazol, an impeded androgen (5 mg/kg 2-3 times daily orally), has been recommended in combination with glucocorticoids in order to reduce the dose of steroid that is needed. Danazol’s most important mechanism of action is probably to reduce MPS Fc receptor/antibody binding affinity. Danazol also competes with glucocorticoids for combination with steroid-binding globulin, increasing the availability of active unbound glucocorticoid. Side effects are uncommon, and include hepatotoxicity and masculinisation of female dogs. However, although some dogs with refractory disease may benefit from danazol, the drug has justifiably fallen out of favour in the past few years, probably because it is very expensive and response to therapy is sluggish and very unpredictable. Chlorambucil, an alkylating agent that does not cause sterile cystitis, can be used (starting oral dose of 0.1 to 0.2 mg/kg/day) as an alternative to long-term cyclophosphamide, particularly in cats and small dogs, although there is little data regarding its efficacy. The drug is typically well-tolerated, although it can occasionally cause anorexia, GI disease and myelosuppression. One advantage of chlorambucil is that it is available in a tablet size that is small enough for use in most average-sized cats. One very expensive new drug that holds promise for the treatment of immune-mediated blood disorders is leflunomide (Arrava), an inhibitor of pyrimidine biosynthesis. Leflunomide, at a starting oral dose of 4 mg/kg daily adjusted to attain a serum trough level of 20 µg/ml, has been used in dogs to successfully treat refractory cases of IMHA and IMT. Leflunomide appears to be very well tolerated. Mycophenolate mofetil (Cellcept) has recently been evaluated in dogs with immune disease, including dogs with myasthenia. Mycophenolate inhibits an enzyme needed for purine synthesis, and has a relatively specific effect on lymphocytes. Mycophenolate can cause significant GI side-effects, and concurrent immunosuppressive drugs may need to be given to enable reduction of mycophenolate doses down to well-tolerated levels. Mycophenolate has the advantage of being minimally myelosuppressive. Anecdotally, mycophenolate mofetil is being used by some veterinarians for refractory canine IMHA. Based on extrapolation from human medicine, a starting dose might be 600 mg /m² per dose given orally twice a day or 400 mg /m² per dose given orally three times daily. The exact dose in dogs has yet to be determined, although some published canine cases of myasthenia gravis responded to an initial mycophenolate dose of 20 mg/kg twice daily, a dose that was eventually successfully tapered to approximately 10 mg/kg twice daily. Mycophenolate has recently become significantly cheaper in cost, and its use has as a result become a little more attractive as an option in refractory cases.

Sometimes, one of the emergency treatments for IMHA or IMT, such as splenectomy, intravenous gammaglobulin or IV vincristine (IMT patients) may ‘kick start’ refractory chronic cases and render standard therapy more effective. Splenectomy, in particular, may on occasion permit significant reductions in immunosuppressive drug dosage. Unfortunately, however, this beneficial effect is inconsistent and unpredictable.

**SECONDARY IMMUNE-MEDIATED BLOOD DISORDERS**

Whenever an underlying cause of IMHA or IMT can be identified and eliminated, ongoing immunosuppressive therapy is often not needed. In contrast, such therapy is unlikely to be able to be discontinued while the underlying cause persists. Since rickettsial infections can be difficult to diagnose, and often have a strong immunologic component, cats with suspected IMHA should also be treated for haemobartonellosis, and dogs with suspected IMT (and, arguably, IMHA) should probably be treated for ehrlichiosis. Medications or vaccines that were given over the few months prior to onset of disease should be avoided in the future when possible. When the original crisis was not associated with recent vaccination, however, there is arguably no need to modify routine vaccinations in the future, since there is little evidence that animals with primary immune-mediated disease have an increased risk of developing secondary IMHA or IMT as well. However, since the cautious reduction of vaccination loads in adult animals is unlikely to do harm, stripping vaccine protocols down to ‘the bare essentials’ is certainly also a reasonable approach.
In the cat, IMHA is often secondary to either feline leukaemia virus (FeLV) or haemobartonellosis (mycoplasmosis). Feline IMT is similarly often associated with FeLV. Cats with IMHA or IMT should therefore always be tested for FeLV. FeLV positive cats with IMHA or IMT often initially respond well to immunosuppressive therapy, although long-term prognosis is poor. Cats with haemobartonellosis typically present with recurring episodes of haemolytic anaemia. During crises, organisms may not be apparent, since affected cells are either destroyed or sequestered in the spleen. Organisms reappear on RBCs during remission of anaemia, and can be discovered by serial blood smears. Cats with apparent IMHA should probably therefore receive oral tetracyclines, even if organisms are not seen on initial smears. Concurrent immunosuppressive therapy in cats with haemobartonellosis is not contraindicated. In fact, since anaemia due to haemobartonellosis has an immune-mediated component, glucocorticoids may arguably even be indicated during acute crises. Similarly, a combination of glucocorticoids and doxycycline is indicated in dogs where IMT may be secondary to rickettsial infection.

PROGNOSIS

Published mortality rates for IMHA in dogs are high, ranging from about one-third to one-half of all presented cases, with death/euthanasia usually attributable to either severe anaemia or pulmonary thromboembolism during the initial crisis, or persistent or recurrent disease or unacceptable drug side effects during chronic therapy. Negative prognostic indicators with IMHA may include the presence of marked jaundice or haemoglobinemia/haemoglobinuria, a poor regenerative response, and a positive slide agglutination. Canine IMT patients do little better than IMHA patients. In one clinical study, mortality rates during the initial episode of thrombocytopenia exceeded 25%. Principal causes of death were either severe gastrointestinal haemorrhage or euthanasia. Recurrence of thrombocytopenia following a period of apparent clinical remission is common. Chronic thrombocytopenia requiring long-term therapy develops in about 25% of canine IMT patients. Mortality rates are high in such patients (over 50%). Unacceptable drug side effects encountered during long-term management of refractory chronic IMT are frequently a major reason for eventual euthanasia.

Hopefully, as our understanding of these particular diseases increases, and our treatment options expand, deaths due to IMHA and IMT will become much less frequent.

DOES LYME DISEASE EXIST IN AUSTRALIA?
A New Search for Evidence of Lyme borreliosis in Australia
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INTRODUCTION
Lyme borreliosis is an enigmatic yet serious infection of humans and dogs that is well recognised in the Northern Hemisphere, most notably in the United States, northern Europe and in certain regions of Asia. Lyme disease is caused by a group of genetically diverse spirochaetes collectively named Borrelia burgdorferi sensu lato. This complex includes at least 3 species known to be pathogenic to humans; Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii, in addition to other genospecies of uncertain, or disputed, pathogenicity. Borrelia organisms are transmitted by Ixodes ticks; along the west coast of the US the distribution of Ixodes pacificus closely correlates with cases of Lyme disease in humans and dogs, and a similar situation occurs with I. scapularis in the Midwestern and northeast States. In Europe the cattle tick Ixodes ricinus is implicated as the major vector for Borrelia burgdorferi sensu lato, in addition to other potentially zoonotic organisms such as Babesia spp. and Anaplasma phagocytophilum, and in Asia I. persulcatus vectors these pathogens.

Ixodes are 3-host ticks, meaning that each developmental stage feeds on separate hosts and that they leave these for molting between each instar. Nymphs, infected during feeding as larvae, are the most important stage for B. burgdorferi transmission to humans, whereas dogs are thought to be infected primarily by adult ticks. Co-evolution of the ticks and their native mammalian hosts has implicated various species of rodents and other wild mammals as the reservoir hosts for Borrelia spp. in its sylvatic life cycle, and both dogs and humans are at greatest risk of accidental infection when they encroach on these natural habitats. Unsurprisingly therefore Borrelia transmission is somewhat seasonal and requires the relevant stages of the ticks to be active at a time that coincides with maximal human activities in rural localities (such as bush walking and hiking).

The clinical and pathological features of Lyme disease in humans vary geographically according to the predominant genospecies. Almost all (>90%) infected humans show some degree of clinical illness in marked contrast with the situation in dogs in which up to 80-90% individuals in an endemic region are asymptomatic. The characteristic sign of recent human infection is a rash that develops at the site of the tick bite as a red spot that gradually spreads into a much larger circle with a characteristic bulls-eye appearance, known as erythema migrans. This is often accompanied by flu-like symptoms such as mild fever, headache and joint pain. After these initial signs some people develop heart, joint or neurological involvement that may result in protracted and debilitating illness.

Clinical signs of borreliosis in dogs, if they occur at all, develop with chronic infection, weeks to months after tick exposure. Some studies suggest breed predispositions, such as Labradors and Golden Retrievers in the US, and Bernese Mountain dogs in Switzerland. Dissemination of spirochaetes within the skin, joints and connective tissues causes local inflammatory reactions that result in pain, swelling and recurrent lameness. Inflammatory arthropathy is reported to occur within 2-3 weeks of experimental infection with Borrelia organisms, and clinicians describe affected dogs as having exercise intolerance, difficulty in negotiating stairs, and shifting lameness. In addition, renal disease, notably glomerulonephropathy, has been associated with Borrelia infection, although convincing evidence of a direct effect is lacking. Indeed, attributing any given clinical sign to Lyme borreliosis in an endemic area is fraught with pitfalls due to the difficulty in determining previous exposure from active infection.

The diagnosis of Lyme borreliosis requires a combination of clinical data and laboratory results, but the lack of repeatable or pathognomonic signs in dogs contributes to uncertainty in clinical case selection for further testing, especially in endemic regions. Tests for antibodies to B. burgdorferi sensu lato have been widely used since isolation and microbial culture of the organism is seldom achievable. ELISA and indirect IFAT are widely used for the diagnosis of Lyme borreliosis (Littman et al., 2006 – ACVIM Consensus Statement) yet interpretation of the results is complicated by the different methodologies and, in Europe and Asia, by the presence of different species of Borrelia. Further specificity is achieved by immunoblot methodologies, which can be tailored to detect immunodominant Borrelia antigens in different geographical regions (Schulthe-Spechtel et al., 2003). For dogs the recombinant peptide C6 was incorporated into in-clinic ELISA-based diagnostic assays (e.g. IDEXX 3Dx & 4Dx Snap kits) and has largely superseded the need for two-tier testing (e.g. IFAT followed by Western blot). It corresponds to an invariable region (IR6) of the VlsE (Vmp-like sequence, expressed) surface protein that is relatively conserved between genospecies of B. burgdorferi sensu lato. Another clinical advantage of this protein is that it is expressed only once the spirochaete is in the mammalian host (i.e. not in the tick) so the test can therefore distinguish between naturally infected and vaccinated individuals. While this assay was developed predominantly for the North American market, it was recently evaluated as a diagnostic test for borreliosis in Europe and was found to possess similar operating characteristics for Borrelia genospecies in that continent (Liang et al., 2003; Gerber et al., 2009).
Doxycycline is recommended for the treatment of Lyme borreliosis in dogs and for the most common forms of this infection in humans. There is evidence that *Borrelia* organisms may persist in dogs after antibiotic therapy and *Borrelia* DNA has been detected in mice after treatment.

**LYME BORRELIOSIS IN HUMANS IN AUSTRALIA**

To date there has been little convincing evidence for endemic *Borrelia* sp. infection in any country in the Southern Hemisphere, despite widespread presence of the *Ixodes* genus south of the equator. In Australia the diagnosis of Lyme disease in humans is controversial, despite an increasing number of people with illness attributable to borreliosis that are seropositive and/or have *Borrelia* DNA detected in their tissues. Opinion in the medical profession Australia about this issue is divided; some doctors and infectious disease specialists believe that Lyme disease is the cause of illness in their patients, while others attribute such symptoms to other (as yet undetected) pathogens or neuropsychological disorders. Most of the research into Lyme borreliosis in Australia was published between 1980-2000; this has included individual case reports (McCrosin, 1986; Lawrence et al., 1986), a survey of samples submitted for serological diagnosis (Russell, 1995) and an investigation into potential tick vectors (Russell et al., 1994). In terms of potential vectors of *Borrelia*, the *Ixodes* genus is represented by approximately 20 species within Australia that are parasitic on native marsupials, reptiles and birds; the best known being *Ixodes holoclycus*, the cause of tick paralysis. However *Ixodes holoclycus* was found to be incapable of transmitting a North American isolate of *Borrelia burgdorferi* in an experimental study by Piesman and Stone (1991). These studies have focused on the coastal strip east of the Dividing Range between southern Queensland and Victoria, with most reported cases originated from the northern beach communities of New South Wales. The collective opinion following these publications was that Lyme borreliosis was unlikely to be endemic in Australia, and this sentiment has persisted with the majority of medical authorities of eastern Australia.

**LYME DISEASE AND DOGS IN AUSTRALIA**

In regions of the Northern Hemisphere where Lyme borreliosis is established, dogs act as sentinels for various tick-borne infections of humans, including Lyme borreliosis, because they are more likely to be exposed to the vectors than their owners (Goosens et al., 2001; Duncan et al., 2004). Dogs in endemic areas are expected to seroconvert on exposure to the pathogens and can be useful in predicting emergent foci of borreliosis in humans (Rand et al., 2011). To date there have been two studies in Australia that investigate the serological status of dogs with respect to *Borrelia* infection. In a serosurvey based in the Brisbane area of Queensland, none of the client-owned dogs (n=57) tested by ELISA (using a North American tick-derived isolate of *Borrelia burgdorferi* sensu stricto as antigen) was seropositive (Baldock et al., 1993), and in a second (unpublished) study by Russell (1995), 6/239 (2.5%) of dogs in NSW were positive (methodology unclear); these seropositive results were attributed to cross-reacting antibodies.

Given recent advances in molecular and serological diagnostic tests, and the multifaceted concern about emerging parasitic zoonoses, it seems timely to revisit the existing data and to consider novel approaches to answering the question about whether Lyme borreliosis exists in Australia. Research designed to detect evidence of *Borrelia* infection in Australia could investigate different potential hosts for these spirochaetes for example; the reservoir(s), the vector(s) and the accidental host(s) such as dogs and humans.

In a study designed initially to target individuals most likely to be exposed to *Borrelia* organisms, pet dogs owned by people diagnosed with Lyme disease were sampled in the first stage of a research project designed to investigate borreliosis in eastern Australia. In a collaborative study with medical professionals, humans with Lyme disease diagnosed in NSW were recruited to this study by providing consent and completing a questionnaire about their diagnosis and the health of their dog(s). Data collected for each dog included its signalment, exposure to ectoparasites (including whether it had ever had tick paralysis), use of ectoparasiticides, and history of travel and general medical health (including lameness). Each dog was examined and ectoparasites (fleas and ticks) removed for later analysis. Blood was collected into EDTA-coated and plain tubes, processed, and analysed for the presence of antibodies to *Borrelia burgdorferi*, *Anaplasma phagocytophilum* (cross-reacting with *A. platys*) and *Ehrlichia canis*, and antigens of *Dirofilaria immitis*, using an in-house rapid immunoblot assay kit (Snap 4Dx, IDEXX Laboratories). Blood samples reacting to the C6 antigen were further evaluated using by quantification (IDEXX Laboratories). Serum was analysed for *Borrelia* antibodies using a line immunoblot (Goettner et al., 2005) adapted for use in dogs (Straubinger pers. com.). Preliminary results of this study (commenced in May 2011) will be presented together with a discussion of proposed future studies into Lyme disease in Australia.

**Acknowledgements:** IDEXX Laboratories kindly donated the Snap 4Dx test kits and have provided limited financial support.


Small Animal Medicine and Feline Chapters

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Cyclosporine, a powerful immunosuppressive agent originally introduced into human medicine for organ transplantation, is regarded as a promising drug for the treatment of many different immune-mediated diseases in dogs. Cyclosporine targets T-cells by binding to cyclophilin, with the resultant cyclosporine/cyclophilin complex inhibiting calcineurin, a calcium-dependent serine/threonine phosphatase. Calcineurin is necessary for T-cell production of cytokines such as IL-2, IL-4 and IFN-γ. Cyclosporine-induced inhibition of cytokine expression inhibits proliferation of activated T-lymphocytes by arresting generation of cytotoxic T-cell clones. Cyclosporine typically does not produce many of the side effects associated with other immunosuppressive agents in dogs, although gingival hyperplasia, mild gastrointestinal signs, hepatotoxicity and nephrotoxicity are occasionally reported. Cyclosporine has the additional beneficial property of being minimally myelosuppressive. The relatively recent availability of a veterinary-approved oral cyclosporine product has rapidly increased the popularity of the drug for treatment of a wide range of immune-mediated diseases in the dog, including immune-mediated haemolytic anaemia (IMHA).

The lipophilic nature of cyclosporine affects drug bioavailability, which has made the use of the drug particularly challenging to clinicians. The oral bioavailability of cyclosporine is highly unpredictable, with wide ranges of blood levels seen in dogs receiving similar doses. Because of this variability, therapeutic drug monitoring of cyclosporine blood levels is usually recommended. Interpretation of cyclosporine blood levels in the individual patient, however, is difficult. There is limited evidence in the veterinary literature correlating cyclosporine blood levels with clinical response for many inflammatory and immune-mediated diseases. Target therapeutic ranges are often anecdotal, and recommendations vary considerably among references with regards to desired blood cyclosporine levels. Clinical response is often the most reliable means of assessing immunosuppression in treated patients. The relationship between clinical response and drug blood levels also appears to be highly variable, with a given cyclosporine level being effective in some patients but not others. Additionally, for some conditions, positive clinical response is often apparent at drug levels well below those typically recommended for immunosuppression. For this reason, while cyclosporine is marketed for the treatment of canine atopic dermatitis, therapeutic drug monitoring is not routinely recommended for this condition. Based on the unpredictable relationship between blood drug levels and clinical response, there is clearly a need to find a better method for monitoring the immunosuppressive effects of cyclosporine, particularly in patients with life-threatening conditions.

CYCLOSPORINE PHARMACODYNAMICS

A biomarker is a molecule that serves as an objective measure of a biological process. Pharmacodynamic assays evaluating selected biomarkers within the immune system have been advocated in human medicine to help determine drug efficacy and make dosing recommendations. For cyclosporine, these assays include quantification of drug target enzymes (calcineurin), cytokines (IL-2 and IFN-γ), and markers of lymphocyte proliferation or activation (CD25 and CD95). These biomarkers are involved in normal and pathological immune responses, so their inhibition serves as a quantitative, objective surrogate for inhibition of immune function. Pharmacodynamic monitoring of biomarkers of immunosuppression offers a more individualized approach to immunosuppressive therapy when blood levels do not correlate well with clinical response in our patients. Pharmacodynamic monitoring of biomarkers of immunosuppression, like cytokines, has yet to be thoroughly explored in the dog. A coordinated series of studies in our laboratory, however, have now established a number of biomarkers of immunosuppression that are promising candidates for development in clinically useful assays for individualising drug therapy in canine patients receiving cyclosporine.

Firstly, we conducted a study that evaluated the effects of in vitro cyclosporine exposure on a panel of molecules expressed by activated T-cells in order to ascertain their potential as biomarkers of immunosuppression in dogs. Blood was drawn from healthy dogs, and peripheral blood mononuclear cells were isolated and activated. Half of the cells were incubated with 200 ng/mL cyclosporine, and the other half were not exposed to cyclosporine. Samples were analysed using flow cytometry, and the expression of the intracellular cytokines IL-2, IL-4, and IFN-γ was evaluated after 6, 12, and 24 hours of drug exposure. We found that each cytokine exhibited a time-dependent suppression profile, and all but one sample activated in the presence of cyclosporine showed lower cytokine expression than untreated controls. We also evaluated the expression of the surface T-cell activation molecules CD25 and CD95 by flow cytometry after 36 hours of drug exposure. Expression of these surface molecules also decreased significantly when activated in the presence of cyclosporine. Our in vitro results strongly suggested that suppressed expression of the markers related to T-cell activation could potentially be utilised as an indicator of the efficacy of cyclosporine therapy in dogs.
Secondly, we conducted an in vivo study to determine whether selected cytokine biomarkers identified by our in vitro incubation work would be reliably suppressed in healthy dogs receiving oral cyclosporine. Flow cytometry was used to measure expression of IL-2, IL-4 and IFN-γ by circulating T-cells in cyclosporine-treated dogs. Cytokine expression 8 days after oral administration of both a high dose and a low dose of cyclosporine was compared to baseline values. The high cyclosporine dose consisted of a starting dose of 10 mg/kg every 12 hours, adjusted to attain established immunosuppressive trough blood drug levels (> 600 ng/ml), and the low dose was the approved atopy dose (5 mg/kg once daily). We found that high dose cyclosporine was associated with a significant decrease in T-cell IL-2 and IFN-γ expression, while IL-4 expression was not affected. Low dose cyclosporine was associated with a significant decrease in IFN-γ expression, while IL-2 expression was not affected. We concluded that our pharmacodynamic assays utilising cytokines as biomarkers of immunosuppression had potential utility as an adjunct to conventional therapeutic drug monitoring in dogs receiving cyclosporine.

Finally, we developed real-time RT-PCR assays that measured canine activated T-cell expression of IL-2, IL-4 and IFN-γ, and conducted in vivo and in vitro studies that compared the timing and magnitude of cyclosporine-induced suppression of these cytokines as measured by both flow cytometry and real-time RT-PCR. We concluded that real-time RT-PCR could reliably measure cyclosporine-induced suppression of activated T-cell cytokine suppression in a manner comparable to the suppression documented in our previous flow cytometric studies. This finding was important because, compared to flow cytometry, real-time RT-PCR is a technique that is much more ‘user friendly’ for diagnostic clinical use.

