A review of information presented at the American College of Veterinary Pathology meeting in Savannah, Georgia November 2007.

The meetings of the American College have become an integral part of the annual diary at TDDS. Essential for keeping at the forefront of diagnostic knowledge and technology, attendance is also a critical factor in the final phase of our resident training for the American Board Examinations. This review of the recent conference highlights new information relevant to the practising veterinary surgeon in the UK.

MOLECULAR DIAGNOSTICS AND INFECTIOUS DISEASES

The meeting opened with fascinating and slightly combative lectures by two world authorities on veterinary infectious diseases, Edward Breitschwert and his former PhD protégé Michael Lappin.

The main drive of Dr Breitschwert's lecture was the potential for molecular diagnostic techniques (PCR) to identify polymicrobial infections or co-infections with many organisms simultaneously. As clinicians we all appreciate that natural disease processes rarely 'follow the textbook'. Dr Breitschwert's contention is that a major reason for the discrepancy between natural presentations of disease and the rigorous descriptions obtained by experimental infection is that in nature we are often dealing with patients infected with several different pathogens at once. Such presentations are hugely complex and may provide surprising outcomes to treatment directed at one but not all the pathogens involved. Molecular techniques allow us to identify concurrent infections and respond accordingly with treatment. Culture and serological methods of diagnosis have been the mainstay of infectious disease investigation but culture often only identifies the organisms which grow easily, and infections with many pathogens including babesiosis, bartonellosis and leishmaniasis may induce no serological signature of immune recognition. A modern view of natural disease will require a greater understanding of the interactions of viruses, bacteria, protozoa and fungi as contributors to complex disease expression.

Having extolled the virtues of PCR, Dr Breitschwert then outlined the pitfalls including contamination of molecular grade water with plant and bacterial DNA, resulting in false positive results, and the lack of standardisation or quality control among laboratories providing molecular diagnostic testing. As a believer in co-infection, Dr Breitschwert was less outspoken on the dangers of identifying clinically irrelevant infection by sensitive molecular techniques. This nettle was firmly grasped by Dr Lappin who assessed the clinical utility of molecular diagnostic assays in cats. Although PCR assays for infectious agents which are carried out under appropriately strict quality control measures are likely to have high sensitivity and specificity for the presence of the agent in the sample, the clinical utility of the assays is difficult to assess because of the limited knowledge of the positive and negative predictive values of such results for clinical disease.

Summary

Herpesvirus DNA can be amplified by PCR from healthy cats and the test has poor positive predictive value for disease. The negative predictive value is also in question because cats which are likely to have herpesvirus-related disease are often PCR negative. This may be due to clearance of viral DNA from tissue by a hypersensitivity reaction. The same difficulty with positive predictive value is also present in PCR testing for calicivirus, Chlamydia felis, Mycoplasma species, Bordetella bronchiseptica, Bartonella species and coronavirus/infectious peritonitis.

Haemoplasma PCR is much more sensitive than cytology for identifying M. haemofelis and other haemoplasmas and these organisms are not cultured. The PCR assays have reasonable positive predictive value for infectious anaemia if they are run in cats with fever and unexplained anaemia. A similar situation occurs with Cryptosporidium. Faecal floatation, acid fast staining and the antigen IFA test are insensitive in cats and PCR is indicated in cats with unexplained small bowel diarrhoea. Positive PCR results for Cryptosporidium felis do not always prove that the agent is the cause of clinical disease. Similar findings apply with the PCR detection of Tritrichomonas fetus in kittens with large bowel diarrhoea.

In comparison with the low positive predictive value of molecular diagnostic tests for disease, cytology frequently provides results with very high positive predictive value for disease. Two examples are illustrated below.
The session on molecular diagnostics continued with two lectures from the medical field describing ground breaking technology in quality control, current and future trends. The final lecture gave us a glimpse of the future by introducing a compact, simply operated ‘patient side’ PCR analyser capable of detecting MRSA with the following slogan: ‘Real-time PCR meets Real-time patient management!’

**SCIENTIFIC ABSTRACTS**

The scientific abstracts session followed the educational symposium on molecular diagnostics. Wide-ranging new information was presented in a series of short papers.

**Summary**

Myelin-like material can be identified cytologically in CSF samples but its presence is not associated with demyelinating disease and is dependent on the site of sampling and the size of the patient.

**Significant elevations in alkaline phosphatase (ALP)** without obvious concurrent disease are often encountered in Scotties and previously were designated benign hyperalkaline phosphatasemia. This study showed that such increases in ALP are more likely to be associated with atypical hyperadrenocorticism involving adrenal steroids other than cortisol, than benign hyperalkaline phosphatasemia.

**Co-infection with five vector-born pathogens**, *Anaplasma marginale*, *Anaplasma phagocytophilum*, *Babesia bigemina*, *Theileria* species and a haemotropic mycoplasma species were responsible for a disastrous fatal outbreak of anaemia in cattle in Switzerland. This paper brought the concepts of co-infection outlined by Dr Breitschwerdt closer to home. We are almost certainly seeing but more likely to be associated with atypial hyperadrenocorticism involving adrenal steroids other than cortisol, than benign hyperalkaline phosphatasemia.

