

NWL NEWS

The Newsletter of NationWide Laboratories

May 2007

Welcome to the NationWide Laboratories quarterly newsletter.

To follow on from our previous newsletters which presented an overview of hyperadrenocorticism and its diagnosis in the dog, we now turn to Cushing's in the cat.

Refresher...

Hyperadrenocorticism (HAC) in the cat

Overview:

Adrenal diseases are uncommon in the cat in comparison to the dog. HAC is most common but has been reported in <100 cats. More than 80% are due to a pituitary adenoma (PD) (rarely adenocarcinoma) and the remaining due to adrenocortical neoplasm (AD) (equal benign and malignant forms). There is no breed or sex predisposition and most are older with a mean age of 10 (4.5 – 15). Cats show most of the clinical signs reported in the dog as well as extreme fragility of the skin which appears to be relatively common. Most (80%) cats with confirmed HAC have concurrent Diabetes Mellitus. Haematology shows a stress leukogram inconsistently and the most frequent biochemical abnormalities are hyperglycemia (about 80%) and hypercholesterolemia (about 50%). Increased alkaline phosphatase is uncommon (32% cases) in comparison to the dog where this is a frequent finding. Urine specific gravity is usually maintained >1.020.

Diagnosis:

Diagnostic imaging including radiography and ultrasonography may be helpful in investigating unilateral (adrenal tumour) or bilateral enlargement (usually PDHAC) of the adrenals. MRI may be useful for investigating a pituitary mass.

Endocrine tests:

Due to the low numbers of confirmed positive HAC in the cat reported to date, all tests below have unknown sensitivity and specificity values. The usefulness of each test is therefore yet to be determined.

ACTH stimulation test:

This is the most useful test for diagnosing HAC in cats. The sensitivity of this test varies from 50-80%. It can give a positive result in cats with non adrenal illness. There are different protocols advocated as to the timing and number of samples. Generally a protocol similar to that used in the dog can be used with a baseline and post sample one hour after injection of

ACTH. Some protocols advocate a 30, 90 or 120 minutes post sample in addition to the 60 minute sample. The protocol used at NWL involves injecting 0.125 mg of synthetic ACTH (Synacthen) i/v (0.25 mg may be used with cats >5 kg). Samples are taken prior to injection and one hour post injection. Generally samples >600 as with the canine test is suggestive of HAC.

ACTH stimulation test	Normal	HAC
Baseline cortisol nmol/l	20 – 270	20 – ?
Cortisol 60 minutes post ACTH	Increase but <400	>400 equivocal >600 consistent with HAC

Low Dose (0.01 mg/kg) Dexamethasone Suppression test (LDDST):

This test is not considered useful in the cat due to high percentage of false positives obtained.

High Dose (0.1 mg/kg) Dexamethasone Suppression test (HDDST):

This is the dosage used in the dog for differentiation of PD and AD HAC. In the cat it is used like the LDDST and the use of this higher dosage this is a useful screening test with a reported sensitivity of 78%. Serum cortisol values in all normal cats and most cats with non-adrenal illness are said to be suppressed with this dose, whilst all cats with AD-HAC and most cats with PD-HAC fail to suppress cortisol at either three or eight hours. However further cases are required to be evaluated to more accurately assess sensitivity and specificity. In the cat this test can not be used for differentiating between PD and AD HAC.

The advised protocol is to take a baseline sample for cortisol and then inject IV, 0.1 mg/kg dexamethasone. Further samples are taken at three or four hours and eight hours.

HDDST	Normal	HAC
Baseline cortisol nmol/l	20 – 270	20 – ?
3 or 4 hour cortisol nmol/l	<40	<40
8 hour cortisol nmol/l	<40	<40

Combined dexamethasone suppression/ACTH stimulation test:

This test has not been sufficiently evaluated. The protocol used by NWL is as follows: Collect basal blood sample for cortisol. Inject 0.1 mg/kg dexamethasone IV. Collect a second sample at two hours. Immediately inject 0.125 mg synthetic ACTH (Synacthen) IV. Collect a third sample at three hours (one hour post ACTH). Measure cortisol on all samples.

Combined HDDST/ACTH	Normal	HAC
Baseline cortisol nmol/l	20 – 270	20 – ?
2 hours cortisol nmol/l	<40	<40
3 hours cortisol nmol/l (1 hour post ACTH)	<400	>400 equivocal >600 consistent with HAC

Higher Dose (1.0 mg/kg) Dexamethasone Suppression test (HiDDST):

This test has been used to differentiate PD and AD HAC cases. However the reliability of this test has not been assessed.

Endogenous ACTH:

As with the HiDDST, this has been used to differentiate PD and AD HAC. Again the reliability is not currently known but normal to high levels of endogenous ACTH support PD HAC whereas low levels are consistent with AD HAC.

Urinary corticoid : creatinine ratio:

This is not widely used in cats but is likely to be sensitive but have a poor specificity. A similar protocol to that used in the dog has been described with urine collection at home. A value of <10 x 10⁶ probably rules out HAC. Almost all cats with HAC will be positive but false positives are frequently seen with nonadrenal illness. Hyperthyroid cats may also have increased ratios.

Infectious Diseases...