Our research to date gets us one step closer to establishing a simple, usable assay that can be offered to veterinary practitioners to allow individualisation of therapy in dogs receiving cyclosporine and, potentially, other immunosuppressive agents. Effective individualisation of therapy should significantly increase treatment efficacy with immunosuppressive drugs such as cyclosporine.

IS CYCLOSPORINE SAFE IN IMHA PATIENTS?
Recent human studies and results of our preliminary research have, unfortunately, suggested that cyclosporine could have the potential to contribute to the development of pulmonary thromboembolism (PTE) in dogs with IMHA. In humans, cyclosporine has been shown to increase platelet procoagulant activity and accelerate thrombin generation. Researchers have used flow cytometry to determine that cyclosporine causes changes in the human platelet plasma membrane, such as increased expression of platelet phosphatidylserine, which result in promotion of platelet procoagulant activity. Cyclosporine also increases platelet production of thromboxane A₂, a prostaglandin generated via the enzyme cyclooxygenase (COX) that activates platelets and causes vasoconstriction. Preliminary work several years ago in our Flow Cytometry Laboratory at MSU also suggested that, as in humans, cyclosporine may increase platelet procoagulant activity in some dogs. In our small preliminary study, flow cytometry was used to evaluate P-selectin and phosphatidylserine as surface markers of platelet reactivity in dogs before and after the administration of cyclosporine. Our preliminary study revealed markedly increased platelet P-selectin expression and mildly decreased phosphatidylserine expression, suggesting that, at the very least, exposure to cyclosporine was causing detectable changes in platelet surface membranes. Preliminary results of a small in-vitro companion study at MSU utilising several in-house analyzers which enable a more ‘global’ assessment of the effects of cyclosporine on both platelets and coagulation, the Siemens Platelet Function Analyzer-100® (which measures platelet function using a membrane aperture) and the Sienco SonoClot® (which utilizes viscoelastometry to evaluate clot formation), also demonstrated that exposure to cyclosporine appeared to decrease the time of canine platelet plug and fibrin clot formation.

Based on both human studies and the results of our preliminary research, we became concerned that the administration of cyclosporine to dogs with IMHA might have the highly undesirable potential to further increase the risk of thromboembolic disease and lead to an even higher incidence of PTE in IMHA patients. Since veterinarians are turning to cyclosporine as a potential life-saver in dogs with IMHA, it could have disastrous consequences if, in fact, the drug predisposed such patients to PTE and actually led to an increased mortality rate. We therefore devised and recently completed a much more comprehensive study at MSU-CVM designed to determine whether cyclosporine actually predisposes dogs to thrombosis. In our comprehensive study, we thoroughly evaluated platelet reactivity and procoagulant activity in eight healthy dogs before and after exposure to cyclosporine. Pre-treatment assessment included a complete blood count, serum biochemistry, urinalysis, prothrombin time (PT), partial thromboplastin time (PTT), plasma fibrinogen levels, antithrombin, von Willebrand factor, D-dimer, buccal mucosal bleeding time (BMBT), flow cytometric evaluation of a battery of markers of platelet function, activation and procoagulant activity (including P-selectin, phosphatidylserine, COX-1 and COX-2), viscoelastometric analysis of clot formation and retraction using the Sienco SonoClot®, evaluation of platelet function using the Siemens PFA-100® with collagen/ADP and collagen/EPI cartridges, and measurement of urine thromboxane B₂ (a metabolite of thromboxane A₂ that is more stable). The dogs were then administered oral cyclosporine at immunosuppressive doses (starting dose 10 mg/kg bid), and doses were adjusted accordingly to reach target immunosuppressive cyclosporine levels as determined by HPLC in our Clinical Pharmacology Laboratory. Samples were collected two hours after administration at peak plasma levels,
one day after initiating treatment, and after cyclosporine blood levels had achieved steady state and been determined to be at immunosuppressive levels for each individual dog, and evaluated using the battery of tests of haemostasis used for pre-treatment assessment. After a wash-out period, the study was repeated using a lower, anti-inflammatory dose of cyclosporine (5 mg/kg sid) that is typically used for canine atopy and the long-term maintenance of IMHA patients. Most of the results of our comprehensive study suggested that cyclosporine does not cause a detectable increase in platelet reactivity or procoagulant activity during the first week of drug administration in dogs. Mean platelet expression of P-selectin, phosphatidylserine, COX-1 and COX-2 all tended to decrease with cyclosporine therapy at both high and low doses, PFA-100® closure times tended to be slightly prolonged, and SonoClot® results were essentially unaffected. Urine thromboxane B₂ levels, however, increased markedly after each cyclosporine dose at both high and low dose rates. This finding is troubling because a very comparable phenomenon is reported in human kidney transplant patients receiving cyclosporine, and increased urine thromboxane B₂ levels are thought to reflect platelet activation and higher rates of post-transplant thrombosis and organ rejection. Clinical trials evaluating the safety of cyclosporine in dogs with IMHA, including assessment of potential prothrombotic effects, are still indicated and warranted, but our preliminary data suggests that cyclosporine may have the potential to predispose such patients to PTE.

What is Canine parvovirus?
Canine parvovirus (CPV2) is one of the smallest viruses and has a single stranded DNA genome. This virus does not contain genes for self-replication and it is dependent on replication in dividing cells. It therefore has a predilection for thymus, intestinal crypt cells and bone marrow cells. Canine parvovirus is genetically similar to feline parvovirus (feline panleukpenia virus or FPV). The virus has no lipid envelope and it is structurally very resistant to drying and many common disinfectants. This means the virus can remain capable of infection for long periods in the environment.

Where did it come from?
Canine parvovirus emerged as a pandemic around 1978-1979. However, the virus may have been present in the dog population prior to this time. The virus is thought to have possibly have arisen from FPV or mink enteritis virus (MEV) by minor mutations that allowed it to replicate in dogs, then further mutations and adaptations occurred resulting in an increased ability to spread in the dog population. FPV by contrast does not replicate in dogs. The first CPV identified is referred to as CPV2 to distinguish it from the previously identified but distantly related parvovirus of dogs, canine minute virus). CPV2 caused a severe pandemic with high mortality in dogs, but was unable to infect cats or mink. A variant virus (referred to as CPV2a) emerged in 1979 and replaced CPV2 worldwide in approximately one year (Parrish et al., 1988). CPV2a differed antigenically from CPV2 and infected both dogs and cats. Another antigenic variant (referred to as CPV2b) with an equivalent host-range was defined by a single substitution at amino acid 426 in the capsid protein gene and spread globally after 1984. Further mutations arose and spread widely, such as a mutation at residue 297 of the capsid protein (See table 1). Currently, another CPV2 with a mutation at capsid residue 426 is spreading globally. This mutation was first described in Italy in 2000 but has since been observed in other countries and most recently in the United States. It is clear from large-scale sequence studies that CPV2 is a highly mutable virus that is undergoing adaptation to its host.

Does CPV2 infect cats?
The original CPV2 isolates did not replicate in cats, but both CPV2a and CPV2b replicate efficiently in cat cells and can also infect cats naturally. Many studies have now been done in cats and in one study about 10% of the viruses isolated from cats with natural parvovirus disease were antigenically indistinguishable from CPV2a or 2b. The capsid protein gene sequence of a 1990 feline parvovirus isolate (“FPV-24”) was essentially identical to the sequence of CPV type-2b viruses from dogs. The question then perhaps what is really an FPV isolate and what is a CPV2 isolate? The loss and reacquisition of the feline host range in CPV2 was most likely due in each case to small numbers of changes in a region of the virus capsid (VP2) where three protein monomers interact (the three-fold spike)1. Importantly, from a clinician’s point of view these CPV2a, 2b isolates may cause disease in cats, usually a mild to moderate enteritis. It is also likely that vaccination of cats with FPV vaccines protects cats to some degree from disease with CPV2 types.

Subsequently other CPV2 types have been isolated from cats and designated CPV2c. These viruses have been also shown to cause diarrhoea in cats and parvovirus enteritis in dogs2. It is now considered that there may actually be a spectrum of CPV2 rather than distinctly 4 or 5 types. In Australia, the predominant type would appear to be CPV2a like although more CPV2 isolates probably need to be studied2.

So how do we know when there is a “NEW” type of parvovirus?
Many veterinarians when examining dogs with severe parvovirus query if the virus is a new type that is more pathogenic than before and maybe not protected by the current vaccines available. These questions are valid and require some understanding of how the CPV and FPV isolates are classified. The investigation and designation of CPV2 and FPV is largely based on determining the DNA sequence (and deduced amino acid sequence) of the VP2 gene. The VP2 gene codes for the capsid protein that forms the surface of the parvovirus necessary for attachment to the host cells. Analysis is usually done by PCR amplification of the VP2 gene and DNA sequence analysis. In this way large studies have been conducted now that clearly show that CPV2 is capable of forming many types and is under evolutionary pressures to change. However, the genetic factors that makes one CPV2 more pathogenic than another have yet to be elucidated.
then increases the load of CPV in the environment thus posing a risk to animals that are unvaccinated. Such an immunity vaccines are registered by the APVMA on the Australian market for canine distemper, canine adenovirus and canine parvovirus that do not provide sterilizing immunity on challenge will shed the virus sub clinically. This virus antibody titres (Mouzin, 2004). Several other studies have confirmed these findings. 5. 6. 7. For the moment it appears that current vaccines in Australia more likely a protective titre can be used to infer protection and the lack of one to infer susceptibility. (However, some dogs that have been vaccinated but do not have a protective titre will not. 6. 5. VACCINE protection against CPV2. Questions of duration of immunity? CPV2 is a disease where antibodies are highly protective against disease. There is a very strong correlation between the vaccine titre and protection from challenge (the gold standard). This means that measuring antibody levels can infer whether a dog is protected against parvovirus or not. However, in contrast with this finding two studies using current CPV2a and 2b followed by challenge with CPV 2c viruses showed adequate protection. 6. 5. For the moment it appears that current vaccines in Australia more than likely would also protect against CPV2c if that were the predominant type. There is no evidence at present of prolonged carriage and shedding of CPV2 in dogs. There is no evidence at present of prolonged carriage and shedding of CPV2 in dogs. However, the general environment is likely to have an unknown status as parvovirus can survive for a long time in the environment and be spread by fomites. So if there are new CPV2 mutants arising we should be seeing vaccine resistant types emerge? What about sub-clinical shedding of virus by dogs with existing antibody titres? An argument against the use of 3-year duration of immunity vaccines has been raised that dogs with existing titres against parvovirus that do not provide sterilizing immunity on challenge will shed the virus sub clinically. This virus then increases the load of CPV in the environment thus posing a risk to animals that are unvaccinated. Such an
argument has yet to be proven in any clinical trial. Furthermore, a dog with a HAI titre that is 1.80 or greater might be expected to shed virus (if at all) for only a few days after sub clinical infection. The argument also is not backed by studies that prove that yearly vaccination is superior to 3-year duration of immunity vaccine with regard to preventing sub clinical shedding. Finally, the source of parvovirus in the environment has never been documented. The role of unvaccinated dogs, feral cats and dogs with existing immunity has not been quantified. The issue of whether people who vaccinate their dogs should pay for yearly vaccines that are not needed for protection but provide herd immunity are those who don’t is also a vexed one. The key issue remains to get universal vaccination of young dogs that are at greatest risk.

So what is the most important issue regarding CPV2?
The most important issue regarding CPV2 remains the adequate vaccination of puppies during the period of maternal antibody decline. The use of high titre MLV virus vaccines is likely to help achieve a successful immunization by 10-12 weeks of age. These vaccines allow for safe early socialization after 12 weeks of age. Protection rates of the vaccines with a finalization time of 10-12 weeks are reported as being very high-approaching 100% 15. Nevertheless, the use of a 16-week CPV2 vaccine to follow may be required by veterinarians not using high titre vaccines. In Australia, the overwhelming majority of vaccine failures would appear to be associated with improper administration of vaccines during the maternal antibody period rather than the emergence of new CPV2 types or not vaccinating adult dogs yearly for CPV2 3.

References
EYELID DYSGENESIS IN THE CAT

A review of the disorder and corrective procedures

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POINTS TO NOTE:

- Foetal eyelid formation and fusion should be complete at approximately half way through gestation.
- The dysgenesis defect in development should be considered a coloboma.1
- The defect occurs in the upper eyelid, usually involving the lateral half of the lid.
- Further colobomatous defects may be found in the uvea - e.g. an iris defect or an optic disc hole.
- Dysgenesis almost always occurs in poorly developed kittens, usually nutritionally stressed.
- Typically, an owner will present an affected kitten found abandoned.
- There may be a genetic link to this problem in Persian cats. Clinical problems are associated with eyelid dysfunction with exposure and trichiasis from misdirected facial hair leading to corneal changes (keratitis).

MANAGEMENT:

- Epilation of trichiasis hairs with epilation forceps can be effective where the defect is small.
- Cryo epilation for a more permanent destruction of facial hairs is poorly effective as skin coat hair is resilient to cryogenic damage.
- Topical lubricants should always be used.
- Surgical eyelid reconstruction can provide good results in re establishing a functional eyelid.
  - Various techniques have been described and are dealt with below.

SURGICAL OPTIONS:

1. **The ROTATING FLAP procedure**2,3

   In this procedure an appropriately wide section of skin and orbicularis muscle is dissected from below the lid margin of the lower eyelid and transposed into a prepared bed in the defective upper eyelid. The surgeon should endeavour to support the skin graft with conjunctiva mobilized from the upper lid or from the lower palpebral conjunctiva. Care must be taken to avoid suture contact with the cornea. A third eyelid flap can be helpful during healing.

2. **LID SPLIT repair**4

   This procedure involves preparing the coloboma area as if it were the site of a tumour removal. The eyelid is then split into a skin side and a conjunctival side. The splitting technique mobilises the skin and conjunctiva so that the conjunctiva and then the skin can be sutured horizontally to close the defect. More skin is then mobilised from the lateral canthus by canthotomy to relieve any tension.

3. **LIP to LID TRANSPOSITION**5

   The hairless commissure of the lips makes for an ideal eyelid margin. Oral mucosa, attached to the commissure, is well accepted into the prepared defect. The procedure is more cumbersome than others described but results are better. The procedure is presented as a Power Point presentation.

References:

FELINE VIRAL KERATITIS AND ASSOCIATED SYNDROMES
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FELINE HERPES VIRUS (FHV-1) FEATURES & EPIDEMOLOGY

- Double stranded DNA virus of the Herpesviridae family & Alphaherpesvirinae subfamily
- 3 major genotypes: C7301, F2, C7805
- The characteristics of alphaherpesviruses are: tight species specificity, short reproductive cycle, cause lysis of infected cells, establish latency in sensory ganglia
- Natural host is domestic and wild felids
- Function of virus host cell surface receptor interaction
- 75-97% of all world's cat population is seropositive; FHV-1 responsible for 45% of all cat URTI
- Following infection 80% of cats develop latent infection
- About 50% of these have potential to shed virus intermittently associated with stress
- 30% will shed virus spontaneously
- Labile in environment, easily killed by disinfectants; lives for up to 5 days in eye wash
- Spread via macro droplets; sneezed up to 1.3 m!!
- Normal cat population tested via nPCR is 1-30% [approx. 12% in many studies] results depend on PCR technique i.e. single round or nested
- Cats with conjunctivitis - 20-50% are PCR positive for FHV-1
- Cats with URTD 14% PCR positive for Chlamydophila felis & 21% positive for FHV-1
- General Rules:
  - Young cats with conjunctivitis in summer months more likely to be Chlamydophila positive
  - Acute onset outside cat more likely to be FHV +
  - Co-infection is uncommon (up to 7 % in one study)

FHV-1 REPLICATION - URT epithelial membranes: Conjunctiva; nasal turbinates, nasopharynx, (corneal epithelium)
- Heparin sulfate glycoprotein host cell surface receptors essential for virus entry
- Entry > Uncoating > Release of viral DNA > Viral gene expression > Transcription > Synthesis of viral protein > Latency or lysis of host cell & enveloping virus
- Glycoprotein gp60 on viral surface elicits host antibody response

INCUBATION - 2-10 days. As early as 24 hours. Can persist for 3 weeks. Severity of disease is dose dependent
- Intermittent shedding = carrier state

PRIMARY INFECTION - Viral cytolysis. Conjunctivitis, hyperaemia, chemosis, serous or purulent discharge (5-7 days post infection), blepharospasm. Conjunctival epithelial sloughing.
- Biphasic corneal ulceration: 1. Dendritic corneal ulceration (pathognomonic for FHV-1) 2. Geographic corneal ulceration with chronicity (epithelial cell lysis & coalesce of lesions)
- Spontaneous recovery in 10-21 days

RECRUDESCENT INFECTIONS - Severity varies widely between individuals & between episodes of disease.
- Conjunctivitis is usually less ulcerative. Dendritic then geographic corneal ulceration. Immune mediated stromal disease common. Delayed virus clearance & chronic stromal exposure predispose to stromal immunopathological disease. Vascular fibrosis – corneal scarring

IMMUNE REACTION TO FHV-1 INFECTION - Gp 60 major target of serum neutralising antibody. Serological levels of circulating antibody not necessary a good indication of disease state. Reflects more important role of cell mediated immunity & less significant role of humoral immune response against FHV-1. Humoral immunity cannot prevent latency or infection. After vaccination - detectable titre for 4 yrs - associated with 50% reduction in clinical signs upon re-exposure

LATENCY - Definition: Period when virus cannot be cultured i.e. non-lytic phase
- During this time FHV-1 expresses LAT gene in trigeminal ganglia but not cornea
- Following acute infection, in approx. 80% of cats, FHV-1 ascends ciliary n. > ophthalmic br. CN V > ciliary ganglia in orbit
- Stress (physiological or pharmacological) results in periodic reactivation.
- Virus is reactivated but the disease is recrudescent
PERSISTENT VIRAL STATE - Recently, a separate disease state, independent of lytic, latent or reactivated viral states, has been suggested for HSV-1. Associated with chronic clinical inflammatory disease. The role that persistent viral state has on chronic immunopathological disease is under investigation.

CLINICAL SYNDROMES ASSOCIATED WITH FHV-1 INFECTION

- **KCS** - definitely occurs, mechanism unknown - most likely secretory ductule fibrosis &/or lacrimal adenitis
- **Corneal sequestration**
  - Most likely a non-specific response to substantial stromal exposure or damage
  - Nasisse reported 55% of sequestra had FHV-1 DNA compared to 5.9% of normal cats
  - Other authors have failed to replicate these numbers
  - Persians & Himalayans had statistically significant lower FHV-1 DNA in sequestra, suggesting non viral aetiology (facial conformation)
  - As a general rule it is thought that corneal sequestration are caused by FHV-1 in up to 80% of DSH, but only 50% in brachycephalic breeds
- **Eosinophilic keratitis or Proliferative keratitis** - Inconclusive link - 76% FHV-1 +ve on PCR in one study
- **Symblepharon** - Mostly young cats. Concurrent conjunctival & epithelial lysis or loss of inhibition of limbal conjunctival stem cell
- **Uveitis** - Well documented in HSV-1 and given the shared biological behaviour of Alphaherpesviruses, probably FHV-1 as well. It has recently been shown that there is local production of antibody direct at FHV-1 in the eyes of some cats with uveitis. FHV-1 can therefore gain access to anterior chamber to stimulate a specific antibody response
- **Dermatitis** - Around nose & periocular skin due to the epithelial tropism of FHV-1

Interactions With Other Agents
- Cats with chronic FHV-1 infection more likely to have co-infection with FIV/FeLV
- Clinical signs more severe but duration of shedding NOT different
- Co-infection with Chlamydophila uncommon (<7%)
- Evidence lacking for a relationship between chronic FHV-1 disease & co-infection with Toxoplasma gondii & Bartonella henselae

THE DILEMMAS ASSOCIATED WITH PCR & DIAGNOSIS

- Copious viral shedding available for PCR during primary infection, but unnecessary because clinical signs characteristic of disease and diagnosis is straightforward
- In contrast, during chronic disease ambiguous clinical signs & elusive viral DNA makes diagnosis difficult
- PCR is extremely sensitive & specific
- 33% of normal cats PCR positive - this needs to be considered when interpreting PCR results
- Three scenarios possible:
  - Presence of FHV-1 DNA may be coincidental (i.e. unrelated to primary disease process)
  - Presence of FHV-1 DNA may be the consequence of another primary disease
  - Presence of FHV-1 DNA may be the cause of the primary disease
- One of the best ways to diagnose FHV-1 is to maintain a high clinical suspicion of its involvement in any cat with surface ocular disease
- PCR sensitivity 97% in acute cases (<1 month) of unvaccinated cats
- PCR sensitivity 56% in acute cases (<1 month) of vaccinated cats
- PCR sensitivity 90% in acute Chlamydophila cases
- 73% sensitivity in chronic (>2 months)

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>FHV-1</th>
<th>FCV</th>
<th>Chlamydophila</th>
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<tbody>
<tr>
<td>Malaise</td>
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<tr>
<td>Sneezing</td>
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<td>Nasal discharge</td>
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<tr>
<td>Oral ulceration</td>
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<td>Ptyalism</td>
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<td>Ocular discharge</td>
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<td>Conjunctivitis</td>
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<td>Keratitis</td>
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THERAPY

- **Topical Idoxuridine**
  - No longer commercially available, compounded medication only
  - Interferes with thymidine for incorporation into viral DNA – thus prevents replication
- **Acyclovir (Zovirax)**
  - A purine nucleoside that inhibits viral replication by interfering with DNA synthesis
  - Little effect on non-infected host cells
  - Thus one of the few non-toxic drugs and can be used systemically (potential BM toxicity)
  - But oral dosing only achieves 1/3 of the plasma concentration required to be effective (ED50)
  - ED50 for FHV-1 >>>>> HSV-1 - Therefore far less effective in FHV-1 cases

- **The Interferons**
  - IFN’s are produced by leukocytes, 14 subtypes
  - Viral infections stimulate IFN production within the extra cellular spaces and through mechanisms not understood prevents the spread of infection (IFN binds to cell receptors preventing infection of neighbouring cells)
  - Therapeutic benefit controversial
  - Limited effects in treating established infections
  - Recent study demonstrated increased severity & duration of disease in SPF cats infected & treated compared to infected non-treated cats - other studies have demonstrated benefits with topical, (oral [digested by peptidases]) & subcutaneous injection
  - Topically, compound with tears into 10,000 IU per drop, applied 4 x daily

- **1% Betadine drops**
  - Solution not scrub
  - Virucidal
  - May be useful in chronic cases unresponsive to IDU therapy
  - Applied topically 6-10 x daily

- **Famciclovir**
  - Prodrug of penciclovir which inhibits viral DNA synthesis
  - Dose 6-10 mg/Kg every 12-24 hrs - been used safely and is well tolerated in cats
  - Clinically been shown to reduce conjunctival hyperaemia, ocular discomfort, excessive tearing and improve healing rates of corneal ulceration

- **Anti inflammatory therapy**
  - Controversial - The use of anti inflammatory medications has been inadequately studied
  - Topical & systemic steroids will exacerbate clinical disease during active primary or reactivated lytic infection
  - CsA 1% specific T-cell inhibitor will suppress viral clearance during lytic phase
  - Indicated only in chronic stromal immunopathological disease with no or low viral replication

- **Lysine therapy**
  - Arginine is an essential amino acid for viral replication
  - Arginine in combination with high levels of L-lysine suppresses FHV-1 replication
  - In vitro 80% reduction in FHV-1 replication in tissue cultures
  - L-lysine may substitute for arginine & act as an antimetabolite
  - L-lysine & arginine competitively inhibit the others transport b/c they may share a common transport mechanism
  - Cats are sensitive to argentine deficiency
  - Exp infected SPF cats receiving 500 mg BID had shorter duration & less severe clinical signs
  - Oral lysine also been shown to prevent virus reactivation &/or shedding in latently affected cats

- **Surgery therapy**
  - Keratectomy may physically remove virus from infected corneas
  - May also help with stromal immunopathological disease
  - Penetrating keratoplasty is used in humans??