**Pre-treatment cancer-related anaemia occurred in dogs** with osteosarcoma and lymphoma. In osteosarcoma this did not affect the remission or survival times but in cases of lymphoma, pre-treatment anaemia (particularly in Stages III and IV with HCTs <35%) decreased median survival and remission times. Greater understanding of the mechanism of cancer-related anaemia is needed to improve the prognosis of these patients.

**Cavalier King Charles Spaniels with the autosomal recessive macrothrombocytosis** and reduced platelet counts have a normal platelet mass (measured as ‘plateletcrit’) and normal bleeding times. True thrombocytopenia is not a reduction in platelet count, but a reduction in the platelet mass or ‘plateletcrit’. Currently the only good analyser of ‘plateletcrit’ is the IDEXX VetAutoRead (QBC).

**Low dose endotoxin administration** causes significant changes in the CBC. In comparison with the saline control, the dogs receiving endotoxin had transient reductions (at 3 hours) followed by increases (at 24 hours) in neutrophil, lymphocyte and monocyte counts. There were increased toxic band neutrophils at 3 hours. Platelet concentration decreased for at least 24 hours. These findings may explain the frequent presence of leucopenia in the early stages of non-specific GI disease.

**In a retrospective study of thrombocytosis in dogs** neoplasia (particularly carcinoma) was the most commonly associated disease state followed by respiratory, endocrine, cardiac, infectious, GI and immune-mediated disease. Steroid use was the most common drug association. Dogs with thrombocytosis had a 5.8% increased risk of thromboembolic disease with high mortality.

**A study of the cytological evaluation of degenerative disc material** in dogs showed that cytology alone is not an effective means of differentiating between degenerative disc material and neoplasia.

**PCR for clonality and phenotype was used on DNA extracted from archived cytology slides of lymphoma and lymph node hyperplasia.** The PCR for clonal rearrangements was both sensitive and specific for distinguishing between lymphoma and hyperplasia and the lymphoma phenotype was accurately determined using cytological specimens.

**D-dimer was retrospectively evaluated as an indicator of coagulation status in cats** and was found not to correlate significantly with any other single coagulation parameter. As yet no particular diagnostic role for D-dimer has been identified in cats whereas in dogs it has a useful role in the diagnosis of thrombosis and DIC.

**Lymphoproliferative disease in ferrets:** a study of 28 cases demonstrated that, as with other species, lymphoma is a heterogeneous group of diseases in ferrets. T-cell, B-cell and Hodgkin-like lymphomas were identified. The majority of presentations were multicentric, but intrathoracic, cutaneous, solitary nodes and GI lymphomas were also noted. The prognosis varied, some of the tumours having a relatively benign course.

**OBESITY - A NEW GLOBAL PANDEMIC**

An entire morning session was dedicated to aspects of obesity which is now a human global pandemic and an increasing problem in pets. The main drive of the session was to view adipose tissue as a highly active metabolic tissue capable of inducing wide ranging systemic metabolic effects many of which become pathogenic when there is excessive adiposity. Adipocytes secrete proinflammatory cytokines whose local and systemic actions lead to a state of systemic inflammation in the obese patient. The long-term repercussions of this include carcinogenesis, insulin resistance/diabetes and cardiovascular disease. Adipose tissue is an endocrine organ releasing powerful hormones including leptin, adiponectin and angiotensinogen. Leptin is pro-inflammatory, pro-oxidant and prothrombotic. Angiotensinogen contributes to hypertension and cardiovascular disease. In addition to the effects of increased adipose tissue, the deposition of fat in non-adipose tissues in obese patients induces lipotoxic effects in hepatocytes, skeletal myocytes, cardiac myocytes and pancreatic epithelium.

**CANCER AND INFLAMMATION - THE STEM CELL HYPOTHESIS**

These meetings often yield one or two defining lectures with real impact. Last year the introduction to a new endocrine system for the control of iron metabolism employing the hepatic hormone hepcidin provided the defining moment (UK Vet Vol 12 No 2). This was the year of the ‘stem cell’. The traditional model of oncogenesis involves dysregulated cell growth resulting from abnormalities in genes which regulate proliferation, apoptosis and invasion. The ‘stem cell’ hypothesis offers an additional concept of self-renewal. This is a cell division in which one resulting daughter cell remains undifferentiated and retains the ability to give rise to other stem cells as well as differentiating cells. Stem cells are difficult to recognise because they are poorly differentiated. They tend to be more resistant to chemotherapy than more mature cells of the same population. They are likely to explain our failure to produce therapies which completely eradicate solid tumours. The more mature cells are debulked by the agent but the stem cells remain with their potential to regenerate the tumour.

Normal tissues contain stem cells and therein lies one of the potential links between inflammation and cancer. Inflammatory
processes such as ulcerative colitis result in an increased rate of stem cell replication due to the need to regenerate tissue in the face of destructive inflammation. The increased stem cell regenerative activity increases the likelihood of tumorigenesis.

The stem cell hypothesis is quite easy to understand but occasionally in science a bold investigator asks an improbable question and when the improbable becomes first probable and then true, a great leap forward is made. This happened when Emina Huang who gave this excellent lecture took stem cells from a biopsy of non-neoplastic human ulcerative colitis and implanted them in an immunocompromised mouse. A colonic carcinoma developed in the mouse. This result suggests that untransformed stem cells present in normal tissues will develop into tumours given the right conditions for growth.

I would like to acknowledge my colleagues at TDDS: Oliver Coldrick, Cora Sommerey, Lindis Fouracre and Maria Pennilla.