Vetnostics

Partners in Practice

Autumn 2015

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- ACTH stimulation and LDDST protocols
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- What's Your Diagnosis?
- Why are pathology request histories important?

Vetnostics

Welcome...

We hope everyone managed to remain safe during the recent extreme weather and that life and business has returned to normal. We tried to maintain our level of service over this difficulty period but apologise if there were any delays.

This edition of the newsletter includes important information relating to zoonotic diseases, ocular pathology rounds, discussion of the importance of clinical histories, ACTH stimulation/LDDST protocols and 'What's Your Diagnosis'.

As always, please contact Dr Doug Hayward (tel. 02 9005 7272 or email doug.hayward@vetnostics.com.au) if you have any requests for future newsletters, questions or any other queries.

Zoonotic Disease - Important Information

Given the zoonotic potential of certain viruses and bacteria and the associated Health and Safety issues/concerns in routine diagnostic laboratories, there are certain situations where Vetnostics cannot undertake preliminary testing.

In this regard, samples from dogs at high risk of Brucella exposure (dogs in contact with wild pigs or fed pig meat/offal, with consistent clinical signs) should not be submitted to Vetnostics. Samples for culture and serology from such cases should be sent directly to NSW DPI EMAI laboratory. Other samples from such high risk dogs will only be accepted by Vetnostics after the consultation with a Vetnostics Veterinary Pathologist prior to submission of samples.

Hendra virus additionally continues to be a differential diagnosis for certain veterinary cases presenting in areas of NSW and Queensland. It is requested that Hendra virus is excluded (specific testing offered at EMAI and Queensland Government labs) where it is considered a clinical differential prior to submission of samples for further testing at Vetnostics.

Moving forward, you will also note an amendment to our Vetnostics clinical pathology and histopathology submission forms - there is a clearly marked area to indicate whether there is any possibility of exposure to zoonotic infections. Please remember to fill in this area of the submission form as well.

Many thanks for your assistance in these situations. Please feel free to contact a Vetnostics Veterinary Pathologist if you have any queries.

ACTH stimulation and LDDST protocols

A common query we receive is the protocols for stimulation testing for diagnosis of hyperadrenocortisim. Below you will find two ACTH stimulation testing protocols (one for use with the aqueous Synacthen solution and the other for use with the depot formulation) as well as the recommended protocol for low dose dexamethasone suppression test.

Standard ACTH (aqueous solution) stimulation protocol

1. Take a 0h blood into a serum tube
2. Inject 5µg/kg Synacthen® IV
3. Take another blood sample 1h later into a serum tube
4. Label sample times clearly on the tubes
5. Tick ACTH stim (code VAS) l on submission form
6. If only submitting the 1h sample, tick cortisol (code COV) but indicate in history that an ACTH stim test performed

Symbion Vetnostics' post-stimulation reference range and thus interpretation is based on this protocol

Depot ACTH (Synacthen® Depot) stimulation protocol

1. Collect a basal (0 hour) blood sample into a serum tube
2. Inject 250ug of Synacthen® Depot IM
3. Collect a further blood sample into a serum tube 60 mins later
4. Label sample times clearly on the tube
5. Clearly indicate on the submission form that Depot Synacthen formulation was utilised as well as indicating dosage and blood sampling times
6. Tick ACTH stim (code VAS) as normal on submission form
7. If only submitting the 1h sample, tick cortisol (code COV) but indicate in history that an ACTH stim test performed

Low Dose Dexamethasone Suppression Test protocol

1. Collect a basal blood (serum) sample
2. Inject 0.01 mg/kg i/v of Dexamethasone
3. Collect two further blood (serum) samples 4 hours and 8 hours later
4. Label sample times clearly on the tubes
5. Tick Dexamethasone suppression test (VDX) on the submission form

What is your diagnosis?

(Answer on back page)

The image here (Figure 1) is of an EDTA blood film from an eighteen month old staffordshire bull terrier cross that presented with a history of seizures. No metabolic cause for the seizures was evident on routine biochemistry and the dog was not anaemic. In the image below (Figure 1), what erythrocyte abnormalities are most prominent and what are the differentials based on the clinical history?