**VACCINATION**
- Controversial
- Serological levels of circulating antibody not necessary a good indication of disease state - reflects the more important role of cell mediated immunity & less significant role of humoral immune response
- Humoral immunity cannot prevent latency or infection
- Vaccination has been shown to result in a 50% reduction in clinical signs upon re-exposure
- Mucosal vaccination (intranasal) may be more useful because it generates rapid cell mediated immunity
  - But it’s associated with high shedding of vaccine virus during recrudescent disease
- In addition, vaccination has not been shown to reduce amount of virus shed or inhibit latency
FELINE ANTERIOR UVEITIS
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The uvea forms the internal vascular structures of the eye. Anteriorly, the uvea forms the iris, centrally the ciliary body and posteriorly the choroid. When uveal tissue becomes inflamed this is called uveitis. If it mainly affects the iris it is called anterior uveitis or iritis, when mainly affecting the ciliary body region it is called cyclitis or intermediate uveitis. Inflammation of the posterior uvea is called choroiditis or chorioretinitis. When inflammatory cells are found in the vitreous we call this hyalitis, hyalitis can only occur when the ciliary body becomes inflamed as the normal vitreous in adult mammalian species is devoid of vessels. Hyalitis is therefore regarded as a sign of uveitis.

AETIOLOGY - Traditional ophthalmology texts suggest that most of the causes of anterior uveitis in cats are thought to be infectious. However extensive serological testing does not always suggest that this is the case. Failure to identify an infectious cause could be due to:
   a) the fact that the cause is not infectious
   b) there is an infectious cause unknown or unidentified by the tests
   c) the limitations of the test have resulted in a false negative result

Infectious - All of the following infectious agents have been incriminated as causes of anterior uveitis in cats:
   • Feline Immunodeficiency Virus (FIV)
     There is no doubt that FIV can be a cause of anterior uveitis in infected cats. The uveitis can be transient or chronic and may persist despite anti-inflammatory therapy, eventually causing secondary glaucoma. The most common ophthalmic manifestation of infection with this virus is thought to be ciliary body inflammation resulting in accumulations of inflammatory cells in the peripheral anterior vitreous giving a “snowbank” appearance behind the lens.
   • Feline Leukaemia Virus (FeLV)
     Feline leukaemia virus is really an uncommon cause of uveitis in cats even in countries such as UK where infection with this virus is more prevalent, however it can cause lymphosarcoma involving the anterior uvea and signs of uveitis will often be seen in association with occurrence of tumours in this part of the eye. The most common sign of FeLV infection is spastic pupil syndrome where abnormal pupil responses may occur due to effects of the virus on autonomic nerves.
   • Toxoplasmosis
     The single cell parasite Toxoplasma gondii can be found in almost all warm blooded animals, usually in its inactive cystic or bradyzoite form. Felidae however, both wild and domesticated, are the only definitive hosts for the parasite in which ingestion of sporulated oocysts will result in production of new oocysts in a gut replicative cycle. The gut replicative cycle however can result in formation of tachyzoites deeper in the intestinal wall - the organism may then get into the bloodstream and result in an extraintestinal clinical infection especially if the immune system is compromised by for instance concurrent FeLV or FIV infection. A properly functioning immune system is thought to be important in suppressing the formation of these tachyzoites. The uvea is one of the sites where an immune reaction to the organism can then localise, causing acute signs of uveitis. In the chronic phase this will often be manifest as a nodular uveitis.
   • Feline Infectious Peritonitis Virus (FIP, Coronavirus)
     After initial infection with coronavirus most cats recover spontaneously, with a small percentage developing persistent infection that causes death months to years later. The virus causes vasculitis and associated pyogranulomatous perivascular inflammation, due to fixation of complement after an antigen antibody reaction. Virus is thought to persist in mononuclear phagocytes. Immunosuppression by other viruses may also be important in disease pathogenesis. Anterior uveitis is one of the manifestations of the so-called “dry” form of the disease. This disease is seen more commonly in younger cats.
   • Fungal
     Fungal causes of uveitis in cats are commonly recognised in North America but not usually in Australia with the exception of cryptococcosis, which can sometimes be implicated as a cause of uveitis – usually these cats will show other signs of infection as well e.g. involvement of the nasal cavity/ paranasal sinuses and granulomatous chorioretinal lesions.

Idiopathic (suspected to be infectious)
The search for other causes of uveitis in cats has mainly been fuelled by the failure to identify traditional causes in many cats with uveitis. Generally these cases would be called idiopathic when no other causes can be found (the definition of “idiopathic” after all)
Possible infectious causes

- **Feline Herpes Virus (FHV-1)**
  HSV-1 is a well documented cause of Herpesvirus in man and EHV-1 has been strongly implicated as a cause of uveitis in horses in at least one paper describing an outbreak of EHV-1 in a group of foals. In one study in cats by Maggs and Lappin, ELISA was used to examine FHV-1-specific antibody concentrations in aqueous humor and serum in cats. All of the cats in the study were seropositive for FHV-1 but intraocular antibody production, as determined by a Goldman-Witmer coefficient (C-value) > 1, was detected only in cats with uveitis. Additionally, a C-value > 8, which is frequently quoted as a more clinically useful indicator of intraocular antibody production, was found only in cats with idiopathic uveitis (i.e. another cause could not be found). So there is some evidence that feline Herpesvirus stimulates a specific local intraocular antibody response in some cats.

- **Bartonellosis**
  There has been a lot of interest in Bartonella spp as a potential cause of uveitis in cats over the last 10 years. This organism is an intracellular parasite and is transmitted between cats by fleas. It is generally thought to be harboured primarily in red cells and possibly in vascular endothelium. The problem with “naming and shaming” this organism as a cause of uveitis in cats is that there are many cats which have high levels of naturally occurring antibody to the virus and few of these cats show clinical signs associated with infection with the virus. More studies are needed to further confirm that Bartonella may be a cause of uveitis in some cats.

Non infectious

- **Lens induced** - When cataracts age they can eventually become hypermature and leak lens proteins into the eye. The internal immune system of the eye recognises lens proteins as foreign and develops an immune response to them. This condition is referred to as lens induced uveitis or LIU. The uvea seems to be more reactive to lens proteins in young animals than old animals and LIU seems not to be as common a phenomenon in the cat as it is in the dog, as cats generally are less likely to form mature cataracts. A problem also arises in that persistent uveitis of any cause can also cause cataract development – these cataracts will develop in the anterior cortex as this is the area closest to the anterior chamber (“sick” anterior chamber fluid is thought to cause changes in the lens proteins closest to the anterior chamber across the anterior capsule of the lens) and sometimes it is hard to know whether the cataracts caused the uveitis or the uveitis caused the cataracts.

- **Traumatic** - Any penetrating trauma to the eye which breaches the capsule of the lens will cause leakage of lens proteins and the induction of an inflammatory response by the uvea. Even if the lens is not breached however, an ocular inflammatory response can occur in response to any significant blunt trauma.

- **Neoplasia** - cases of intraocular lymphoma commonly present with signs of intraocular inflammation

OPHTHALMIC SIGNS OF ACUTE UVEITIS

- **Anterior chamber flare** - When the blood aqueous barrier breaks down, inflammatory cells and proteins leak through vessel walls in the uvea and form microscopic particles in the anterior chamber. These particles will visible with slit lamp microscopy. This is sometimes referred to as “particulate flare” to distinguish it from “non particulate flare” which forms a diffuse “smoke haze” like appearance to the anterior chamber – the most likely cause of non-particulate flare is suspended lipid in the anterior chamber. There appears to be a genetic predisposition to hyperlipidaemia in some Burmese cats and some of these cats may also have a liquid aqueous. There is also some thought that the lipid may in turn be able of inducing an inflammatory response on its own.

- **Anterior chamber fibrin** - In severe inflammation, large amounts of soluble fibrinogen may also leak into the anterior chamber and clot to form fibrin.

- **Miosis** - The release of prostaglandins in uveitis causes contraction of smooth muscle of the pupillary constrictor and pupil miosis.

- **Keratic precipitates** - Inflammatory cells and proteins released from the iris and ciliary body can eventually settle and adhere to the endothelial surface – these aggregates form localised opacities, usually inferiorly on the endothelial surface. If other immune reactions such as complement fixation occur on the endothelium then localised stromal oedema may also be seen in association with endothelial dysfunction.

- **Changes in iris colour** - When vessels on the anterior stromal surface of the iris become injected during inflammation the iris will change colour from its usual tan (or blue colour in colour dilutes) to a pinkish red colour.

- **Hypotony** - Uveitis is associated with release of inflammatory mediators into the eye formed by the breakdown of phospholipids in cell walls. These include prostaglandins and leukotrienes. Prostaglandins in particular are well known for their ability to lower intraocular pressure – it is thought that they open up an alternative “uveoscleral” drainage pathway in the eye increasing the egress of aqueous fluid from the eye. This pressure lowering effect of prostaglandins can be negated by the pressure increasing effects of obstruction to aqueous drainage pathways by the chronic formation of posterior synechia to the lens and the formation of
fibrovascular membranes over the iridocorneal angle. Elevation of intraocular pressure is commonly seen, especially in cases of poorly controlled chronic anterior uveitis.

- **Vitreal flare** - Egress of inflammatory cells from the ciliary body into the vitreous can cause “vitreal flare”. This will appear as a white cloudiness immediately behind the lens peripherally and seems to be commonly associated with FIV infections.

### OPHTHALMIC SIGNS OF CHRONIC UVEITIS

- **Ruberosis** – anterior stromal neovascularisation. Production of vascular endothelial growth factors (VEGFs) inside the inflamed eye will result in the chronic vascularisation of the anterior surface of the iris giving the iris a permanently red appearance.

- **Iris nodules** - Small grey nodules are sometimes seen on the anterior surface of the iris – these are actually small localised collections of lymphocytes and sometimes plasma cells. These are usually permanent although their appearance will often be diminished by chronic use of topical corticosteroids.

- **Posterior synechia/pigment remnants on anterior lens capsule** - When fibrin is released from the anterior uveal vessels in acute anterior uveitis, it will sometimes stick the posterior pigmented epithelium of the iris to the lens capsule. When the inflammation is controlled permanent posterior synechia (adhesions between the iris and the anterior lens capsule) may occur or if the adhesions breakdown eventually small remnants of uveal pigment may be left on the anterior capsular surface.

- **Vitreal debris** - Inflammatory cells and proteins released into the anterior vitreous from the ciliary body can eventually result in vitreal flare or haze with permanent opacification in this region.

- **Lens Luxation** - Chronic inflammatory destruction of lens zonules around the ciliary processes may eventually result in lens instability and subluxation or luxation of the lens.

- **Glaucoma** - A common sequel to chronic anterior uveitis in cats is the development of secondary glaucoma. This occurs because of the development of chronic fibrovascular membranes within the eye which eventually grow across the iridocorneal angle eventually occluding it and reducing aqueous outflow.

### DIAGNOSIS

- **Ophthalmic examination** - Since uveitis is a clinical disease of the eye, identification of some or all of the classical ophthalmic signs mentioned above is the most important criterion in diagnosis of this disease.

- **Tonometry** - Because of the IOP lowering effects of prostaglandins within the eye demonstration of low pressure is supportive of a diagnosis of uveitis however not too much importance should be placed on IOP readings as many cats will develop elevations of IOP associated with obstruction to aqueous flow in the more chronic phases of the disease.

- **Serology** - Serology is not always very useful in establishing a diagnosis of a definitive cause of uveitis.
  - Traditionally it has been used in the diagnosis of toxoplasmosis IgG and IgM - The presence of significant IgG antibodies to *T. gondii* in a healthy cat suggests that the cat has been previously infected and now is most likely immune and not excreting oocysts. The presence of significant IgM antibodies to *T. gondii*, however, suggests a recent or current active infection.
  - The diagnosis of FIP has always been more complicated by the common cross-reactivity of the infectious peritonitis virus with other enteric coronaviruses. Unfortunately confirmatory serological tests for FIP generally have low diagnostic value and there is a reasonably high incidence of serological false negatives i.e. cats infected by FIP virus which are negative on serology. These cats may have been previously exposed but have good cell mediated immunity. In one study cats with clinical signs suggestive of FIP, the presence of lymphopaenia, hyperglobulinaemia, and an antibody titer of 160 or greater had a positive predictive value of 89%, whereas the absence of all of these findings had a negative predictive value of 99%. The only definitive diagnostic test for this disease seems to be histopathology of affected tissues with demonstration of the typical pyogranulomatous vasculitis.
  - Latex cryptococcal antigen testing (LCAT) is also useful in the diagnosis of cryptococcosis.

- **“Anterior chamber” serology**
  Obtaining more evidence that the organism is actually inside the uveitic eye is probably the way we need to go in diagnosis of infectious causes of uveitis. This is the gold standard in research where attempts are being made to implicate a particular organism as a cause of uveitis. The problem is that the process of anterior chamber fluid collection is involved and this process is not commonly performed in clinical practice. The Goldman-Witmer coefficient (C value) is used to detect the significant presence of antibodies to the organism inside the eye by showing that the local intraocular immune system is producing relatively greater amounts of antibody to the organism than the immune system outside the eye. \[GW = \text{Goldmann-Witmer coefficient} = \frac{X}{Y}\] where \(X\) = specific antibody in aqueous or vitreous divided by total IgG in aqueous or vitreous; and \(Y\) = specific antibody in serum divided by total IgG in serum. It is commonly accepted that a C value >8 suggests significant local antibody production to an organism within the eye?
Workup for systemic disease - In association with serological investigations a more general workup will sometimes identify other illness which could be implicated as a cause of the uveitis.

TREATMENT

Anti-inflammatories

- Corticosteroids - The most appropriate treatment for an inflammatory disease like uveitis is obviously anti-inflammatories, and corticosteroids are usually more useful as they block more inflammatory pathways. As high systemic doses can be immunosuppressant a balance has to be struck between anti-inflammatory and immunosuppressive doses especially when treating FIV, FeLV or FIP positive cats. Topical corticosteroids such as Prednefrin Forte (1% Prednisolone Acetate – Allergan) or Maxidex (0.1% dexamethasone – Alcon) are most commonly used in veterinary ophthalmology practice in Australia. These drugs are useful in treating anterior uveitis but the further posteriorly the inflammation occurs the less useful topical drugs become. In a normal cat SID dosage with Prednefrin Forte is adequate for long term control of anterior uveitis – persistent more frequent dosage will result in systemic absorption and associated side effects.

- Non steroidal - NSAIDS are generally less useful in treating uveitis in cats because they are usually a poor second runner in suppressing the CMI response in the uvea, however clinicians may occasionally use drugs such as Acular (ketorolac) in the treatment of chronic uveitis in cats. On occasions the development of a superficial corneal ulcer may preclude the use of topical corticosteroids, but the use of topical NSAIDs may also retard ulcer healing as well making them a poor alternative. Cats are also more sensitive to the effects of systemically absorbed NSAIDs on renal function making drugs such as Voltaren ophtha totally contraindicated for cats. Ultimately if it is inappropriate to use topicals then the use of oral corticosteroids is probably the best option.

Mydriatics - While traditional ophthalmology texts suggest that mydriatics such as atropine are useful in treating anterior uveitis (because spasm of the parasympathetically innervated pupillary constrictor and the ciliary muscle is a significant cause of pain in uveitis) their usefulness in cats is debatable, except perhaps for very short periods when there is extreme miosis causing pain in very acute disease. Their use in chronic uveitis would be totally inappropriate and cats are renowned for salivating profusely after topical administration of atropine, an unpleasant side effect which many owners do not enjoy watching!

Glaucoma medications - As discussed previously, some cats with chronic anterior uveitis will eventually develop secondary glaucoma. Often the use of anti-inflammatories will reduce the IOP again when used on their own but sometimes glaucoma drugs such as Trusopt may be needed to control IOP long term. Xalatan is inappropriate, as being a prostaglandin, it is pro-inflammatory and the receptors seem to be different in cats making it of little use for control of glaucoma in cats.

Antimicrobials: Clindamycin, Azithromycin, Doxycycline - There are a few useful antimicrobials which can be used in the treatment of specific infectious causes of uveitis:

- Clindamycin can be used to treat acute clinical cases of toxoplasmosis.
- Azithromycin seems to be the drug of choice in treating bartonellosis in cats
- Doxycycline: there has been a lot of interest in the use of this drug in treating uveitis in cats in recent years. Doxycycline is not only antibacterial but it has some other very interesting anti-inflammatory properties which can be exploited in treating uveitis – in particular doxycycline inhibits Interleukin 1 and is also a potent nitric oxide synthase inhibitor. A dose rate of around 5mg/kg BID (1/2 a 50mg tablet BID for an adult cat) seems to be useful in the treatment of idiopathic uveitis – anecdotal experience and discussions with other veterinary ophthalmologists suggests that this drug can achieve a complete long term resolution of the disease without the need for continuing medication in some cases. NOTE: the tablet form of the drug can form a dry powder which can stick to and irritate the oesophageal wall so be careful using tablets: I suggest using the paste form or “wetting” the oesophageal wall by syringing water before giving the tablet.
UPDATES ON THE DIAGNOSIS OF FELINE INFECTIOUS PERITONITIS (FIP)
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DEFINING FIP
FIP is a diagnosis more feared than cancer as no effective treatment has been established and unlike many neoplastic diseases, it more frequently affects young cats in their most vibrant years. Coupled with the dread of this diagnosis is the complexity, inaccuracies and confusion that surrounds it, generally due to an overextension of the limitations of many of the purported ‘FIP tests’ and an overreliance on circumstantial evidence. To determine what tests are appropriate or inappropriate for diagnosing FIP, it is essential to have a clear understanding of what the disease is and where the virus can be found within normal healthy cats. It is confusion over this latter point that has resulted in misleading diagnostic tests, many of which are still on the veterinary diagnostic market.

The clinical presentation of FIP is diverse, ranging from body cavity effusion to none at all; neurological disease to focal GIT disease to upper/lower respiratory disease to multiple body systems affected. Almost any body system can be affected, singly or in combination. While FIP is classically seen in cats less than 2 years of age (>50% of cases), it has no age barriers. The bimodal age distribution described by others was not apparent in our analysis of 300+ confirmed cases through our diagnostic lab (publication in submission). Pedigree cats are certainly over-represented (70% of cases) but the disease also occurs in domestic crossbreeds. While cats with yellow, sticky, high protein body effusions are usually straightforward to diagnose through cytological analysis of fluids, protein analysis (serum and fluid) and direct immunofluorescence (DIF) to detect FIPV within macrophages, more atypical cases are seen for which clinicians need to be careful not to dismiss the diagnosis of FIP or over interpret circumstantial evidence and incorrectly diagnose FIP.

There are important principles we must consider when assessing the diagnostic value of FIP tests:

1) Feline Coronavirus, namely Feline Enteric Coronavirus (FECV) is ubiquitous in the cat community, infecting >30% of pet cats, >80% of cattery confined cats.
2) FECV is not confined to the GIT tract and several researchers have detected it using qRT-PCR, living in small numbers in the haematopoietic tissue or blood of cats.
3) Currently FECV is serologically/antigenically indistinguishable from Feline infectious peritonitis virus (FIPV).
4) The best way to differentiate them is by their biological behaviour. Only FIPV has the capacity to produce large numbers of FIPV within macrophages (detectable by immunodiagnostic techniques) and produce the pathology we recognise as FIP.
5) There is still great debate about the genetic differences between FECV and FIPV. Pedersen (2009) recently reported that mutations within the 3c region of the FIPV genome are important determinants of virulence but this is contested by others. If this is found to be a consistent genetic mutation across all FIP cases, then it may lead to FIP specific diagnostic tests. However extensive research looking into healthy vs. unhealthy non-FIP vs. FIP confirmed cats would be needed to ensure mistakes of the past are not repeated.

CONFIRMING FIP
Currently, definitive diagnosis of FIP in cats with suggestive clinicopathological findings, can only be achieved when FIPV is detected within the macrophages of tissue or fluid samples, using immunohistological or immunocytological techniques.

Detection of FIPV within macrophages of tissue or body cavity effusions:

1. **Immunohistochemistry** is used in the diagnosis of FIP to detect FIPV laden macrophages within tissue sections. It confirms the histological and clinicopathological suspicion of FIP and is mandatory for definite diagnosis of FIP. Only in FIP, are the number of FIPV within macrophages sufficiently high enough to be detected by this method. Therefore unlike nucleic acid amplification techniques (e.g. RT-PCR) which are extremely sensitive, it will not detect small numbers of FIPV or FECV within healthy tissue. It also provides the added benefit of detecting FIPV within the landscape of the pathology seen. Interpreted in the context of suggestive history, it is 100% specific for FIP.
2. **Direct immunofluorescence (DIF)** is used to detect FIPV-laden macrophages within fluid samples most frequently abdominal effusions, but also thoracic, pericardial and occasionally CSF. It is 100% specific however its sensitivity ranges from 57% to 80%.
3. **Histology** has always been essential in the diagnosis of FIP. Fibrinous-granulomatous serositis with protein-rich effusions into body cavities, granulomatous-necrotising phlebitis and periphlebitis and granulomatous inflammatory lesions in several organs can be seen. In dry FIP, perivascular macrophages are often surrounded by dense infiltrates of mainly B lymphocytes and plasma cells within the surrounding tissue.
SUPPORTIVE DIAGNOSTIC TESTS REQUIRING CAUTIOUS INTERPRETATION

In addition to clinical presentation, routine haematology, biochemistry, cytology of effusions etc, the following diagnostic tests have been proposed to offer additional support for FIP, but each has limitations requiring careful consideration by the veterinarian:

1. **Serum protein electrophoresis**: Taylor et al (2010) published an extensive retrospective review of serum protein electrophoresis results for 155 cats with a range of different diseases. Polyclonal gammopathies were seen with many different infectious/inflammatory conditions other than FIP, indicating the lack of specificity in FIP diagnosis. Most cats with FIP (35/41) had elevated gamma globulin but this was seen in a many other diseases.

2. **Albumin/globulin ratios**: Serum a/g ratio <0.8 and effusion a/g ratios of <0.4 have a high probability (>90%) of FIP (Hartman et al 2003). However there are other possibilities for elevated globulin.

3. **Alpha-1-acid glycoprotein (AGP)**: is an acute phase protein that rises during infectious and inflammatory conditions. It is not specific for FIP. Duthie et al (1997) determined that levels >1.5 g/L in serum, plasma or effusions were highly valuable in distinguishing FIP from other diseases with similar clinical signs. Giordano and colleagues (2004) found that cats with FIP had AGP of 2.72 +/- 1.46 which was significantly higher than clinical normal SPF cats or FCoV exposed cats (AGP ~1.20 +/-0.6). Further research is required using a wide spectrum of disease and non-diseases states.

4. **Rivalta test**: Hartman et al (2003) reported this test had high positive predictive (0.86) and negative predictive indexes (0.97). We routinely perform this test with all our DIF tests offered as it’s easy to do but it doesn’t offer any value over routine cytological analysis of effusions.

5. **Nucleic acid amplification (aka PCR)**: In most infectious diseases, establishing a molecular technique that amplifies nucleic acid is the ultimate in diagnosis. This is not the case with FIP for a variety of reasons. Firstly, the genetic differences between FECV and FIPV are still contentious, so differentiating the viruses by molecular methods is currently not possible, although Pedersen’s recent work in the 3c area of the virus is promising. Secondly, it was incorrectly assumed that FECV did not escape from the GIT, so RT-PCR methods that amplified feline coronavirus from blood, effusion or tissue was assumed to be FIPV. However the work of Kipar (2006, 2010) has shown that healthy cats infected with FECV produce a monocyte associated viraemia within a week of infection and that this viraemia may reoccur at different time points. She has also found that the virus can be found in tissue macrophages in healthy cats. Quantitative RT-PCR techniques are available at UC Davis, USA and Auburn University. However, further research is needed looking at the persistence of FECV in various body tissues and fluids of a wide cross section of the cat population. Until this is available the results of all molecular methods have to be viewed within the context of the cat’s clinical presentation and it is not a stand-alone diagnostic test.

6. **Serology**: The limited value of serology in the diagnosis of FIP is frequently overreached. This has resulted in the unnecessary euthanasia of cats due to ignorance on the part of diagnostic labs and veterinarians. The reasons for this are:
   a. At the present time, FECV and FIPV are serologically indistinguishable, so any test that claims it is FIP specific is false;
   b. Indirect immunofluorescence is the gold standard for measuring antibody titres of FCoV. However there are no guidelines on the cell line that should be used as the base of the test. Researchers or laboratories using different cell lines in their test, produce radically different antibody titres using the SAME SERUM sample. This was clearly established by Kummrow (2005). Therefore the often misquoted titres of 1:1600 based on Hartmann’s work cannot be used as cut-off points for FIP diagnosis for several reasons 1) in Australia a totally different method is used and >60% of healthy cattery confined breeding cats exceed this magical number using their exact method (Norris unpublished results), 2) Studies need to include a large number of healthy cats as controls to determine the positive predictive value of a test when the test is used more broadly, as it is in reality. A standardised technique needs to be established and tested across a wide population of cats (healthy vs. non-healthy non-FIP vs. FIP cats) and labs need to stop using interpretations based on different methodologies to the ones they are performing.

CENTRAL CRYPTOCOCCOSIS IN A BLIND CAT
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Acute sudden onset of blindness in association with multifocal neurological signs in cats is a diagnostic challenge. Rapid and accurate diagnosis is crucial to preserve ocular function and prevent neurological deterioration. Systemic disease should always be suspected in these cases, with ophthalmic examination providing a vital clue with regards to diagnosis. Delayed treatment may result in permanent damage to the eye or brain.

Cryptococcosis is the most common cause of systemic mycotic disease in cats. Inhalation of spores and sinonasal disease, with or without localised infiltration to surrounding structures, are recognized as being fundamental in both pathogenesis and clinical findings in cats with this disease. Primary brain cryptococcomas are rare and the route by which they attain entry into the brain is still speculative. Long term aggressive medical therapy with a combination of fungistatics and fungicidals, improves long term outcomes for these patients, although the prognosis still remains guarded.

Monitoring using conventional serum antigen titres is well recognised. With the development of advanced imaging techniques such as magnetic resonance imaging (MRI), primary brain lesions and the changes associated with treatment can be sequentially observed.

This case report presents a cat with central Cryptococcus which showed clinical neurologic improvement and reduction in serum antigen titres in response to treatment, but maintained pathologic changes on sequential MRI examinations over 15 months. Visual recovery was identified several weeks after treatment commenced, and has been maintained.

References:
HYPERLIPIDAEMIA AND THE FELINE EYE
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Hyperlipidaemia refers to abnormally elevated levels of lipids or lipoproteins in the blood. Lipoproteins are the transporters of cholesterol and triglycerides in the circulation. In cats, it is the large lipoproteins such as chylomicrons (CM) and very low density lipoproteins (VLDL) that transport the majority of exogenous and endogenous triglycerides, respectively. An increase in CMs or VLDLs will produce a visible lipaemia which can be observed directly in the lumen of blood vessels or within the anterior chamber. Retinal vessels will appear visibly lipaemic when plasma triglyceride levels are in the region of 28 mmol/l.

Lipid aqueous occurs when there is a breakdown of the blood-aqueous barrier with a concurrent hyperlipidaemia; the inflamed blood vessels then leak large lipoproteins directly into the normally clear aqueous humour. The appearance ranges from spectacularly white or creamy in the presence of elevated chylomicrons, to a more subtle opalescence when hypertriglyceridaemia is present without accompanying hyperchylomicronaemia. Treatment and investigation includes stabilisation of the blood-aqueous barrier and blood sampling for lipoprotein analysis after at least 12 hours fasting.

A proportion of Burmese cats in Australia have delayed triglyceride clearance compared to other cats. Burmese cats are certainly the most common breed reported to present with lipid aqueous, with the average age at presentation of 6.5 months, but any breed of cat may be affected. However, in general, affected cats are most commonly less than 12 months of age.

In any cat presenting with lipid aqueous it may be difficult to distinguish whether:
1. Uveitis caused leakage of lipids from blood into aqueous
2. Uveitis was caused by increased lipid levels
3. The ocular signs reflect the leakier nature of iridal capillaries in immature cats as opposed to adults

Systemic hypertension is a common and important disease in humans. The clinical manifestations of systemic hypertension are seen in all organs although the eyes, kidneys, heart and brain are particularly susceptible. The importance of this condition in veterinary medicine is now also apparent with particular reference to older cats. In cats, the diagnosis of hypertension has generally been accepted as systolic blood pressure measurements exceeding 160-180mmHg. Presentation and diagnosis often occur late in the disease and usually following major organ damage, which may reflect a failure to identify animals with the disease although poor awareness among owners may also be a factor.

The histopathological changes associated with hypertension are well reported in experimental models of the disease. In the eye the apparent predilection for retinal vessels in humans led to the term hypertensive retinopathy to describe the clinical disease. However choroidal involvement is now well documented suggesting that this term is too narrow. The cat retina has a huge demand for energy although all ocular structures are at risk. Any disruption to the supply of nutrients can result in retinal damage. With time hypertension leads to vessel wall damage with breakdown of the blood ocular barrier ultimately resulting in exudation and haemorrhage with retinal detachment and ischaemia. The most commonly reported presentation in cats is acute ocular disease with animals often presenting with sudden onset vision loss due to retinal detachment. Retinal arterioles can be visualised easily and non-invasively and share similar anatomical and physiological properties with cerebral and coronary microcirculation. Therefore fundus exam can be used to ascertain the health of the ocular circulation. In cats, breakdown of the blood ocular barrier is not necessarily restricted to the posterior segment with hyphaema also a relatively common presentation. Anterior signs include hyphaema, secondary glaucoma, and iris aneurysms. However both the posterior and anterior segment signs can be indicators of other vascular disease and are not necessarily specific for systemic hypertension. In people there is no significant relation shown between retinal microvascular changes detected by qualitative examination of the fundus and prognostically validated markers of target organ damage as a result of hypertension. Efforts to classify ocular signs and correlate them with grades of severity of hypertensive disease have been shown to not be consistent and consequently should not be used in cats. Therefore the absence of fundus lesions should not necessarily rule out the presence of hypertensive disease in other target organs. Of most importance is to recognise the presence of disease and then to pursue investigation to try and identify the underlying cause.

Hypertension is generally classified as essential or secondary hypertension. The pathogenesis of essential hypertension is unknown in cats, although a primary age-related form of feline hypertension has been proposed with most studies of hypertension in cats reporting affected animals are greater than 10 years of age. However underlying disease is invariably difficult to rule out due to target organ damage. The most frequent form of secondary hypertension is due to renal disease. Renal insufficiency is a common finding but can be both a cause and effect of elevated blood pressure. Other conditions cited as predisposing to hypertension include hyperthyroidism and hyperadrenocorticism. Once hypertension is diagnosed in a feline patient, therapies should be directed to address the underlying disease as well as instigating anti-hypertensive medication. The two most commonly used medications for treating feline hypertension are amlodipine and angiotensin-converting enzyme inhibitors.

Early recognition of hypertensive disease remains an important step in the risk stratification of hypertensive patients. Therefore ophthalmic examination should form part of any physical examination, with particular attention given to clinical examination in older cats and ocular signs are one of the indicators of target organ damage in hypertensive disease. Careful examination of the eye and identification of early hypertensive changes should not only help to prevent visual loss as a result of this disease but also help minimise the risk to other target organs. Identifying and targeting subsets of hypertensive patients at highest risk could help reduce the incidence of permanent blindness and other debilitating diseases.
FELINE MYCOBACTERIAL KERATITIS
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The purpose of this presentation is to present two cases of feline mycobacterial keratitis seen at Animal Eye Care. Both cases required a keratectomy and long term medical therapy. Despite being clinically normal for several months following surgery, both cats appeared to suffer recurrences of the infection. Keratectomy lesions from both cases were submitted for histopathology and culture and sensitivity. Modified Ziel Nielsen staining demonstrated stout acid fast organisms contained within the phagocytes confirming a diagnosis of mycobacterial keratitis. Fresh tissue was submitted for culture but identification of the organism was unsuccessful. Identification of the organism was performed using PCR amplification and sequencing of bacterial DNA. The closest match (99.2%) was obtained with Mycobacterium sherrisii (Accession no. AY353699).1

Feline mycobacterial keratitis is a relatively rare condition in comparison to feline mycobacterial skin disease. Although it has not been documented to occur concurrently it shares some similarities with the skin condition. Like the skin condition, the cornea develops a granuloma, requiring extensive surgical excisions and long term antimicrobial treatment in order to ensure a cure.2 It has been speculated that the route of infection for most feline mycobacterial disease is fight wounds contaminated with saprophytic mycobacterium.3

A histological diagnosis is made by identifying mycobacterial organisms within lesions. Both of these cases had sheets of macrophages and deep penetration of the infection. Identification of the exact species is difficult as culture and sensitivity is often unsuccessful, however PCR is a more valuable tool.

Treatment for feline mycobacterial keratitis has two primary components. The first is extensive surgical resection and the second is protracted medical therapy.4,5,6 Medical therapy is further divided into topical and systemic components. In human patients triple –drug regimens are used with some of the medications being used hourly.7 Some of the topical antimicrobials used in order of increasing effectiveness in humans include amikacin, clarithromycin, levofloxacin, ofloxacin, gatifloxacin and moxifloxacin.8 Systemic antimicrobials are not routinely used in human mycobacterial keratitis, but when required, clarithromycin and doxycycline are the drugs of choice.7,8 Topical and systemic steroids are definitely contraindicated in the treatment of mycobacterial keratitis. They result in a protracted healing time.

Mycobacterial keratitis remains an uncommon but challenging condition in feline ophthalmology.

THE FELINE ORBIT
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ANATOMY
Significant Periorbital Structures - Diseases here may compress or invade the orbit.
- Sinuses - frontal, maxillary.
- Teeth - especially upper fourth premolar (carnassial).
- Ramus of the mandible – when the mouth is opened the ramus moves anteriorly and compresses the orbital soft tissues.

Intraorbital Structures - Globe, nerves (II-VI), blood vessels, gland of the 3rd eyelid, lacrimal gland, zygomatic salivary gland, extraocular muscles, masticatory muscles, fat, and periorbita.
Bone and sinuses incompletely surround the orbital soft tissues in domestic animals. Alterations in any of the bony, or soft tissues, also alters the remaining tissues and usually the position of the eye.

Extraocular muscles - Suspend the globe in the orbit and provide ocular motility.
- Four rectus muscles:
  o Dorsal, ventral and medial (innervated by CN III – The Oculomotor Nerve)
  o Lateral (innervated by CN VI – The Abducens Nerve)
- Two oblique muscles:
  o Dorsal (innervated by CN IV – The Trochlear Nerve)
  o Ventral (innervated by CN III – The Oculomotor Nerve)
- Retractor bulbi (innervated by CN VI – The Abducens Nerve)

DIAGNOSTIC TECHNIQUES
Physical Examination – cheap and effective
- Look, feel, retropulse the globe, open the mouth - pain on opening the mouth is typical of inflammatory orbital disease, whereas neoplasia tends to be non painful.
- Check nares for passage of airflow or haemorrhage (no air flow or haemorrhage may indicate sinus disease)

Radiography
- Take films before surgery on the orbit to avoid confusing artefacts.
- Often unhelpful unless bone or sinus involvement
  - Plain films
    o Useful if bony or sinus disease is suspected but can be difficult to interpret.
    o Usually need general anaesthesia.
    o Soft tissue does not show up well on plain films but the globe can be approximated by placing a thin metal ring (or bent paperclip) in the conjunctival cul-de-sac.
    o Plain films rarely give an exact diagnosis but can be very helpful in suggesting a prognosis. (Bony involvement suggests a poorer prognosis).
- Positive contrast radiographs = seldom performed.

Computerized Tomography/Magnetic Resonance Imaging
- Soft tissue resolution is enhanced and multiple cross-sectional views make them more useful than plain or contrast radiographs.
- CT/MRI is also very helpful in planning surgery of the orbit.
- Disadvantages are limited availability, need for general anaesthesia, and expense.

B scan Ultrasonography = helpful in soft tissue disease and in guiding a FNA of a small or localized lesion
- Do before surgery on the orbit.
- Does not image bone well.
- Does not require general anaesthesia.

Aspiration and cytology
- An aspirate or biopsy is usually required to get an accurate diagnosis in orbital disease. Disadvantages are possible damage to important orbital structures (optic nerve), infection, failure to indicate the full extent of the pathology, the need for anaesthesia or sedation (some patients), and getting an unrepresentative sample.
- Usually failure to yield a diagnosis is because the lesion is small or localized, unrepresentative tissue was sampled, or lack of tissue architecture to aid in diagnosis. Neoplasia can be missed if only the necrotic centre of a tumour is aspirated.

Surgical Exploration of the Orbit - Necessary if less invasive techniques fail to yield a diagnosis.
- It allows you to fully assess the extent of the disease, it can be therapeutic as well as diagnostic, and provides representative tissue for pathology.
Disadvantages include general anaesthesia, the need for advanced surgical training, infection, potential disfigurement or damage to vital structures, time involved and the expense.

**CLINICAL SIGNS OF ORBITAL DISEASE**

- **Exophthalmos (protrusion of the globe)** results if orbital volume or pressure is increased caudal to the equator of the globe. Clinically one must differentiate exophthalmos from "apparent exophthalmos" due to shallow orbits in brachycephalic breeds, enlarged palpebral fissures, facial paralysis, and buphthalmos (large globe) in glaucoma.
- **Enophthalmos (sinking of the globe)** results if orbital volume or pressure is reduced, or if orbital volume is increased anterior to the equator of the globe.
- **Strabismus (deviation of the globe)**
- A differential diagnosis list is created by considering what pathologic processes can affect the few tissues in the orbit and produce the observed deviation in the globe.

**CONGENITAL/INHERITED ORBITAL DISEASE**

**Shallow orbits** - Brachycephalics
- Prominent globes due to decreased orbital space.
- Bilateral exophthalmos.
- Wide palpebral fissures - lagophthalmos (inadequate lid closure).
- **Predisposes to:** Inadequate tear distribution • Exposure • Ulceration • Traumatic proptosis
- Can perform lateral or medial canthoplasty to shorten palpebral fissure if necessary
- Topical lubricants (e.g. visco tears)

**Microphthalmos** - Abnormally small eye. Unilateral or bilateral enophthalmos.
- Usually (although not always) associated with other congenital abnormalities, e.g. microcornea, persistent pupillary membranes, cataract, retinal dysplasia, retinal detachment etc.
- Vision may or may not be affected depending on associated ocular abnormalities
- If blind "wandering" nystagmus.
- **Cause**
  - Sporadic: Teratogenic influences on dam during early pregnancy. Griseofulvin in cats can cause anophthalmia.
  - Hereditary predisposition (proven or suspected).

**ACQUIRED ORBITAL DISEASES**

Always try for an accurate diagnosis prior to therapy.

**Exophthalmos - differential diagnosis:**

- **Proptosis** - Exophthalmos with contracture of the eyelids behind the globe.
  - Hit by cars and facial bite wounds are the two most common causes.
  - Greater force is required to proptose cat eyes than dogs, so the prognosis for feline eyes following this is generally poorer than in dogs.
  - **Treatment:** Emergency replacement of the globe +/- tarsorrhaphy (suture lids together). Note sutures emerge through meibomian gland openings (not in contact with globe). Systemic steroids to reduce inflammation of the optic nerve.
  - **Prognosis:**
    - For vision: generally hopeless in cats
    - For the eye: Depends on how many extraocular muscles are ruptured because blood supply to the anterior segment comes in with the extraocular muscles. < 3 muscle ruptured – reasonable prognosis for the eye, >3 muscles ruptured – poor prognosis for the eye (ischaemia of the anterior segment, phthisis bulbi).
    - If eye survives, generally has lateral strabismus as the medial rectus is the shortest extraocular muscle and tends to rupture first in cases of proptosis
  - Although a proptosis is easy to diagnose, radiographs/US may also help determine the extent of disease.