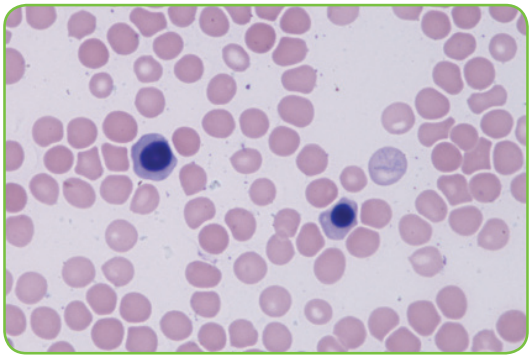

Figure 1: Wright-stained blood film (x100 oil) >

Ocular Pathology Rounds

Feline Diffuse Iris Melanoma—a clinical and pathological comparison of cases with early and late (extensive) presentation

Dr Karen Dunn BVSc (Hons) **Consultant Veterinary Ocular Pathologist**

Feline Diffuse Iris Melanoma (FDIM) is the most common primary ocular tumour diagnosed in cats, and usually arises from progression of pigmented iris freckles or naevi over a variable, but usually lengthy period—most cats are middle-aged or older at the time of diagnosis.

Cases of FDIM may present with a history of irregular iris pigmentation or hyperpigmentation which has been observed by the owner, and may have progressed over a variable period, in some cases over several years. There is often some distortion of the iris surface associated with bulging of pigmented tissue, and there may be pigment deposition on the anterior lens capsule. Figure 1 (courtesy Dr Ida Gilbert, UK) shows a typical case in a 13 year old DSH with extensive iridal pigmentation involving almost the circumference of the iris, but frequently sparing the iridocorneal angle. There is no glaucoma at this stage. Histologically, early cases of FDIM show partial to full thickness involvement of the iris, without (or with minimal) involvement of the iridocorneal (drainage) angle, and lack neoplastic infiltration of the ciliary body and sclera (Figure 2: H&E, 10x).

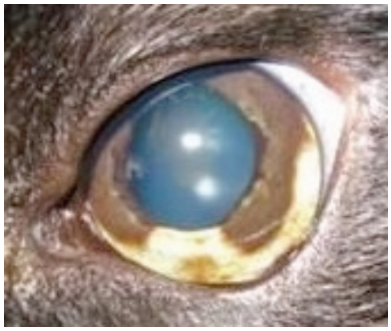

Figure 1: *Focally extensive iridal pigmentation, involving almost the circumference of the iris, but frequently sparing the iridocorneal angle. DSH, 13y, FN. Clinical image courtesy Dr Ida Gilbert, BVSc CertVOphthal MRCVS.*

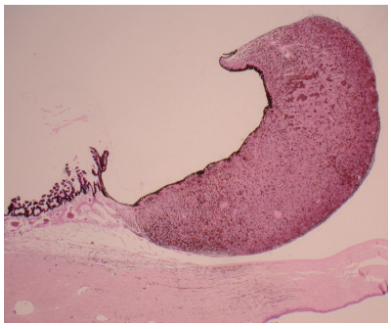

Figure 2: *Full thickness infiltration of the iris leaflet by pigmented neoplastic melanocytes—note that the iridocorneal angle is open and unobstructed in this section from a relatively early case of FDIM. British Shorthair, 5y, MN, H&E stain, Magnification 10x.*

Advanced cases of FDIM will often present with gross thickening and distortion of the iris, often with a 'velvety' texture to the iris surface, and an irregular pupil or dyscoria. Figure 3 (courtesy Dr Georgie Fricker, UK) , demonstrates a typical case of advanced FDIM in a 9year old Persian cat. Cats with advanced FDIM will usually have elevated intraocular pressure or glaucoma, and may show signs of ocular pain, although cats with glaucoma do not always exhibit obvious signs of discomfort. Advanced cases show full thickness involvement of the iris stroma with associated iridal distortion, and there is involvement of the ciliary body and the iridocorneal angle—obstruction of the iridocorneal angle by tumour tissue is responsible for the development of glaucoma. Figure 4 (H&E, 12x) is from a 13year old Australian Mist cat, displaying marked scleral localisation of tumour tissue, with intravascular aggregates of pigmented cells consistent with neoplastic cells noted.