- **Orbital cellulitis/retrobulbar abscess**
  - Relatively common
  - Often diagnosed by physical examination (look for swelling behind last molar) and the presence of pain on opening the mouth. Some need a full diagnostic work-up including aspiration/cytology with a culture and sensitivity.
Enophthalmos

- Rapid onset
- Unilateral
- Pain opening mouth - look for redness/swelling behind last upper molar tooth
- Protrusion 3rd eyelid
- Conjunctival erythema/chemosis/discharge
- Febrile/anorexia.

Causes

- Foreign body entry via conjunctiva, eyelid or soft palate
- Infection - extension from: nasal, frontal, maxillary sinus, oral cavity = tooth root abscess, lacrimal or zygomatic salivary gland
- Rarely orbital neoplasia necrosis.

Rx

- Initial medical treatment with broad systemic antibiotics combined with systemic steroids with ocular lubricant to avoid exposure keratitis, OR can consider surgical drainage via mouth - but exercise caution.

C/S cytology of any drained purulent material (commonly no purulent discharge)

Drainage Method:

- Incise with a scalpel blade approximately 1 cm behind the last molar tooth. The incision should not be deep. Then insert a fine pair of straight haemostats dorsally for a short distance into the ventral posterior orbit. Gently open blades to establish drainage.
  - Potentially dangerous procedure. Do not probe too deep. Never use sharp instruments.
  - Follow up with oral broad spectrum antibiotics.
  - Rx Following drainage back to normal 2-3 days.

Sequeiae

- Exposure keratopathy
- Atrophy of periorbital tissue enophthalmos

Recurrence

- Exophthalmia less obvious
- Draining fistulas
- Check chronic infections other sites
- Retained FB
- Myotic infection.

Neoplasia

- primary, metastatic, or an extension from surrounding tissues (most commonly sinuses). A full work-up including a CT / MRI scan and surgical exploration may be needed. The prognosis is often poor as they are often malignant and quite extensive at initial presentation. Therefore an aspirate and a skull radiograph showing bony lysis may be enough.

Cats - of 21 cases of orbital neoplasia, only about 15% were primary. 85% were secondary (lymphosarcoma / oral, nasal, adnexal SCC)

Fibrosing orbital sarcoma – rare, initially unilateral, present with reduced ocular motility and acquired upper lid entropion, corneal ulceration may occur, exophthalmos or enophthalmos may also develop. Generally leads to enucleation / exenteration but generally the other eye also develops similar symptoms in 9-12 months, can occasionally see other lesions e.g. oral sarcoma.

Orbital emphysema - Following orbital surgery or after a fracture into a sinus. History and physical examination (gas crepitation) are usually diagnostic.

Cavernous sinus syndrome – the cavernous sinus is a structure located behind the orbital foramen. Several nerves including CN III, IV, V, VI and the sympathetic nerve travel through this foramen, so multiple nerve dysfunction occurs. Note that the optic nerve does not pass through the cavernous sinus so vision is not affected. Aetiology includes infections, vascular thrombosis, neoplasia, idiopathic

Zygomatic mucocele - extremely rare in cats, (may also see enophthalmos if anterior to the globe). Often diagnosed by aspirating a fluctuant cyst and finding a viscous, saliva-like fluid. Ultrasound helpful. Surgical removal (referral procedure)

Retrobulbar haemorrhage - following trauma or in patients with bleeding disorders. Usually history of bleeding elsewhere aid diagnosis. Aspiration and cytology is usually unrewarding since it can be difficult to differentiate iatrogenic haemorrhage from pathologic. Large amounts (>0.5ml) of non-clotting dark blood would suggest a haematoma but doesn't rule out haemangioma/haemangiosarcoma.

Enophthalmos - differential diagnosis:

- Ocular pain with globe retraction.
- Dehydration with loss of orbital fluid.
- Emaciation with loss of orbital fat.
- Periorbital fractures or trauma. Haemorrhage from the nostril suggests the sinus may have been invaded, making the fracture an open fracture. This has a higher infection rate and more complicated course.
- Horner's syndrome. Rarely seen as enophthalmia alone, usually concurrent third eyelid prominence, ptosis and miosis.
  - Due to loss of sympathetic tone to the eye
  - Clinical Signs
    - Papillary miosis on affected side (anisocoria – unequal pupils)
    - Protrusion of the 3rd eyelid secondary to enophthalmos and loss of smooth muscle tone
    - Ptosis (laxity) of the upper eyelid
    - Enophthalmos
    - Peripheral vasodilation – warmer pinna
    - Sweating on the face and neck of horses
  - The efferent sympathetic pathway controlling the iris dilator muscle is a three-neuron path with the Central neuron (First order Horner’s) beginning in the hypothalamus descending to the pre-ganglionic cell bodies located in the gray matter of the spinal cord at T1 T2 and T3 or T4. The pre-ganglionic neuron (Second order Horner’s) exit’s via the segmental ventral thoracic roots to join the thoracic
sympathetic trunk and extends rostrally via the cervical sympathetic trunk to synapse in the cranial cervical ganglion located medial to the tympanic bullae (Third order Horner’s). The now post-ganglionic fibres exit the middle ear, eventually join the trigeminal nerve and enter the globe via the long ciliary nerve.

- Common causes = Lesions or injury to the cervical and thoracic spinal cord, brachial plexus root lesions or avulsion, mediastinal tumours affecting the thoracic or cervical sympathetic trunk, soft tissue injury to the neck, otitis media, retrobulbar contusion, iatrogenic (thyroidectomy, bulla osteotomy). Many are idiopathic.

- Dx: Topical 10% Phenylephrine applied to both eyes and time taken for pupillary dilation is noted - First order and normal eye - dilation in 60-90 minutes - Second order - dilation of abnormal eye in 45 minutes - Third order – dilation of abnormal eye in 20 minutes

- Rx: Depends on the cause. Most idiopathic Horner’s will spontaneously resolve in 6-8 weeks.

- **Microphthalmia or ptithisis bulbi** = a small eye.
- **Space occupying lesions anterior to the globe.** Maxillary sinus abscess, an anteriorly located zygomatic salivary gland mucocele etc. A full diagnostic work-up as in patients with exophthalmos may be needed.
- **Post-operatively** due to loss of orbital contents or following contraction of surgical scar tissue.
Since the first observations of penicillin resistance in *Staphylococcus aureus* following its introduction in the 1940s, the spectre of impending antibiotic-resistant superbugs has haunted the corridors of hospitals throughout the world. In the past, rigorous development and introduction of new classes of antibiotic have always kept a step ahead of the evolution of resistance. However, great fears are held for the future as the marked increase in prevalence of multi-drug resistance in Gram-positive (G+ve) (*S. aureus*, *Enterococcus spp.* and *Streptococcus pneumoniae*) and Gram-negative (G-ve) pathogens (*Escherichia coli*, *Enterobacter spp.*, *Salmonella spp.* and *Acinetobacter baumannii*) has coincided with an unprecedented global decline in investment in new anti-infective drugs, leading to suggestions by infectious disease specialists that we are rapidly heading towards a post-antibiotic age (IDSA, 2010).

There are few currently registered alternatives for MDR bacterial infections, forcing human clinicians to consider older generation drugs such as colistin with considerable toxic side-effects. In addition, there are fewer classes of novel therapeutics moving through the drug development pipeline. Following recent comprehensive interviews of 13 leading pharmaceutical companies with registered anti-infective products, only four new antimicrobial compounds (ceftaroline, cefotibiprole, dalbavancin and the aminomethylcycline PTK-0796) were in the advanced stages of clinical development (note: ceftaroline was approved by the USA FDA on Oct 29, 2010), with the majority having only narrow-spectrum activity against G+ve organisms (Boucher et al., 2009). Furthermore, of eight new antimicrobial compounds in stage 2 clinical trials or later, only one represented a novel class of drugs with a unique mechanism of action. There were over 300 anti-infective medicines in Stage 1 of development, however, a large majority are previously approved compounds or their derivatives that are being studied for new indications.

If the future human anti-infective formulary cupboard is sparse, the veterinary formulary cupboard is bare. As the prevalence of multidrug-resistance in animal-specific pathogens increases (Weese, et al., 2008), greater regulation on the usage of antimicrobials in animals and registration of new products mean that veterinarians are increasingly reliant on the traditional classes of antimicrobial agents. The risk of transfer of MDR zoonotic organisms from animals to humans has also led to calls for further restrictions on the usage of some recently registered drugs such as the fluoroquinolones, third generation cephalosporins and clavulanic acid-potentiated beta-lactams (Faires et al., 2010).

**Epidemiology of resistance development in humans and animals**

Much of the evolution in resistance development is being driven by changes in the epidemiology of key MDR organisms. Once only restricted to human hospitals and aged care facilities, methicillin resistant *S. aureus* (MRSA) are now being isolated from the community in alarming proportions (Nimmo et al., 2006). Furthermore, community-acquired MRSA strains are more likely to carry the Panton–Valentine leukocidin (PVL) toxin, a virulence factor linked to skin and soft tissue lesions as well as a rapid, fulminating, necrotizing pneumonia with significant associated mortality. A change in MRSA ecology occurred recently with strains becoming host-adapted in several key animal species including livestock, horses and companion animals and regular cases of human-to-animal and animal-to-human transfer are documented (Loeffler and Lloyd, 2010). This has important consequences for strain transmission and public health. These ecological shifts together with rapid development of resistance to new drugs developed specifically for MRSA such as linezolid confirm that new MRSA anti-infectives are urgently needed (IDSA, 2010). Furthermore, hospitals that use vancomycin for treating MRSA then have to contend with outbreaks of vancomycin-resistant enterococci (VRE) infections in their patients, once again with limited antimicrobial choices (Lai, 2010).

The global emergence and spread within the community of highly virulent MDR Gram-negative (G-ve) bacteria such as *E. coli* O25b:ST131 confirms that bacterial pathogens can simultaneously evolve both virulence and resistance determinants (Plattel et al, 2010). Echoing recent MRSA epidemiology, *E. coli* O25b:ST131, a major cause of urinary tract and bloodstream infections in humans, has now been isolated from extraintestinal infections in companion animals (Plattel et al., 2010), though prevalence data would suggest that in the case of companion animals, the direction of transmission is predominantly human-to-animal. The increasing significance of *E. coli* O25b:ST131 and other MDR Enterobacteriaceae with combined resistance to fluoroquinolones and extended spectrum beta-lactams and carbapenems is another worrying trend (Sidjabat et al., 2006; Gibson et al., 2010), especially considering there have been few recent breakthroughs in the development of G-ve spectrum anti-infectives apart from incremental advances in the carbapenem family (IDSA, 2010).

These changes in the epidemiology of key MDR pathogens highlight that AMR is no longer an issue just for hospitals and the medical system. It is now an ecological issue, with selection pressures operating from the widespread usage of antimicrobial agents in many diverse environments. Investment in new classes of anti-infective needs to be urgently upscaled. The Infectious Diseases Society of America has recently developed an ambitious campaign calling for 10 new antimicrobial agents to be registered by 2020. In addition, new drug classes that do not make it to the human arena
should be considered for domestic animal therapies to avoid some of the cross-transmission resistance issues highlighted above (Chauvin et al., 2002). Within the veterinary sphere, we must get smarter with our antibiotic choices, infection control procedures and resistance surveillance and opportunities for zoonotic transmission of strains between humans and companion animals. Australia has been lucky in that resistance emergence has not occurred in this country to the same extent as overseas, but continued vigilance regarding the emergence of resistant strains of *Staphylococcus* and *E. coli* is required to take advantage of this situation.

**CHARACTERIZATION OF MRSA STRAINS FROM AUSTRALIAN VETERINARIANS**

During four major veterinary conferences held in Australia in 2009, (the Australian Veterinary Association Annual Conference Darwin, May; the Australian Association of Pig Veterinarians Conference Melbourne, June; the Australian College of Veterinary Scientists annual Science Week meeting Gold Coast, July; and the 31st Bain Fallon Memorial Lectures on equine medicine and surgery Maroochydore, July), 771 veterinarians provided nasal swab samples for MRSA culture as part of an AVA-sponsored survey. An epidemiological analysis of the survey was recently published in the Australian Veterinary Journal (Jordan et al. 2011). Amongst the 771 respondents “industry and government veterinarians” (controls) had the lowest prevalence of MRSA carriage at 0.9%. Veterinarians with horses as a major area of work emphasis had a prevalence of 11.8% (13 times that of controls) and those who indicated that their major emphasis was horses only had a prevalence of 21.4% (23 times that of controls). Veterinarians with dogs and cats as a major activity had a 4.9% prevalence (five times that of controls). Prevalence rates for other major activities (pigs, dairy and beef cattle, avian and wildlife) were also elevated but were estimated from smaller numbers of respondents.

Complete characterization of the MRSA strain collection from Australian veterinarians is still ongoing. However preliminary data based on a rapid clonal complex typing technique are shown in Table 1. In addition, none of the isolates were confirmed to carry the PVL toxin associated with severe MRSA infection.

The results show that CC22 is predominant in companion animal veterinarians, whereas CC8 is predominant in equine only practitioners. A small number of strains belong to CC88, a clonal group not usually associated with animals. A small number of strains belong to major CCs identified in humans (CC1, CC5) and two strains could not be typed by this method.

Companion animal practitioners colonised with an internationally well recognised clonal group (CC22) have a nasal carriage rate that is not dissimilar to that of human healthcare workers. Nevertheless, with the highest proportion of veterinary graduates working exclusively in companion animal practices and the number of MRSA infections in animals increasing, further epidemiological investigations are now warranted. Similarly, the unusually high prevalence of CC8 in equine only practitioners should be a call to action to identify risk factors for nasal carriage (such as the physical handling of antibiotics), improve infection control practices in equine hospitals and on studs and provide more industry guidelines on MRSA prevention strategies.

**Table 1: Clonal complex typing results for MRSA isolates (n=45) obtained from Australian veterinarians (by major work area).** *CC1; **CC5; ***CC could not be determined

<table>
<thead>
<tr>
<th>Major work area</th>
<th>CC8 (n=24)</th>
<th>CC22 (n=13)</th>
<th>CC88 (n=4)</th>
<th>Other (n=4)</th>
<th>Total (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Animals (SAs)</td>
<td>3 (12.5%)</td>
<td>10 (76.9%)</td>
<td>1 (12.5%)</td>
<td>13 (28.9%)</td>
<td></td>
</tr>
<tr>
<td>Horses</td>
<td>15 (62.5%)</td>
<td>1 (7.7%)</td>
<td>2 (50%)</td>
<td>19 (42.2%)</td>
<td></td>
</tr>
<tr>
<td>Mixed Animals (SAs and Horses)</td>
<td>3 (12.5%)</td>
<td>1 (7.7%)</td>
<td>1 (12.5%)***</td>
<td>5 (11.1%)</td>
<td></td>
</tr>
<tr>
<td>Mixed Animals SAs (no Horses)</td>
<td>2 (8.3%)</td>
<td>1 (25%)</td>
<td>3 (6.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td>1 (12.5%)***</td>
<td>1 (2.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herd Health</td>
<td>1 (25%)</td>
<td>1 (2.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research</td>
<td>1 (7.7%)</td>
<td>1 (2.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry</td>
<td>1 (4.2%)</td>
<td>1 (2.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vet Student</td>
<td>1 (12.5%)**</td>
<td>1 (2.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**References:**
INTRODUCTION
Sepsis is a condition that is characterized by a whole-body inflammatory state (called a systemic inflammatory response syndrome or SIRS) and is caused by an infection. SIRS may develop secondary to the immune response to microbes in the blood, urine, lungs, skin, or other tissues. “Septicaemia”, is an older term for the presence of sepsis. Multiple organ dysfunction may occur as a result of the host immune/inflammatory response. The early recognition of sepsis is key to a successful treatment regime and understanding of the patho-physiological mechanisms at play will aid in the selection of appropriate therapy. (Read reference #10).

CLINICAL SIGNS
Sepsis is characterized by the presence of acute inflammation present throughout the entire body. Fever is common, leukocytosis or leukopenia may occur. Vomiting is often a common prodromal sign. Clinical signs of sepsis may reflect the organ of origin of the infection. However, the host's immune response to the infection causes many of the clinical signs of sepsis, resulting in hemodynamic consequences and damage to organs. SIRS is characterized by tachycardia, increased respiration rate, abnormal white cells such as band neutrophils and toxic change. Sepsis is differentiated from SIRS of other origin by the presence of a microbial pathogen.

SIRS is characterized by the widespread activation of acute phase proteins that interact with the complement system and coagulation systems, which cause vascular damage, and hence organ damage. Various neuroendocrine counter-regulatory systems are then activated as well, often compounding the problem. This process may progress to multiple organ dysfunction syndrome (MODS) and eventually death. MODS has been defined as altered organ function in an acutely ill patient such that intervention is required to maintain homeostasis.

Common Clinical Scenarios for Sepsis in Animals
1. Gastrointestinal perforation (large intestine worse than small intestine)
2. Gastric dilation with volvulus
3. Severe pancreatitis
4. Rupture of abdominal abscess (pyometra, prostatic abscess, pancreatic/hepatic/renal abscess)
5. Pyothorax
6. Pneumonia
7. Neutropenia due to bone marrow suppression/failure/neoplasia
8. Extensive thermal burns
9. Endocarditis
10. Pyelonephritis

SIRS Criteria
- Fever/hypothermia (temperature >39.2 < 37.8 C)
- Tachypnoea, with compensatory respiratory alkalosis (PaCO₂ < 32)
- Tachycardia
- Neutrophilia or neutropenia
- Oliguria/anuria
- Increased lactate (with increased base deficit)
- Clinical signs of organ dysfunction (e.g. jaundice, oliguria/anuria, pulmonary oedema, coma)
- Hypoxemia (decreased SPO₂ or decreased PaO₂)
- Vomiting and intolerance to feeding (suggesting ileus)
- Coagulation changes includes thrombocytopenia, increased APTT/PT or ACT and fulminant DIC
- Hyperglycaemia
- Declining mental status.

SEPSIS
Isolation of bacteria by culture or identification in body fluids and accompanying some of the signs of SIRS listed above. Any combination of the signs listed above should be considered alarm signs suggesting the possibility of sepsis being present and inducing SIRS and should induce a thorough re-assessment of the patient and the search for an inciting bacterial infection. For example, the recent onset of bowel surgery accompanied by fever, white cell changes, increased lactate and increased bilirubin would be a cue to consider leakage of bowel contents and that sepsis and initiation of the SIRS had occurred.
Monitoring of patients in ICUs considered at risk can be followed using the below parameters:

- $\text{PaO}_2$ (SPO$_2$ - easier but less accurate)
- Lactate and blood gases
- Bilirubin
- Platelet count/ACT
- Glucose
- Creatinine and urine output
- Glasgow coma score

In human ICUs a PIRO scheme uses risk factors

PIRO stands for predisposing factors (P) Infection (I) Response (R) Organ dysfunction (O)

<table>
<thead>
<tr>
<th>PIRO score</th>
<th>Laboratory</th>
<th>Clinical Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predisposing factors (1-4)</td>
<td>None identified yet in dogs/cats</td>
<td>Age, pre-existing co morbidity Diabetes, cirrhosis, neoplasia Immune suppressive therapy.</td>
</tr>
<tr>
<td>Infection (1-4)</td>
<td>Microbe identification and sensitivity</td>
<td>Site of infection</td>
</tr>
<tr>
<td>Response (0-1)</td>
<td>WBC, lactate,</td>
<td>Temperature, HR, RR, Blood pressure</td>
</tr>
<tr>
<td>Organ dysfunction (1-4)</td>
<td>$\text{PaO}_2$, Bilirubin, Creatinine, platelet count, ACT</td>
<td>Urine output, Glasgow coma scale</td>
</tr>
<tr>
<td>Total PIRO Score.</td>
<td>0-13</td>
<td></td>
</tr>
</tbody>
</table>

**TREATMENT OF SEPSIS**

Includes administration of intravenous (iv) fluids therapy, appropriate broad-spectrum antibiotics, restoration of tissue oxygen delivery, removal of the source of infection and lung- kidney protective strategies.

**Fluid therapy and Sepsis:** Volume resuscitation of a patient with hypovolaemic shock or sepsis is an essential component of patient care. Massive amounts of intravenous fluid are often administered to replace intravascular volume deficit and to minimize complications attributed to hypovolaemia such as tachycardia, hypotension, acute kidney injury, and multiple organ failure.

“Goal-directed therapies” focused on restoration of normal blood pressure and organ perfusions have been advocated in the management of critically ill patients. This is clearly different to administration of fluids at “shock” rates. In human ICUs, early goal-directed therapy, which is instituted in the initial phase of management of patients with severe sepsis or septic shock, has been shown to improve overall survival. In human ICUs the assessment of hemodynamic response to volume resuscitation and vasopressors (Dopamine/Dobutamine CRI) is often based on specific hemodynamic and oxygenation parameters such as mean arterial pressure, central venous pressure, and central venous oxygen saturation, not solely on symptoms and physical findings and pre determined fluid rates.

In contrast to the notion of aggressive and liberal volume resuscitation, a growing body of evidence in the human literature strongly suggests that fluid overload may be detrimental to critically ill patients. In the veterinary literature, relatively little attention has been paid to the consequences of fluid overload in the precipitation of respiratory failure, increased cardiac demand, and peripheral oedema. Recent studies on human patients with acute lung or kidney injury have reported that fluid overload has been associated with adverse outcomes. Although uniform definitions of fluid overload and well-designed randomized clinical trials are lacking, there seems to be a need to avoid overzealous crystalloid fluid resuscitation in a subset of human patients.

What we can take from this is that input and output in patients should be closely monitored, and loss into “third spaces” should be taken into account. Vital signs, findings from physical examination, and thoracic radiographs are of great importance in assessing the volume status of the patient. This is combined with assessment of blood pressure/CVP and urine output. Although it is often difficult to take the decision to monitor central venous pressure, the placement of a central venous catheter (monitoring CVP) and the monitoring of urine output are strongly encouraged in septic patients.

**Broad Spectrum Antibiotics:** Best given via the IV route initially to achieve high continuous blood levels.

Options:

1. Gram positive (amoxicillin 20 mg/kg TID) or 1st gen cephalosporin (cefazolin) at 15-15 mg/kg TID, Cefoxitin at 30 mg then 15 mg/kg IV q 4 hours and Gram negative antibiotic (Aminoglycosides) such as gentamicin 6.0 mg/kg IV SID (cats at 4.0 mg/kg) or amikacin 20mg/kg IV SID (Cats 10-12 IV BID). **Use aminoglycosides for <7 days.** Anaerobic bacteria use (metronidazole at 10 mg/kg TID).
2. As above but use enrofloxacin at 10 mg/kg IV BID in dogs and (use 5 mg/kg IV SID cats) instead of Aminoglycoside.
3. Alternate use is ticarcillin/clavulanic (Timentin) acid IV at 50 mg/kg QID IV (slow over 30 mins) plus metronidazole at 10 mg/kg IV TID and possibly aminoglycoside.
4. Imipenem-cilastin 5 mg/kg IV TID.

**Use of Vasopressors:** Vasopressors are required if the mean arterial blood pressure falls below a mean of 70mm Hg. Dobutamine 5-20 µg/kg/min and Dopamine 1-10 5-20 µg/kg/min CRI are commonly used.
Additional treatments:
1. **Supplemental oxygen administration** is determined on the basis of results of arterial blood gas analysis (i.e., alveolar-arterial gradient in partial pressure of oxygen > 10 mm Hg) or results of pulse oximetry (Spo2 < 95%) or if results of clinical assessment indicated a need for oxygen supplementation.
2. **Coagulation dysfunction** if PT or PTT/ACT are > 25% above the upper reference limit, if platelet count was ≤ 100,000/μL, or both. Treatment with fresh frozen plasma or whole blood as required to correct APTT/PT. FFP at 20 ml/kg dog and 10 ml/kg cat IV.
3. **Stomach protection.** Pantoprazole, Ranitidine 2 mg/kg IV BID, carafate PO.

**KEY POINTS**
- ICU monitoring for SIRS criteria is important to successful outcomes.
- SIRS in itself is not an endpoint nor a diagnosis but a process that ultimately leads to MODS/death.
- Animals with changes suggesting SIRS should be thoroughly assessed for sepsis.
- Animals with sepsis and SIRS will benefit from:
  1. Fluid therapy and monitoring of inputs and outputs using blood pressure/CVP/urine output measurements combined with lactate and blood gas measurements. Overzealous fluid therapy is likely to lead to adverse outcomes particularly with regard to lung function.
  2. **Broad-spectrum intravenous antibiotic therapy** is required to cover gram positive/gram negative and anaerobic bacteria.
  3. Identification of the cause of sepsis and rectification of its origin is most likely to be associated with a successful outcome.
  4. Prolonged time until the discovery of sepsis is likely to lead to irreversible activation of the inflammatory/immune and coagulation system whereby the body’s response to infection is essentially fatal. **Successful outcome in veterinary patient is largely affected by early identification of SIRS and sepsis** and sticking to the criteria during exam of the animal.

**References:**
NOVEL APPROACHES TO TREATING FELINE INFECTIOUS PERITONITIS (FIP) AND OTHER TRICKY VIRAL DISEASES
JACQUELINE NORRIS BVSc MVS PhD MASM GRAD CERT EDUC (Higher)
University of Sydney, NSW

This seminar will outline in detail the progress and current status of FIP treatment and make comparisons to the progress/status of FHV treatment outlined by the following speaker, Professor Richard Malik.

EVIDENCE BASED VS TRIAL-AND-ERROR APPROACHES TO FIP
FIP appeared in the 1950s, years before the first official report in 1963. Despite the advances in veterinary medicine, a successful treatment for FIP and a quick easy universally applicable definite diagnostic test for the same disease remain the holy grails of feline medicine. Several immunomodulating drugs and some anti-viral drugs have been used to treat cats with FIP but none to date in the peer reviewed literature can be considered ‘successful’ using an evidence based approach. There is likely to be some success reported shortly with Polyprenyl Immunostimulant, currently under research investigation by Professor Albert Legendre.

At least three limiting factors have underpinned the currently poor clinical outcomes seen in the treatment of FIP worldwide. Firstly, despite many studies we do NOT have a clear and thorough understanding of the exact pathogenesis of disease in naturally occurring cases. Secondly, case reports or case series outlining successes or failures in FIP treatment have until recently, lacked control groups. Thirdly, many of the case studies until recently include patients that have not been definitely diagnosed by the current benchmarks of histological, immunohistological or immunocytological techniques and in fact some have quite scant clinical evidence of FIP.

Hartmann and Ritz (2008) compiled a comprehensive historical review of all reported treatments used for FIP and detailed the pros and cons of each. This paper is well worth a read as it summarises the exact case details of each study and whether a control group or more importantly a definite diagnosis of FIP was made. Treatment trials on naturally occurring or experimentally induced cats with FIP have included the immune modulating agents [glucocorticoids, cyclophosphamide, human interferon, feline omega interferon, ozafrel HCl, tylosin, promodulin] or antiviral agents (ribavirin, melphalan). It is completely understandable that a variety of drugs have been used in the past in a desperate attempt to treat what we all fear is an untreatable disease. However studies in the future need a more systemic approach and as a minimum must include cases that have been definitely diagnosed through histological, immunohistological or immunocytological techniques.

The transition to an evidence based approach to the diagnosis of FIP started with Ritz and colleagues (2007) randomized placebo-controlled double-blind treatment trial in which 37 cats with FIP (36/37 with effusive FIP, one with dry FIP) were treated with feline omega interferon and glucocorticoids or glucocorticoids and placebo. In all cats, FIP was confirmed by histology and/or immunohistochemical or immunofluorescence staining of FCoV antigen in effusion or tissue macrophages. All cats received glucocorticoids, either as dexamethasone in case of effusion (1 mg/kg intrathoracic or intraperitoneal injection every 24 h) or prednisolone (2 mg/kg orally every 24 h). In addition, cats received either placebo or interferon-omega at 10^6 U/kg subcutaneously every 24 h for 2 to 3 weeks and subsequently once every week coupled with oral prednisolone 1mg/kg sid 2 weeks reducing to eod for 1 to several months and then ceasing. In the three cases in which treatment was seen to improve clinical signs, the effusion (pleural and peritoneal) subsided and appetite improved. One cat survived 9 months before FIP re-emerged and the cat was euthanased. In this case within 3 months, treatment was reduced to feline interferon alone and when this drug went through a temporary production shortage and the cat had to be taken off the drug there was a noticeable return of pleural
effusion which was again eliminated when feline interferon treatment was recommenced. The remaining two cats are still alive at >18 months post diagnosis and now remain on feline interferon every 4-8 weeks.

Legende and Bartges (2009) published a small but promising study of only three cases of dry FIP treated with Polyprenyl Immunostimulant, an investigational veterinary biologic manufactured by Sass & Sass, Inc comprising a mixture of phosphorylated, linear polyisoprenols. Two of the three cats were vastly improved on the drug and at the time of that publication, were still alive and on treatment 2 years after diagnosis. The third cat survived 14 months but was treated for only 4.5 months. The agent has low toxicity and is orally absorbed. The substance is thought to upregulate biosynthesis of mRNA of Th-1 cytokines. These researchers have embarked on a more extensive study funded partly by the Winn Feline Foundation and are due to release these results at the annual conference in June 23rd, 2011. While it is not suitable for all cases of FIP, some dry FIP cases have been successful treated but the details remain elusive at the time these notes were written.

Finally, there has been considerable discussion on internet feline medicine groups such as VIN on pentoxifylline (Trental), a methylxanthine derivative thought to reduce vasculitis in cats and other species and thought to have a role in improving the comfort and survival time of cats with FIP. In a recent placebo controlled study completed by Hartmann’s group soon to be published, there was no positive effect seen with the closely related drug propentofylline in cats with FIP in terms of survival time, quality of life, or any clinical or laboratory parameters.

IN VITRO TESTING OF ANTIVIRAL DRUGS SUITABLE FOR TREATMENT OF FIP

Feline infectious peritonitis is started by a feline coronavirus however the resulting pathology is considered immune mediated and as such successful treatment is likely to involve the use of immunomodulating agents and antivirals, unless successful immune modulating agent(s) can be found that limit FIPV replication as well as the immune mediated damage.

Our research group has recently established a three tiered method for assess the effect of any treatment on replication of FIPV in vitro using qRT-PCR of intracellular virus (detecting viral genome and M and N mRNA), TCID50 infectivity assay of extracellular virus, and direct IFA staining for viral antigen (McDonagh et al, 2011). All treatments are performed in triplicate within each experiment and the experiments are repeated three times. The results are expressed as geometric mean with 95% confidence interval from three independent experiments. PCR viral genomic and mRNA copy number are normalised to the housekeeping gene GAPDH copy number.

We have used this three tiered approach to assess the effectiveness of short interfering RNAs (siRNAs), on FIPV replication. RNA interference (RNAi) is a highly conserved post transcriptional gene silencing mechanism found in most euukaryotes that has recently attracted significant attention due to its potential therapeutic use. RNAi can be induced in mammalian cells by introducing short interfering RNAs (siRNAs),≈ 21 nucleotide RNA duplexes. Introduced siRNAs are incorporated into an RNA induced silencing complex (RISC) enabling it to bind to and target complementary sequences. Upon binding, an endonuclease within the RISC cleaves the targeted mRNA resulting in sequence specific gene silencing. The in vitro efficacy of RNAi as an antiviral has been demonstrated in a range of important viral pathogens from diverse viral families, including HIV-1, influenza, SARS coronavirus, and FMDV. For some viruses these finding have been extended to in vivo animal models, where induction of a virus specific RNAi response results in reduced viral load and associated pathology.

We evaluated the antiviral potential of a panel of eight siRNAs targeting four conserved regions of the FCoV genome using an in vitro assay. The most effective siRNAs were further characterised by examining the effect of multiplicity of infection and siRNA concentration on their inhibitory effect. The information gained from this pilot study was an essential first step to the development of an effective RNAi based treatment for FIP and provided an effective platform for assessing a wide range of potential anti-FCoV therapeutics in vitro. We demonstrated the effectiveness of specific siRNA mediated RNAi in substantially inhibiting FCoV replication in vitro, without cytopathic effects in a feline cell line. This has provided important preliminary data on the potential effectiveness and safety of these antiviral agents, leading the way for extension into in vivo animal models and as a potential therapy for FIP.

Our research group is using the established three tiered method described above to assess the effectiveness of a range of novel treatments on replication of FIPV in vitro. Our focus has been to use drugs that are widely available, have known pharmacokinetic in cats and have been considered safe. There are 10 drugs currently under consideration and testing.

TREATMENT OF FELINE HERPESVIRUSES

In the following seminar, Professor Richard Malik will give a detailed outlined of how our understanding of FHV treatment has evolved slowly over the last several decades but recently catapulted due to the use of famciclovir. Detailed reports of successful clinical cases (Malik et al 2010), extensive in vitro studies and now more extensive
clinical trials, coupled with the recent removal of patent from this drug, have completely transformed treatment of adult cats and kittens with FHV.

USING FAMCICLOVIR TO TREAT FELINE HERPESVIRUS TYPE 1 INFECTIONS IN CATS: AN EVOLVING STORY

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THE START
In 2002, Dr Helen Powers was invited to speak at the Australian College of Veterinary Scientist’s “Science Week”. Helen had a fascinating career path. She initially pursued an interest in equine medicine, completing an internship and residency in large animal medicine at Cornell University. After time in both equine and small animal practice, she had an epiphany and retrained in veterinary dermatology, with Professors Peter Ihrke and Stephen White at UC Davis, and adjunct faculty like Thelma Lee Gross. Helen currently runs a busy dermatology referral service in San Francisco.

Helen presented a number of talks to the Dermatology Chapter of the College, but the one that sticks in my mind was her description of the dermatitis associated with feline herpesvirus-type 1 (FHV-1). There were two reasons why her talk got our attention. First, the audience by and large, was not aware of the entity. Lesions typically consisted of inflamed and often ulcerated regions in the vicinity of the nares. Usually there is a recent history of an upper respiratory tract (URT) infection in the weeks prior to the development of lesions; nasal signs typically resolve, however cutaneous lesions persist. Although this condition had first been described in the 1970s (the first paper having been penned by Associate Professor Margret Sabine), the clinical features were not initially well characterised, and the diagnosis relied upon on detecting viral inclusion bodies, a method fraught with difficulty. A 1999 paper redefined the syndrome, established the prominent role of eosinophilic inflammation and used the polymerase chain reaction (PCR) to establish the diagnosis in cases that fitted the clinical picture but in which inclusion bodies were absent. This work has subsequently been repeated by several other groups (including Jacqui Norris and Kate Bosward from the University of Sydney, where the test is offered commercially). Nowadays, detecting FHV-1 antigen in affected tissues using immunohistology is proving to be the easiest way to confirm a presumptive clinical diagnosis. A fascinating feature of FHV-1-associated dermatitis is the prominence of eosinophils in the inflammatory infiltrate, which prior to the development of PCR and immunohistology, often led to an erroneous diagnosis of eosinophilic granuloma complex and inappropriate therapy with corticosteroids, progestins or other immuno-modulatory agents, often with disastrous results.

Secondly, Helen introduced the audience to a new drug – famciclovir – which she said was proving very useful in the management of FHV infections in cats, including herpes-associated dermatitis. Graciously, Helen credited the initial use of this drug to veterinary ophthalmologists in California, and her colleague Carlo Vitale, originally from Erie (Pennsylvania), who also trained with the dermatology group at UC Davis. We were excited that there was a drug that could be used to treat FHV-1, because up until then acyclovir, the drug that had revolutionized treatment of systemic and local α-herpesvirus infections in people, could not be used in cats because of the risk of an adverse drug reaction, in particular life-threatening hepatotoxicity. Subsequent key word searches through CAB and Medline using the terms famciclovir and cat didn’t reveal any papers in the peer-reviewed literature. Interestingly, famciclovir was often mentioned on internet list serves especially amongst veterinary ophthalmologists. So we made a mental note to trial this drug in presumptive FHV-1 cases where conventional therapy (L-lysine, idoxuridine eye drops etc) had failed or was not practical.

TOPICAL ACICLOVIR THERAPY
The first potential case was a cat with FHV-1 associated dermatitis seen by Miriam Meek and Paul Gotis Graham who at that time (2004) owned Rose Bay Veterinary Hospital. The patient presented for non-healing ulcers on the nasal bridge, associated with eosinophilic inflammation but without viral inclusion bodies. We were all keen to trial this cat on Famvir®, but while we were waiting for the drug to be delivered to the local pharmacist, Mim started the cat on a topical human cold sore cream (acyclovir 5% w/v; Zolaten®), and the cat ulcers got better before we could try the much more expensive Famvir! Good for the cat!

PRELIMINARY EXPERIENCES WITH FAMCICLOVIR
Several months later, we got another chance to trial famciclovir. A Devon Rex cat with urticaria pigmentosa being treated with cyclosporine developed recurrent bouts of FHV-1 associated conjunctivitis and ulcerative keratitis. This led to the development of a small corneal sequestrum. We e-mailed Helen and got “her” dose for Famvir® and treated the cat. Its keratitis and conjunctivitis improved markedly, the amount of brown material around the eye’s decreased, and after a few weeks, the corneal sequestrum fell off. First wicket for Famvir®!

Over the next three years, we had the opportunity to trial famciclovir in a variety of cats with different types of FHV-1 infection. Cats with various skin and ophthalmic manifestations were treated, including cases with recurrent conjunctivitis (non-responsive to doxycycline systemically and chloramphenicol topically), ulcerative keratitis (with
dendritic or geographical ulcers) and corneal sequestra. Several of these cats were managed by Drs Naomi Lessels and Peter Howe at Leslie Street Veterinary Clinic in Umina. Dr Sarah Webb trialled famciclovir in a cat with severe destructive rhinosinusitis. Glyn Boobyer used the drug extensively in “rescue kittens” with naso-ocular disease (“cat flu”). Ildiko Plaganyi used famciclovir in kittens with viral URT disease at the Lort Smith Hospital in Melbourne. Aine Seavers (Oak Flats Veterinary Hospital) experimented with using higher doses given for shorter periods in cats with recurrent Herpesvirus keratoconjunctivitis. Sally Ann Williams (Auchenflower Veterinary Hospital) and Anne Fawcett and Angela Phillips (Sydney Animal Hospitals, Inner West) treated additional cases with ulcerative keratitis and/or corneal sequestra, and Mandy Burrows (Murdoch University) alerted several colleagues in WA of the value of Famvir for FHV-1 related dermatitis. All these studies were greatly facilitated by the donation of a substantial amount of free product by the human division of Novartis, the company that first developed this drug. Special thanks to Barry Frost who facilitated this.

Our preliminary experiences with famciclovir could be best described as empiric and anecdotal. When we started treating patients, pharmacokinetic data was lacking, and we extrapolated dose rates form human medicine, and the experiences of Helen Powers and Carlo Vitale. Helen was conservative, and started with 62.5 mg per cat once day, increasing to 62.5 mg twice daily (for an average size adult cat); this was the dose which we used most commonly for a couple of years. Carlo routinely used twice this dose in the cases he treated in his busy private clinic in California. In order to encourage other practitioners to start using Famvir®, we wrote up the first series of cases and submitted the manuscript to the Journal of Feline Medicine and Surgery. We were fortunate that both the reviewers and the editor (Andy Sparkes) were generous and flexible enough to see the usefulness of the work, despite its limitations.

DAVID MAGG’S SCHOLARLY CONTRIBUTIONS

Around the time we were collating our case material for publication, the first paper by ex-patriot Australian David Maggs group from UC Davis on the pharmacokinetics of famciclovir and its primary (active) metabolite penciclovir appeared in the American Journal of Veterinary Research. It turned out that the pharmacokinetics of famciclovir in the cat were complex and non-linear. Doses of 90 mg/kg every 8 hours produced optimal blood levels that inhibited (in vitro) the type strains of FHV-1 used by Maggs and colleagues. Such high doses were uniformly well tolerated by healthy research cats and kittens, and kittens with experimentally induced FHV-1 infections. A series of papers concerning this work are currently making their appearance in the literature, and most seem to appear in the American Journal of Veterinary Research. The only side effect of high doses of famciclovir reported to date is transient (reversible) loss of renal concentrating ability in a very small number of cats.

Despite the rigorous work from Davis, it is clear that much lower doses of famciclovir do indeed work in vivo, and only time will tell the optimal dose rate and dose interval for this drug in clinical practice. Our evolving experience is that utilisation of higher doses on a mg/kg basis produces a faster response, and some feline patients only respond to high doses. The drug is very well tolerated by cats. It is also remarkably well tolerated in human patients, including children and patients with HIV infection. Currently, the dose rate we recommend is largely dictated by the issue of cost, as the drug is expensive, and Maggs’s recommended dose is just too expensive for most Australian owners, especially if a long course of therapy is required. The author therefore currently recommend a dose of 125 mg twice daily for adult cats (weighing 3.5 to 5 kg). For kittens, we recommend 25-50 mg/kg every 8 to 12 hours. Such doses are more affordable in kittens, as they are smaller, and usually require shorter course of therapy for treatment of primary viral rhinosinusitis. A generic formulation of famciclovir (EZOVIR®) has recently become available in Australia. It is made by Alphapharm, a very reputable Australian company that makes a wide range of generic medicines. Famciclovir in this form is approximately 25% less expensive than the original formulation, and to date our experience has been that the generic drug is just as effective as the original. Our hope is that veterinary wholesalers like Provvet and Cenvet will start listing this medication routinely.

OTHER THERAPEUTIC CONSIDERATIONS

Most cats with FHV-1 infections have secondary bacterial infection of either the conjunctiva and/or sinonasal cavity. In our view, it is therefore prudent to treat concurrently with an antibacterial agent, and we suggest the routine use of doxycycline monohydrate (5 mg/kg orally twice daily with food; Vibravet® tablets) for this purpose. Doxycycline has a broad spectrum of activity, with coverage of Bordetella bronchiseptica, Pasteurella multocida and obligate anaerobes, plus many Staphylococcus and Streptococcus isolates. Importantly, it is also active against the ocular pathogens Mycoplasma felis and Chlamydia felis. Thus, if empiric therapy is undertaken, combination therapy with famciclovir and doxycycline should have all primary feline ocular pathogens “covered”, and this may be more cost-effective than obtaining a definitive diagnosis using the multiplex PCR for feline oculo-respiratory pathogens available through the University of Melbourne or IDEXX. The anti-inflammatory effects and metalloproteasase inhibitory action of doxycycline are useful also in this setting.