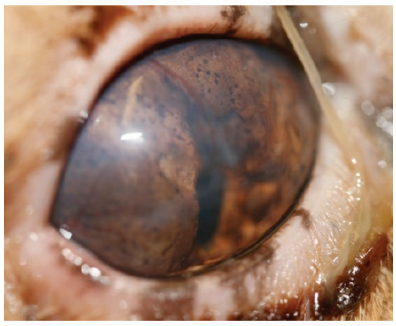

Figure 3: *Clinically advanced case of FDIM with dramatically thickened iris leaflets expanding into the anterior chamber, and with an irregular pupillary aperture or dyscoria. Persian, 9y, MN, clinical image courtesy Dr Georgie Fricker BVSc CertVOphthal MRCVS, UK.*

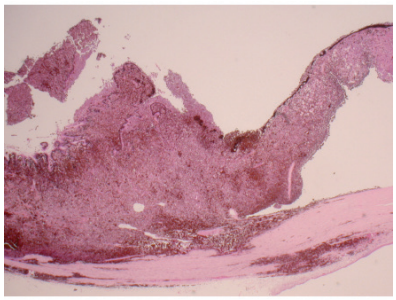

Figure 4: *Low power view shows dense neoplastic infiltration of the iris leaflets, ciliary body and the iridocorneal angle by neoplastic melanocytes in an Australian Mist cat (13y, FN). Note the disruption of the anterior border layer of the iris, and the significant scleral localisation of tumour tissue. H&E stain, 12x Magnification.*

Prognosis in FDIM cannot be reliably correlated with the degree of pigmentation, pleomorphism, or mitotic activity. However, clinically and histologically advanced cases of FDIM that involve the iridocorneal angle and ciliary body may be associated with a poorer prognosis (measured by reduced survival time following enucleation). Cases with tumoural extension beyond the iris to involve the ciliary body and the iridocorneal angle are often referred to as 'extensive' FDIM by pathologists. Extensive FDIM is more frequently associated with clinical glaucoma, and with scleral localisation of tumour tissue on histopathology. Scleral localisation can be passive, associated with, amongst other things, elevated intraocular pressure, and in rare cases may result in orbital implantation with recurrence after enucleation. Where there is intravascular localisation, as seen in the case of the Australian Mist cat with extensive FDIM illustrated here, distant metastasis to abdominal organs or lungs is possible, and the prognosis should be somewhat guarded. The overall rate of confirmed metastatic disease in FDIM is low, however under-reporting of metastatic complications is very likely, as metastases may not be evident on scans at the time of enucleation, and may take some months or even years to develop. Euthanasia of such cases at a later date without post-mortem (and therefore without further investigation or definitive diagnosis of the cause of terminal decline in an elderly cat) is likely to be common, and the incidence of metastatic disease may be much higher than that currently reported.

For further information on this ocular pathology service, please contact Vetnostics, or visit **www.FocusEyePathLab.com.**

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Why are pathology request histories important?

Dr David Taylor – Veterinary Pathologist

A recent commentary in JAVMA (Vol. 244, No. 4, February 2014) sought to highlight the relevance and importance of supplying a concise history when completing pathology request forms.

The obvious goal is clear communication. Without a pertinent history and clinical context, our interpretation of diagnostic test results can be incomplete and at worse, misleading. This is not a unique issue with many pathologists and clinical pathologists agreeing that perhaps 50-80% of sample interpretation is context driven.

The commentary put forward several practical suggestions to assist in capturing critical information. These include documenting all patient information, including owner and animal name, species, age, sex, breed and weight. This is relevant because different species develop different tumours and diseases and some disease processes are more common in certain breeds and age groups.

Submission pots should be labelled with pet and owner names, and any sample identification that relates to the request. Handwriting should be legible. Because Vetnostics operates from photocopies and scans, poor handwriting is often not legible in these situations. While adding highlighting to the request may seem to be a good way to emphasize important information, it can often appear as black banding that obscures the text when the request is scanned/photocopied. Use of abbreviations should be restricted to those that are known to be widely understood. If a vet delegates the responsibility for completing request forms to technicians and nurses, they should provide training in proper methods for completing these forms and then review what was written. Ultimately the vet is responsible for ensuring that the information is complete and correct, and a marginal history on a document of record may not reflect well on the competence of the vet.

A concise and pertinent history can be developed by answering 6 simple questions, that a vet familiar with the case should be able to complete in 2-3 minutes.