Doxycycline monohydrate is well tolerated orally, and the tablets can be ground up in a pill crusher and mixed in with tasty food. The paste formulation is convenient to use in kittens, but is more expensive for adult cats, and pilling or
One of the key findings from David Maggs’s experimental work concerning FHV-1 in kittens and cats is that the number of goblet cells in the conjunctiva and therefore the concentration of mucin in the tear film is reduced by FHV-1 infection. Indeed, it takes the tear film many months to recover from a bout of viral conjunctivitis. Accordingly, there is benefit in using hyaluronic acid eye drops as adjunctive topical therapy for treating FHV-associated conjunctivitis and keratitis, to improve the quality of the tear film. These can be obtained from BOVA Compounding Pharmacy, but are expensive. Lacri-Lube® or chloramphenicol eye ointment twice daily represent good alternatives when owners cannot afford the expensive contemporaneously prepared medication.

Chronic sniffer cats may benefit from the administration of famciclovir in concert with either clindamycin, doxycycline or amoxicillin clavulanate, especially when high doses of famciclovir are used and the combination is continued for a protracted period, typically 2-3 months. Unfortunately, many cases only improve partially or transiently, presumably because some of the structural damage within the sinonasal cavity referable to Herpesvirus is irreversible, resulting in ongoing secondary bacterial infections despite therapy.

Cats with unilateral or bilateral otitis media, which present with signs of peripheral vestibular disease (head tilt and/or Horner’s syndrome, or wide excursions of the head, respectively), appear also to benefit from combination therapy using famciclovir and one of the aforementioned antibiotics.

**FUTURE DIRECTIONS**

Without doubt, we will develop a better “feel” for the therapeutic efficacy and therapeutic indications for famciclovir over the next 10 years. We certainly need information of *in vitro* susceptibility of a larger number of strains of FHV-1 to this agent, and to correlate this with clinical efficacy *in vivo*. We need to know how long a course of therapy is generally required for its various indications, including primary rhinosinusitis, conjunctivitis and keratitis.

The authors strongly recommend routine treatment of kittens with viral respiratory disease with both famciclovir and doxycycline, especially if naso-ocular signs are present. In cases where Calicivirus is the primary agent, famciclovir will be of no benefit, and instead omega-interferon (Virbagen®; Virbac) is indicated, should the owners have sufficient financial resources (Interestingly, co-infection with both FHV-1 and Calicivirus is not uncommon).

**Our great hope is that timely therapy using high doses of famciclovir will prevent many of the adverse long-term sequellae of FHV-1 disease including symblepharon, recurrent conjunctivitis and ulcerative keratitis, stromal keratitis, eosinophilic conjunctivitis, some cases of corneal sequestra, and especially the “chronic sniffer syndrome”, where disruption of normal anatomy, mucosal barriers and viral persistence establish a lymphoplasmacytic rhinitis, with variable secondary bacterial invasion. We suspect that a reduced prevalence of FHV-1 associated rhinosinusitis will also reduce the prevalence of invasive mycotic diseases of the nasal cavity, such as cryptococcosis, aspergillosis and sino-orbital disease due to *Neosartorya* species.

In cases where cats are prone to reactivation of FHV-1 associated signs with stress (such as boarding, parturition, lactation, hospitalisation, corticosteroid administration) it might be possible to prevent this happening by prophylactic use of famciclovir, or failing that, timely implementation of high dose therapy administered by the owners or carers at the first signs of viral recrudescence. Persistence of virus in the trigeminal ganglion means that “Herpes is forever”, although our experience had been that the frequency of recurrent episodes tends to decrease over a cat’s lifespan.

Finally, administration of famciclovir to queens starting immediately after parturition, and up until weaning, might decrease the frequency with which kittens are infected with FHV-1 in the perinatal period. This strategy, combined with timely administration of appropriate vaccines, may greatly reduce the frequency of FHV-1 disease in future generations of cats, especially pedigree cats kept in catteries and crossbred kittens raised in shelters.
SUMMARY: CURRENT RECOMMENDATIONS:

1. For treating FHV-1 associated dermatitis, use topical cold sore cream (Zovirax® or Zolaten®) combined with famciclovir orally.
2. For treating conjunctivitis and keratitis due to FHV-1, use famciclovir in concert with doxycycline (5 mg/kg twice daily) plus topical hyaluronic acid eye drops or Lacri-Lube/chloramphenicol eye ointment twice daily.
3. For treating acute or chronic rhinosinusitis, or middle ear disease, combine famciclovir plus either doxycycline, clindamycin or amoxicillin clavulanate.
4. For most adult cats, 125 mg Famvir or its generic equivalent, orally twice daily is a reasonable starting dose, although some cats will do better if higher doses are used.
5. For kittens, use 30 to 50 mg/kg orally twice daily for acute viral respiratory disease, in concert with one of the aforementioned antimicrobials.
6. The duration of therapy is yet to be determined definitively, but generally courses should be in the order of 2 weeks for acute disease, and until the resolution of signs in chronic cases.

References:
LOWER RESPIRATORY TRACT INFECTIONS IN CATS
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Bacteria, parasites, fungi and viruses cause lower respiratory tract infections (LRTIs) in cats. Most cats with LRTIs have pneumonia (inflammation of the lung parenchyma), although occasionally, pathology is limited to the airways. Parasitic, bacterial and fungal LRTIs can be diagnosed by routine investigation of LRT disease. Ante-mortem diagnosis of LRTI relies on analysis of all the patient details: signalment, history, physical examination findings, radiography, cytologic and microbiologic assessment of bronchoalveolar lavage (BAL) fluid or pulmonary fine needle aspirates (FNAs), histopathology (if available) and response to appropriate therapy. BAL (and to a lesser extent, FNA) cytology and microbiology are usually the mainstays of ante-mortem investigation of LRTI. However, the cytologic and microbiologic analyses by the laboratory have to be of an excellent standard, and appropriate requests from clinicians are essential. Only the veterinarian handling the case can make a diagnosis of LRTI.

General comments
There have been three detailed retrospective studies on feline LRTIs: one from Switzerland by Bart and others (2000; 245 cats), one from United States of America (USA) by McDonald and others (2003; 39 cats) and one from Australia by Foster and others (2004; 21 cats). The American and Swiss studies both analysed LRTIs that were histologically confirmed at necropsy.

Signalment and Clinical Signs
No breed or sex predilection has been noted for LRTIs in cats although males were 2.4 times more likely to have LRTIs than females in one study. Reported signs vary depending on study inclusion criteria: necropsy versus surviving clinical cases. Whilst coughing was the most common clinical sign in one study, lack of coughing does not preclude LRTI with only 8% of fatal cases coughing in another study. Dyspnoea, tachypnoea, coughing, increased tracheal hypersensitivity and/or nasal discharge may be noted but in one study of fatal cases, 36% of cats lacked any clinical signs referable to the respiratory tract.

Haematology, serum biochemistry and radiography
Haematology and serum biochemistry are of little help in the diagnosis of LRTI. Cats with LRTIs may have increased globulin and total protein concentrations. Lack of consistent radiographic abnormalities also contributes to the difficulties in diagnosing LRTIs in cats. All lung patterns have been represented radiographically and in 23% of cases confirmed by necropsy in one study, there were no radiographic abnormalities. Lack of radiographic abnormalities was also reported in a cat with confirmed cryptococcal LRTI.

Bronchoalveolar lavage (BAL) and lung fine needle aspiration (FNA)
The gold standard procedure for airway sampling is BAL via bronchoscopy however, it is not always possible due to lack of availability of equipment or financial or time constraints. For non-bronchoscopic BAL, use a short-acting anaesthetic agent and pass a sterile endotracheal tube using a laryngoscope to try and minimise oropharyngeal contamination. Place the cat in lateral recumbency with the most affected side down and pass a sterile dog urinary catheter (6-8 French gauge) carefully through the endotracheal tube until it wedges in a distal airway. Flush two, 5-10 ml aliquots of warm sterile saline through the catheter and retrieve as much as possible from the catheter by syringe aspiration. I also collect fluid that is coughed through the sterile endotracheal tube during anaesthetic into a sterile container to ensure adequate sample volume for assessment (a “back-up” sample). I have terbutaline, with dose pre-calculated (0.01 mg/kg IV, IM or SC) “on-hand” in case of acute bronchoconstriction and maintain an intravenous (IV) catheter until full recovery to enable IV drug administration if necessary. Lung FNA may also be indicated to investigate lesions identified by thoracic radiography or ultrasonography. Clinical complications are rare after careful lung FNA.

Cytology
Cytology can be performed on BAL fluid or lung FNAs. BAL fluid analysis has not been standardised. Many studies and most commercial laboratories report total cell counts (which can be influenced by the amount of lavage fluid delivered and retrieved) and use cytospin preparations for cytology. When performing total cell counts and preparing cytocentrifuged smears, mucus is routinely excluded from the analytic material. However, many of the cells in the BAL fluid are trapped within the mucus strands and not performing direct smears of mucus can result in failure to detect Aelurostrongylus abstrusus. Practitioners and laboratories can prepare smears in-house and perform cytology as follows. For each sample, two drops of lavage fluid containing mucus are placed on a microscope slide and unstained wet preparations are examined microscopically for the presence of parasites. Then, multiple squash preparations of mucus from each lavage specimen are air dried, stained with Diff Quik and analysed for the presence of mucus, oopharyngeal squames, oopharyngeal bacteria, respiratory pathogens, epithelial cells and inflammatory cells. Inflammatory cell numbers are subjectively
assessed as many, moderate, few or none and a differential white cell count performed. BAL fluids can be classified by the predominant inflammatory cell type, if greater than or equal to 50% of the total, or described as mixed, if no cell type predominates.\textsuperscript{5,6} If sufficient material is present, it is recommended that several smears are examined from each sample. If no mucus is present, a centrifugation technique is required for smear preparation as mucus-free samples may contain abnormal cells; cytospin preparation is ideal for this. If bacteria are identified, a Gram stain should be performed; acid fast stains may also be necessary for some bacteria. Total cell counts are not necessary as reference ranges have not been validated for this method of sample collection.\textsuperscript{5,6}

The usual lower airway response to pulmonary bacterial or protozoal infection, when BAL is performed in this manner, is neutrophilic unless there is concurrent immunosuppression.\textsuperscript{2} Bacteria consistent with the culture results should be observed cytologically in bacterial LRTIs except those with mycoplasmosis and occasionally mycobacteriosis. Mycoplasmas do not usually take up Diff Quik or Gram stain although small Gram-negative stained “flecks” have occasionally been observed in cytologic preparations of BAL fluid from which mycoplasmas are cultured.\textsuperscript{6}

BAL fluid cytopathology performed on cytocentrifuged samples does not always correlate with the type of pulmonary disease identified on histopathology. However, histopathology correlated reasonably well with cytology in one study in which a number of cats had inflammatory LRT lesions.\textsuperscript{7} One cat with feline infectious peritonitis in this study had normal BAL cytology, which was not representative, but BAL cytology in three other cats (one with toxoplasmosis, two with focal bronchitis) was more sensitive than histopathology.\textsuperscript{7} Cytologic specimens may be more sensitive for detection of microscopic focal lesions and toxoplasma tachyzoites but less sensitive when lesions are confined to the interstitium.\textsuperscript{7} BAL may demonstrate LRT parasites. However, faecal floatation and faecal analysis by the Baermann technique should routinely be performed in cats with possible LRTIs.\textsuperscript{7}

### Microbiology and Parasitology

Airways in healthy cats are not sterile. The bacteria reported as occurring in low numbers (less than 2x10\textsuperscript{3} colony forming units/ml) in the airways of healthy cats include Escherichia coli and species of Pasteurella, Pseudomonas, Staphylococcus, Streptococcus and Micrococcus. Anaerobic bacteria and mycoplasmas have not been isolated from the lower airways of healthy cats.\textsuperscript{10,11} The most common bacteria causing feline LRTIs in the three retrospective studies of LRTIs were B. bronchiseptica, Pasteurella spp., Mycoplasma spp., Streptococcus spp. and E. coli. Other agents identified as causing infectious pneumonia in these studies were other bacteria (including mycobacteria, Salmonella typhimurium, Pseudomonas spp., anaerobes), viruses (herpesvirus, coronavirus), lungworm (Aelurostrongylus abstrusus, Eucoleus aerophilus (previously Capillaria aerophila)), protozoa (Toxoplasma gondii) and fungi (Cryptococcus spp., Candida albicans, Mucor sp., Aspergillus sp.).\textsuperscript{1,2,3} Other aetiological agents, including avian and swine influenza viruses, have also been identified in case reports, small case series and textbooks.\textsuperscript{6}

### Histopathology

Lung histopathology is occasionally performed ante-mortem. Samples can be obtained via ultrasound- or computed tomography-guided core-biopsy or surgical excision (via thoracoscopy or thoracotomy).\textsuperscript{5}

### TREATMENT

Due to the diversity of causes of LRTI in cats, empirical therapy in cats with signs of LRT disease is not recommended and appropriate investigation should be performed in all cases. A brief overview of treatment of bacterial, parasitic, fungal and viral LRTIs is provided.

#### Bacterial LRTI

- It is probably worth treating all cats with LRT disease with an appropriate antibiotic for mycoplasmas whilst awaiting results of cytology and culture. In proven cases, given that chronic persistent infection has been documented in humans, that some cases of mycoplasmal LRTI have been reported to have recurrent doxycycline-responsive coughing or need continuous treatment,\textsuperscript{3} and the finding that 42 days may be required for treating feline URT mycoplasmal infections,\textsuperscript{12} then it may be prudent to treat mycoplasmal LRTIs with doxycycline 5 mg/kg BID PO for a minimum of 6 weeks.\textsuperscript{6}
- Bordetella bronchiseptica - The currently recommended drugs for treatment of bronchopneumonia due to B. bronchiseptica are doxycycline, trimethoprim-sulphonamide and fluoroquinolones.\textsuperscript{6}
- Streptococcus species - Procaine and benzathine penicillin (long acting penicillin) have been used both in treatment of cases and prophylactically in cats.\textsuperscript{6,12} Penicillin doses are usually provided in Units. The mg/kg conversions of these doses for the various preparations of penicillin G are provided in DC Plumb’s Veterinary Drug Handbook.\textsuperscript{14} In humans, clindamycin is regarded as the drug of choice in streptococcal infections. Clindamycin is also reported to be valuable in the treatment of streptococcal infections in cats.\textsuperscript{13} Fluoroquinolones are not effective for streptococcal infections.
- Escherichia coli - Lower respiratory tract infections due to E. coli, whilst relatively uncommon, would appear to be associated with high mortality, especially in kittens.\textsuperscript{6} Early aggressive broad spectrum antibiotic treatment is likely to be required.
- Pasteurella species - These bacteria are usually susceptible to the aminopenicillanic derivatives.\textsuperscript{6}
• *Salmonella* species - Antibiotic sensitivity profiles are required for effective treatment.

• *Eugonic Fermenter-4 (Neisseria species)* - There is only one report of successful treatment of LRTI due to EF-4 bacteria. The cat was treated with marbofloxacin 5 mg/kg q 24h PO for 28 days, in conjunction with amoxicillin-clavulanate 20mg/kg q12h PO for the first 6 days.6

• *Mycobacterium species* - Management of mycobacterial infections is dependent on the species isolated. Mycobacteria have a well-documented capacity to develop resistance to various antimicrobial agents and combination antimicrobial therapy is recommended when treatment is being considered.15

Parasitic LRTIs

• *Aelurostrongylus abstrusus* - A single dose of ivermectin 400 µg/kg SC cannot be recommended based on evidence-based medicine. Two doses of 300-400 µg/kg SC are required if using ivermectin.6 Imidacloprid/moxidectin (Advocate; Bayer) applied topically once proved 100% effective in 12 cats16 which makes this the treatment of choice in most countries. Emodepside/praziquantel (Profender; Bayer) had 99% efficacy in another study. Topical selamectin has only been effective in two of four cats in which its use has been reported.18,19

• *Eucoleus aerophilus* (previously *Capillaria aerophila*) - Two doses of abamectin (Avomec; Merial) 300 µg/kg SC, two weeks apart, have proven efficacious.20 Topical treatment with Advocate® is also worth considering but its efficacy is unknown.

• *Toxoplasma gondii* - Diagnosis for respiratory toxoplasmosis is poor. Sole clindamycin therapy cannot be recommended on evidence-based medicine.6,25 Clindamycin (12.5 mg/kg q12h PO) in conjunction with pyrimethamine (0.25-0.5 mg/kg q12h PO) would currently appear to be the treatment of choice, especially in cats which have had had ciclosporin treatment.6 It is perhaps also worth considering the use of triazines such as toltrazuril, which have shown promise in the treatment of equine protozoal myeloencephalitis.22 Diclazuril and pyrimethamine in mice with experimentally induced acute toxoplasmosis had a synergistic effect on survival23 and toltrazuril has been shown to be effective against the intestinal developmental stages of *T. gondii* and reasonably effective against extraintestinal stages of *T. gondii* in cats.24

Fungal LRTIs

Treatment is dependent on the fungal agent identified. Fungal causes of LRTI include *Cryptococcus* spp., *Histoplasma capsulatum*, *Sporothrix schenckii*, *Aspergillus* sp., *Mucor* sp., *Candida* spp., *Coccidioides immitis*, *Blastomyces dermatitidis*, *Ochroconis gallopavum*, *Paecilomyces lilacinus* and *Cladophialophora bantiana*.6 Siamese and Abyssinian cats appear to have an increased prevalence of cryptococcosis25,26 and possibly, histoplasmosis.27 Siamese cats also featured prominently in an early description of blastomycosis cases.25 Neither histoplasmosis nor blastomycosis have been reported in cats in Australia.


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Small Animal Medicine and Feline Chapters
FELINE ORBITAL ACTINOMYCOTIC MYCETOMA: *STREPTOMYCES CINNAMONEUS*

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The orbit is a protective boney fossa separating the eye from the cranial cavity. It provides a foramina for blood vessels and nerves. Feline orbits are classified as incomplete or open as they are incompletely encompassed by bone. Only the medial third of the orbital wall is osseous with the remainder formed by a thick dense fibrous ligament (orbital ligament) spanning between the frontal and zygomatic bones (periorbita).

Retrobulbar abscess is a relatively common condition, due to this incomplete encasement in both cats and dogs alike. It develops when diffuse inflammation consolidates into a discrete mass.

Clinical signs manifest as changes in both volume and function of orbital structure. Exophthalmos is the predominant finding with other secondary findings of third eyelid protrusion, discharging sinuses, periorbital swelling, pain on manipulation of tissue structures surrounding the orbit, inability to retropulse the eye, conjunctival hyperaemia, ocular discharge, exposure keratitis, and lagophthalmos. Visual impairment may also occur. Proposed aetiologies (Table 1) are diverse with most not being clinically apparent at the time of diagnosis. Retrobulbar abscesses may be sterile or infectious in origin with multifarious infectious aetiologies being previously described in veterinary literature.

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<th>Proposed aetiologies for retrobulbar abscessation</th>
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<tbody>
<tr>
<td>Foreign bodies</td>
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<tr>
<td>Bite wounds</td>
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<tr>
<td>Extension of infection (tooth root, sinuses, salivary gland (zygomatic, lacrimal))</td>
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<tr>
<td>Trauma</td>
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<tr>
<td>Haematogenous</td>
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<td>Idiopathic</td>
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*Table 1. Proposed aetiologies for retrobulbar abscessation*

*Streptomyces* spp. are well known for their medical importance in the human and animal world with biologically active metabolites being used to produce antibiotics (clavulanic acid, tetracycline, chloramphenicol, streptomycin, and neomycin), antifungals (Amphotericin B) and antiparasitic (ivermectin) compounds. Additionally, they have provided enzymes which are required for the natural turnover of many macromolecules in soil. The organism is slow growing and prevalent in tropical and subtropical regions where it exists in soil rich organic matter. It has a complex lifecycle and low pathogenicity. Infrequently, they have been encountered in association with serious human and animal infections with modern disease description in humans dating back to the 19th century. Pathogenic medical species include *Streptomyces somalienses* and *Streptomyces sudanensis*.

Streptomyces infection is rare in animals. In humans it is characterized by the production of a mycetoma with the disease having precedence in developing countries. Inoculation is via disruption to the mucosal barrier, usually through trauma with the bacteria slowly growing through production of densely packed mycelium causing fibrosis. Lesions appear as subcutaneous swelling and grow in size spreading to surrounding tissue and bone. Abscessation may be associated with infection.

A number of single case studies in human literature have highlighted the potential for the development of systemic disease with the heart, lungs and central nervous system being potential targets for the organism.

This case report presents a cat with progressive retrobulbar disease refractory to medical treatment. Advanced imaging and biopsy of tissue initially failed to identify the causative agent or the pathogenesis. Failure of medical therapy, lead to surgical exenteration of the eye with subsequent histopathology and culture and sensitivity successfully identifying the causative organism as *Streptomyces cinnamoneus*. Follow-up long term medical therapy has seen control of the disease with no further recurrence.