What is the primary reason for evaluation? A concise statement of the primary reason the animal was brought to you provides overall context of the problem. It is not sufficient to note only the tissue source or location where the sample was collected.

What is the duration and frequency of the problem? This helps to establish the clinical importance of the problem. Avoid use of terms such as acute and chronic as they are open to interpretation and lack specific meaning. A statement of "vomiting for 2 months, 3-4 times a day, usually after eating" provides more meaningful information than "chronic vomiting".

What are the objective clinical findings? These are clinical signs that are relevant and that can be observed, described, measured or quantified. Terms such as small, large etc are open to interpretation and should be replaced by objective measurements. This is particularly useful when submitting small samples from large lesions. An example would be cytologic evaluation of a lymph node. Predominately small lymphocytes in a FNA are more likely to be considered neoplastic if taken from a 3cm diameter lymph node in a Cavalier, and benign if from a 3cm lymph node from a Great Dane.

Actual values for relevant tests and changes over time should be provided. An ALT value of 1000 may be more important than a value of 150, and both are more important than the term "elevated liver enzymes".

What are the differential diagnoses? Pathologists often find it helpful if they can understand what you consider the underlying problem to be. It may surprise you to learn that some vets feel the need to conceal this information for fear that it will bias the result. Creating a list of differential diagnoses is foundational to the diagnostic process. A differential diagnosis list allows us to rule these considerations in or out.

What specifically was sampled? In many cases this will be obvious. However for small samples and cytologic samples this isn't always true. A common problem occurs with request forms that have marks made on the picture of the animal. A mark on the abdomen could mean a lesion in the skin, subcutis, muscle, mammary gland, peritoneal cavity, intestine, liver, spleen, kidney, ovary, uterus, adrenal gland, pancreas and lymph nodes.

What is the appearance of the tissue or lesion? This includes objective size, colour, consistency and texture, as well as whether the lesion is discrete or ill-defined, fixed or mobile. Results of imaging studies are also very useful, especially when evaluating bone biopsy samples. These details provide clues to the behaviour of the lesion.

Next Newsletter... Sample considerations and expectations.

If you would like a copy of the JAVMA commentary please email me at david.taylor@vetnostics.com.au

What is your diagnosis answer:

The most significant finding is the metarubricytosis (nRBC 44/100 WBC in this case) despite no anaemia and presence of basophilic stippling - see figures 2 and 3. This animal had a blood lead level of 5.81 umol/L (Reference range < 1.71 umol/L).

Figure 4 is an image of an abdominal radiograph which revealed a radiopaque foreign body. A lead fishing sinker (Figure 5) was removed at surgery.

Lead levels dropped over the following weeks post gastrotomy without requirement for chelation therapy and with associated improvement in clinical signs.

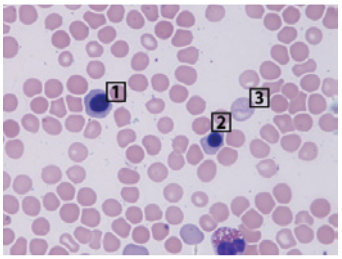

Figure 2: *Wright's stained blood film (x100 oil). Markers 1 and 2 indicate metarubricytes and marker 3 indicates basophilic stippling.*

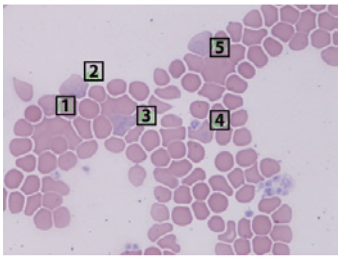

Figure 3: *Wright's stained blood film (x100 oil) Markers 1-5 indicate basophilic stippling, particularly easy to visualise in the tail of the blood film in this case.*

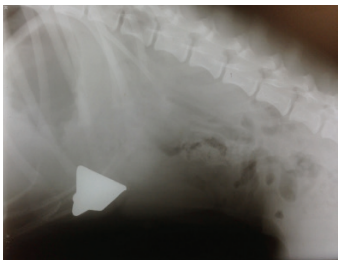

Figure 4: *Abdominal radiograph showing radiopaque foreign body.*

Figure 5: *Lead fishing sinker post gastrotomy.*