**References:**

Toxoplasma gondii infection is common within the feline population. Serological surveys have demonstrated exposure rates of between 32% and 52% in domestic cat populations in Australia and North America\(^1,2,3\). Seroprevalence has been shown to increase with age reflecting an increased risk of exposure over time\(^1,2,3\). A recent study found seropositivity (IgG) was 12-fold higher for cats aged > 12 months old compared with cats aged < 6 months old\(^4\). Seroprevalence is not significantly different between owned and unowned cats\(^4\) or between cats from urban versus rural environments \(^3\). Cats can become infected with \emph{T. gondii} through carnivorous feeding and the ingestion of bradyzoite tissue cysts within an infected intermediate host, or less commonly by the ingestion of sporulated oocysts in the faeces of infected cats. Despite high exposure rates, most cats are able to mount an effective immune response that contains, but does not eliminate, the infection\(^5\). Clinical toxoplasmosis, though rare, may develop in cats exposed to overwhelming numbers of infective \emph{T. gondii}, those that are immunocompromised, or those with reactivation of latent infection due to immunosuppression\(^6\).

Definitive diagnosis of clinical toxoplasmosis requires detection of \emph{T. gondii} tachyzoites or DNA in body cavity effusions, bronchoalveolar lavage fluid or tissue biopsies by cytological, histological or immunohistological methods. Clinical presentation, laboratory findings and response to anti-toxoplasma therapy may assist in the ante-mortem diagnosis of toxoplasmosis\(^7\). Serology is a useful adjunct to the diagnosis of toxoplasmosis, though interpretation can be complicated. Following infection, \emph{T. gondii}-specific immunoglobulins IgM, IgG and IgA can be detected in serum. Positive IgM titres develop within 1 to 2 weeks post-exposure and are usually negative by 12 weeks\(^8\). Positive IgG titres develop 2 to 4 weeks post-infection and peak within 2 to 4 weeks after initial detection\(^9\). IgG levels persist for at least six years, and probably for life, due to persistence of \emph{T. gondii} antigen within tissues\(^10\). Maternally derived IgG antibodies in kittens typically wane by 8 to 12 weeks of age. Positive IgA titres develop 4 to 6 weeks post-infection and persist for at least 26 weeks\(^9\). Measurement of both IgG and IgM simultaneously followed by a second IgG titre 2-4 weeks later is recommended as an aid to diagnosis of active toxoplasmosis\(^9\).

![Figure 1: Appearance of \emph{T. gondii}- specific serum antibodies in experimentally infected cats](image)

Serological tests that detect \emph{T. gondii} antibodies include the enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), Sabin-Feldman dye test (DT), indirect haemaggulination test (IHA), latex agglutination test (LAT) and modified agglutination test (MAT). Of these, the MAT is the most sensitive and IHA is the least sensitive. The two most commonly used diagnostic tests are the ELISA and IFA, which are reported to have a similar sensitivity\(^5\). Reported titres can vary with the type of serological test used and between diagnostic laboratories, requiring that the laboratory provide some interpretation as to what is considered a significant titre. Generally, a positive IgM titre greater than the least significant level of reactivity for that assay (usually > 1:64) or a fourfold increase in IgG levels in paired serum samples taken 2–4 weeks apart is considered diagnostic of clinical toxoplasmosis\(^7\).

Interpretation of serological tests can be notoriously difficult. Negative serological test results suggest that the cat has not been exposed to \emph{T. gondii}. However, false-negative IgM titres can occur in peracute infections since an IgM response does not develop until 2 weeks post-exposure in the majority of cats. Also, only 80-90% of cats infected with \emph{T. gondii} have a detectable IgM response and cats with concurrent feline immunodeficiency virus (FIV) infection may not develop an IgM response\(^8,11\). Furthermore, there may no increase in IgM titres in cats re-infected with \emph{T. gondii}.
several months after primary infection. Clindamycin administration to cats with toxoplasmosis may blunt IgM responses resulting in lower titres.

Positive serological test results suggest that the cat has been infected previously. IgM is occasionally detected in the serum of chronically infected cats and so does not always correlate with recent or active infection. Persistent IgM titres have been documented in cats co-infected with FIV and in cats with ocular toxoplasmosis. In cats with concurrent FIV-infection, this is attributed to a delayed antibody class shift from IgM to IgG. Also, administration of high doses of glucocorticoids to cats that are latently infected can increase IgM titres and can diminish the IgG response. A single positive IgG titre indicates exposure but not active infection. IgG antibody titres can persist for at least six years following primary infection and can remain high (>1:30,000) and simply reflect the presence of T. gondii antigen within tissues. A rising antibody titre may not be able to be demonstrated, as many cats have mild clinical signs and may not be serologically evaluated until antibody titres have already reached maximal levels. Also, in cats with reactivation of latent infection, IgG titres rarely increase.

INTRODUCTION
In the search for less toxic and more effective cancer therapies, an important goal has been to develop agents that specifically target some characteristic unique to cancer cells and absent from normal cells. As a generalization, most chemotherapeutics are most active against rapidly dividing cells or cells in particular phases of the cell cycle and this gives a relative advantage against tumour cells compared to most normal cells. However, rapid turnover is not unique to cancer cells, and the effect on rapidly dividing normal populations – bone marrow, GI mucosa, reproduction and wound healing - results in the main side effects of chemotherapy.

The first step in the development of a targeted therapy is to identify a tumour-specific target, and the next step is to develop a treatment that exploits it. The search for a target has been underway for decades, and while a target that is absolutely unique to cancer still remains elusive, some promising advances have been made. Once a target is identified, possible approaches to exploiting it include immunotherapy directed against a tumour-specific antigen, and using an inhibitor to block a critical metabolic pathway. Two recent breakthroughs in veterinary oncology – the 2 “Hot Topics” to be discussed in this lecture - each use one of these approaches.

ONCEPT
Immunotherapy and melanoma
Immunotherapy seeks to harness aspects of the immune system in recognizing and attacking cancer cells; using either nonspecific immunomodulators such as cimetidine, TNF and IL-2, or liposome encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE); or a specific approach such as a monoclonal antibody (passive immunotherapy), or a vaccine (active immunotherapy) against a tumour-specific antigen. Attempts at cancer immunotherapy of various cancers in humans and other animals have met with mixed success.

One cancer for which immunotherapy is increasingly being considered an important component of therapy is melanoma, with agents including interleukins, interferons, other cytokines, adoptive immunotherapy, and vaccines all under investigation. Recent advances include better understanding of the value of immune checkpoint proteins such as CTLA4, PD1 and CD40 and using monoclonal antibodies directed against these; and increased understanding of dendritic cells and their use in cancer vaccine development. However significant challenges remain to clinically effective melanoma vaccination in humans, including dysfunctional host immune responses and tumour cell immune evasion.

Canine melanoma
In dogs, oral melanoma is the most common oral malignancy. Unlike cutaneous melanomas, which are often benign, oral melanomas are usually malignant. Aggressive local growth and distant metastasis are common, but recent studies suggest not uniform. Some studies have found a WHO staging scheme prognostic, with significantly longer survival after surgery alone for dogs with stage I (<2cm diameter) tumours (median = 17 months) than for dogs with stage II or III (primary tumour >2 cm, or any bone invasion, or lymph node metastases) disease (median = 5.4 months). One study found that dogs with melanoma of the lip lived a median of 25 months (1-year survival = 57.4 %; 2-year survival = 34.3%) which was significantly longer than for dogs with melanoma at other oral sites (7 months). Shorter average survival times are seen for dogs with tumours that are larger, have high numbers of mitotic figures per 10 high-power fields (mitotic index), have marked nuclear atypia and more inflammation or necrosis: these characteristics are added into a numerical score with >10 being considered malignant.

While approximately 85% of cutaneous melanomas are benign, subungual (nail bed) melanoma is a notable exception and is often also highly malignant. As for oral melanomas, pathology is important in determining the likelihood of malignant behaviour in cutaneous melanoma. One study made a distinction between the behaviour seen for melanomas of the feet and the lips, from those seen elsewhere in the skin and oral cavity, respectively. Dogs with melanoma of the feet or lips had a median survival of 22 months with a 30% death rate due to melanoma; which was better than for dogs with oral melanoma, but worse than for dogs with cutaneous lesions. In one study, the median survival after surgery for dogs with digital melanoma was 12 months, with 13% alive 2 years after surgery.

Surgery remains the mainstay of local treatment for melanoma. Most dogs with oral tumours are euthanized because of progression or recurrence of local disease, but if surgery is aggressive from the outset, it may prolong survival as well as provide palliation. Likewise for subungual tumours, digital amputation is warranted. Radiation therapy has been shown to improve local control of intraoral melanoma if complete surgery is not anatomically possible.
If local control can be achieved, the primary problem becomes the risk of metastasis. When metastatic disease occurs in patients with digital melanoma, it usually occurs within 6 months (median 5.3 months) of treatment. The growth rate of melanoma metastases may vary, and it is probably this variation, rather than the time at which metastasis occurs, that determines survival time if local disease is controlled. Some dogs may tolerate pulmonary metastatic disease with very little apparent impact on their quality of life for some months. The best chemotherapeutic responses reported for melanoma are for platinum drugs. One chemotherapy study reported responses (PR and CR combined) in 7 of 25 dogs (28%) with unresectable oral melanoma, including one dog with a complete response lasting nearly 3 years. Overall median response duration was 5.5 months. Similar response rates have been seen in established metastatic disease. As chemotherapy is always more likely to be successful when disease is microscopic, the outcome in the adjuvant setting would be expected to be somewhat more favourable. A combination of radiation therapy and chemotherapy used as an adjunct to incomplete surgical excision was reported in 39 dogs with incompletely resected oral melanoma. Only six dogs (15%) had local recurrence (within a median time of 4.6 months) and 20 dogs (51%) developed metastatic disease within a median time of 10.2 months. Median survival time for all 39 dogs was 12 months.

**Xenogeneic murine tyrosinase DNA vaccination (Oncept) for dogs with melanoma**

In early 2010 the USDA gave full licensure to a xenogeneic human tyrosinase DNA vaccine to treat dogs with melanoma, following a period of conditional licensure starting in 2007 during which the vaccine was available to veterinary oncologists in the US for trials. The vaccine is now available in the US as Oncept, produced by Merial, for use by veterinary specialists practicing oncology. The vaccine is given as an initial series of 4 treatments, using a specifically designed needle-less transdermal injector, 2 weeks apart, and then a booster every 6 months.

DNA vaccination is used because DNA is relatively inexpensive and easy to purify in large quantity. The gene is cloned into a bacterial expression plasmid with a constitutively active promoter. The plasmid is introduced into the skin or muscle where dendritic cells present the transcribed and translated antigen in the proper context of major histocompatibility complex and co stimulatory molecules. The plasmid also contains immunostimulatory sequences that may act as an adjuvant in the immune response.

The Oncept vaccine encodes the melanosomal glycoprotein tyrosinase, which is essential in melanin synthesis. In mice, immunization with xenogeneic human DNA encoding tyrosinase family proteins induced antibodies and cytotoxic T-cells against melanoma cells, but immunization with mouse tyrosinase-related DNA did not, showing that xenogeneic DNA vaccination could break tolerance against a self tumour differentiation antigen.

The first developmental studies of the vaccine in 9 dogs with stage 2, 3, or 4 dogs treated at 3 different dose levels of human tyrosinase plasmid DNA. That study reported that toxicity was limited to mild local reactions at injection sites. One dog with pulmonary metastases (stage IV disease) had a complete clinical response for 11 months, and 2 other dogs with stage IV disease had survivals of 14 months and over 19 months in the face of significant bulky metastatic disease. Two dogs with locally controlled stage II/III disease had survivals of between 16 and 17 months with no evidence of melanoma on necropsy. Four other dogs were euthanized because of progression of the primary tumour. The Kaplan-Meier median survival time for all nine dogs was 13 months. In a subsequent study, a large number of dogs were vaccinated at varying dose levels of human or mouse tyrosinase or GP75, with or without additional of human GM-CSF. Overall survival time for 33 dogs with loco-regionally controlled stage II-III melanoma across the xenogeneic vaccine studies was nearly 19 months. This was better than historic controls. In addition, dogs vaccinated with human tyrosinase were shown to have 2- to 5-fold increases in circulating antibodies to human tyrosinase. A later data analysis showed that dogs which received any melanoma vaccine had a median survival time of >31 months (median not reached) for stages I and II, >54 months (median not reached) for stage III, and just under 8 months for stage IV.

Most recently, a study of 58 dogs with digital melanoma demonstrated overall median survival time of nearly 16 months for dogs treated with xenogeneic DNA vaccine following complete loco-regional control, with a 1-year survival rate of 63%. Median survival time for dogs with metastasis was 3.5 months versus nearly 18 months for dogs without metastasis (P < .0001). Oncept is labelled for use in the treatment of dogs with stage II or stage III oral melanoma and for which local disease control has been achieved. This means that the primary tumour may be of any extent, as long as it is locally controlled using surgery and / or radiation; and that if lymph node metastases are present, the involved nodes have also been removed or irradiated. Dogs with distant metastases are excluded from the label indication. Therefore the goal of vaccination would be prevent local recurrence and delay or prevent the onset of metastases.

As noted above, occasional responses have been seen in macroscopic melanoma. However responses in bulky disease are rare and Oncept is not recommended as a treatment for macroscopic disease.
The use of Oncept in cats has only been reported anecdotally to date.

Table 1. Summary of median survival times of dogs with malignant melanoma with different treatments.

<table>
<thead>
<tr>
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<th>Surgery alone</th>
<th>Surgery + RT + chemo</th>
<th>Surgery +/- RT + Vaccination</th>
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<tbody>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stage I</td>
<td>17 months</td>
<td></td>
<td></td>
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<tr>
<td>Stage II-III</td>
<td>5.4 months</td>
<td>12 months</td>
<td></td>
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<tr>
<td>Lip</td>
<td>25 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not lip</td>
<td>7 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digital - all</td>
<td>12 months</td>
<td>16 months</td>
<td></td>
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<tr>
<td>+ mets (stage IV)</td>
<td></td>
<td>3.5 months</td>
<td></td>
</tr>
<tr>
<td>- mets (stage II-III)</td>
<td></td>
<td>18 months</td>
<td></td>
</tr>
<tr>
<td>All malignant – stage II-III</td>
<td></td>
<td>19 months</td>
<td></td>
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<tr>
<td>Stage I</td>
<td></td>
<td>&gt;31 months</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td></td>
<td>&gt;31 months</td>
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<td>Stage III</td>
<td></td>
<td>&gt;54 months</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
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<td>8 months</td>
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**PALLADIA**

**Tyrosine Kinases**

Cancer cells rely on proteins that regulate signal transduction, cell survival and cell proliferation; and several key members of this regulatory family that have been identified in various cancers are tyrosine kinases (TKs). Several inhibitors of these proteins (tyrosine kinase inhibitors, TKIs) have now been approved for cancer treatment and have shown significant clinical efficacy.

Tyrosine kinases bind ATP to add phosphate groups to key tyrosine residues on themselves (autophosphorylation) and on other proteins, leading to intracellular signalling and alterations in gene transcription which affect cell proliferation, differentiation and survival. TKs expressed on the cell surface (receptor tyrosine kinases, RTKs) bind growth factors that regulate their activation. These RTKs include Kit, Met and EGFR, all of which are known to be dysregulated in various forms of cancer. Some, such as VEGFR, PDGFR, and FGFR are also important in promoting angiogenesis. TKs are often abnormally activated in malignant tumours through mutation, overexpression, and the generation of fusion proteins from chromosomal translocation. The best-characterized example is the Bcr-Abl fusion in chronic myelogenous leukaemia leading to chronic activation of the TK Abl, a key step in the malignant transformation in this disease. Another example, Kit, is normally expressed on haematopoietic stem cells, melanocytes, and mast cells. Dysregulation occurs in human systemic mastocytosis, acute myelogenous leukaemia, gastrointestinal stromal tumours (GISTs), and other cancers. Kit mutations have been documented in up to 30% of canine mast cell tumours (MCTs) as well as in canine GISTs and in feline MCTs.

**Tyrosine Kinase Inhibitors**

Various strategies have been explored to exploit this target, but the most successful is the group of drugs called small molecule tyrosine kinase inhibitors (TKIs). These drugs block the ATP binding site of kinases, acting as competitive inhibitors, so the kinase is not able to phosphorylate itself or initiate downstream signalling.

The prototype drug in this group and the first to be used clinically is imatinib (Gleevec / Glivec). Imatinib was designed specifically to target the constitutively active Bcr-Abl fusion protein in CML and is extremely successful clinically. For individuals in the chronic phase of CML, imatinib provides a molecular response for the majority of patients. Imatinib also blocks ATP binding of Kit, providing an overall response rate of 55% plus another 25% - 30% stable disease in GIST (compared to approximately 5% with previous chemotherapy). There are now more than 8 TKIs FDA approved in the US for use alone or in combination for CML, GIST, ALL, non small cell lung cancer, pancreatic cancer, breast cancer, and renal cell carcinoma. More recently TKIs are being investigates for their role in nonmalignant proliferative diseases such as atherosclerosis, rheumatoid disorders, and pulmonary fibrosis.

In dogs, imatinib is associated with idiosyncratic hepatotoxicity in some dogs, although this is not as common as was initially thought. It has been provided responses in dogs with MCTs, including systemic mast cell disease. In cats, a phase I clinical trial in 9 cats with a variety of tumours showed no evidence of hematologic or hepatic toxicity and only mild GI toxicity, and a complete response in 1 cat with systemic mastocytosis. The major drawback to Glivec use in veterinary medicine is its extremely high cost (~$3800 COST for 30-day supply for a 30 kg dog!).

Small Animal Medicine and Feline Chapters
Two TKIs have undergone registraional studies in dogs with mast cell tumours:

**Masitinib (Masivet / Kinavet; AB Science)** primarily targets Kit. Masivet is approved in Europe and the UK for dogs with recurrent or non-resectable grade 2 or 3 MCT regardless of c-kit mutation status, and is being launched as Kinavet in the US, however AB Science have indicated that they have no plans to enter Australia.

- A placebo-controlled randomized double blind phase III clinical trial in 202 dogs with non-metastatic grade 2 or 3 MCTs showed a 12-month complete response (CR) rate of 11.1% (vs. 2.9% with placebo (not significantly different)), and “controlled disease” (overall response rate (ORR) plus stable disease (SD)) of 23.1% (vs. 5.9% with placebo (significantly different)). Dogs with Kit mutations had 27.3% CR (vs. 0% with placebo (not significant), and 31.8% “controlled disease” (vs. 0% with placebo (not significant). The study did report significantly prolonged time to progression (TTP) of 118 days (vs. 75 days with placebo), which was greater in dogs with Kit mutations (230 days vs. 42 days with placebo). 22
- A more recent study reported improved survival in dogs with non-resectable MCTs compared with results for the placebo, with 59 of 95 (62.1%) and 9 of 25 (36.0%) dogs alive at 12 months and 33 of 83 (39.8%) and 3 of 20 (15.0%) dogs alive at 24 months, respectively; and median overall survival times of 20.5 months and 10.7 months. 23
- A recent study evaluated safety of Masitinib in healthy cats and found proteinuria in 10% of cats and neutropenia in 15% of cats, as well as an increase in serum creatinine concentration and adverse gastrointestinal effects in some cats; however masitinib was considered tolerated in the majority of cats. 24

**Palladia - Toceranib** (Palladia; Pfizer Animal Health) is similar to sunitinib, in that it is active against members of the split-kinase RTK family (VEGFR, PDGFR and Kit), and has both anti-angiogenic and anti-tumour activity.

- In a placebo-controlled randomized study in dogs with non-resectable grade 2 and 3 MCTs, 86 Palladia treated dogs had an overall response rate (ORR, complete response (CR) plus partial response (PR)) of 37.2% (7 CR, 25 PR) compared to 7.9% (5 PR) with placebo (n=63). Following placebo escape, the total ORR for all 145 dogs receiving Palladia was 42.8% (21 CR, 41 PR); dogs with Kit mutations had an ORR of 82% (vs. 55% in dogs without mutations), and dogs without lymph node metastasis had higher response rate (67%, vs. 46% in dogs with metastasis). 25

The pharmacokinetics of Palladia in dogs has been reported recently. 26, 27 Since its commercial release in the US, Palladia has been used with some efficacy in a wide variety of diseases in both dogs and cats. There are multiple abstract reports of Palladia use in a variety of indications in the 2010 Veterinary Cancer Society Annual Conference Proceedings, and overall use of Palladia by US-based specialists is best summarized in the abstract by Johannes et al. 28 There have been several small reports of Palladia use in cats. In general Palladia is found to be well tolerated in cats, with the most recent report showing a limited adverse event profile and some efficacy in a variety of tumours. 29

As targeted therapy, it would be hoped that the toxicity of TKIs would be minimal; however they should best be viewed similarly to other chemotherapeutics in terms of prevention and management of side effects. Both toceranib and masitinib can be associated with gastrointestinal side effects that can be significant, and each has specific side effects. Management requires good supportive care and the judicious use of treatment breaks or adjustments to the dose or schedule, as with other chemotherapy drugs.

**CONCLUSION**

The advent of effective tumour vaccine strategies and TKIs in veterinary oncology has generated a huge amount of excitement and anticipation. Although no single agent can be expected to replace all other treatments; the addition of a new, well documented active agent is always most welcome and will allow some patients to be treated that otherwise would not be able to respond to therapy. As they become commercially available in Australia over the coming years, these new agents will undoubtedly find their place in therapy of veterinary cancer patients and are likely to become a valuable component of treatment for many animals.