Part I – Leishmaniasis

Leishmaniasis is an infectious disease of people and wild and domestic animals caused by protozoans of the genus *Leishmania*. In recent years, a significant increase in the number of infected dogs has been registered in the southern European countries where human cases are principally found in children and immunocompromised adults, who become secondarily infected. Infected dogs serve as the reservoirs of the disease for people in many countries where leishmaniasis is endemic. Interest in the disease became important in non endemic areas because of the ever-increasing traffic of tourists and immigrants accompanied by their pets.

Leishmania are transmitted among canine and to humans by blood-sucking sand flies of the genus *Phlebotomus*. In the vertebrate host, *Leishmania* undergo phagocytosis by the macrophages/monocytes in which they transform to and multiply as amastigotes, rupture out of these cells, and infect new cells. Repetition of this process leads to the dissemination of amastigotes and their presence in several tissues: skin, spleen, bone marrow and lymph nodes.

It is a chronic condition, and severely affected animals not always survive even if they receive treatment. Although the dog seems to be much more susceptible than man, more than half of the infected animals remain asymptomatic. Dogs easily carry this chronic insidious disease unnoticed.

Interleukin-2, tumour necrosis factor, interferon- γ , CD8+ and/or CD4+ cytotoxic T cells are needed for protective immunity to leishmaniasis in dogs. These phenomena are lacking in non-resistant dogs. T-lymphocyte regions in the lymphoid organs become depleted, and antibody-producing B lymphocytes, plasma cells, histiocytes, and macrophages proliferate resulting in generalised lymphadenopathy, sometimes hepatosplenomegaly, and consistent hyperglobulinaemia. The immunoglobulin response is non protective and detrimental: production of autoantibodies gives rise to

immune-mediated thrombocytopenia and anaemia; deposition of circulating immune complexes in the walls of blood vessels causes vasculitis, polyarthritis, uveitis and glomerulonephritis. Dogs may also show generalised nodular skin lesions and alopecia and increased bleeding tendency. The **clinical findings** are a consequence of these pathologic processes: **weight loss, diarrhoea, vomiting, polydipsia, anorexia, rhinitis, sneezing, epistaxis, melaena, lymphadenopathy, skin involvement, abnormal nails, cachexia, abnormal locomotion and conjunctivitis.**

The **laboratory diagnosis** is based on finding a set of abnormal results: **hyperglobulinaemia, hypoalbuminaemia, proteinuria, anaemia (frequently Coombs' positive), thrombocytopenia, high ALT and ALP activities, azotaemia, leukopenia, demonstration of amastigote forms in lymph node or bone marrow aspirates, positive antibody titre and PCR.** Serologic testing may verify the presence of antibodies, but do not prove or disprove active disease. PCR may be negative in diseased dogs.

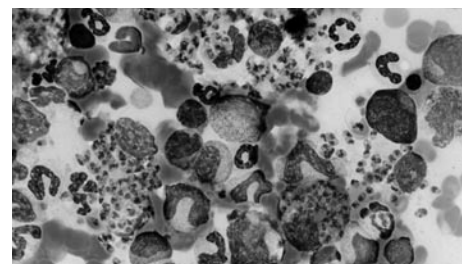
Leishmaniasis is more resistant to therapy in dogs than in people, and *Leishmania* organisms are rarely completely eliminated. Relapses necessitating retreatment are the rule. Treatment with the following drugs produced clinical improvement: pentavalent antimonials, allopurinol, combinations of these two, amphotericin B, amanosidine and pentamidine,

Decreasing antibody titres are usually used to monitor therapy, but results are not consistent and relapses occur frequently despite a negative test result.

The prognosis mainly depends on renal function at the start of treatment. In dogs with serious renal insufficiency, the prognosis is very poor.

Leishmania on cytology:

Ovoid or round, non-flagellate forms measuring 2.5-5.0 um long X 1.5-2.0 um wide.



Therapeutic monitoring: Potassium Bromide KBr

Currently the majority of reports are with reference to canine therapy. However its use in the cat is now receiving more attention.

KBr in the DOG:

This is the recommended add-on antiepileptic drug to phenobarbitone. Its addition may allow better or complete control of canine refractory idiopathic epilepsy which is insufficiently controlled by phenobarbitone (PB) alone. It may also reduce the incidence of hepatotoxicity by allowing a reduction in PB dosage. NaBr can be used in cases with adrenal insufficiency (Addison's) or renal disease.

Therapeutic range of KBr in dogs with concurrent PB treatment is 1.0 – 2.0 mg/ml (100-200 mg/dL). Reports advise to aim at achieving steady-state trough serum concentrations of 25 ug/ml PB and 1.5 – 2.0 mg/ml KBr.

KBr in the CAT:

Also recommended as a first choice add-on drug to PB. Similar steady-state serum levels of PB and Kbr as stated above in the dog are recommended.

Species	Mean T _{1/2}	Time to achieve steady state concentrations (days)
Dog PB	24 – 92 hours	14 – 21
Dog KBr	25 – 46 days	83 – 120
Cat PB	43 hours	14
Cat KBr	11 – 21 days	56 – 105

Answers to Case Question 2...

CLINICAL DETAILS:

Case 2 - eight year old male neutered Yorkshire Terrier presents with polydipsia, polyuria, thinning of the hoar coat, pot bellied appearance and lethargy.

Diagnostic tests:

- **Haemogram** – *Neutrophilia, lymphopenia, monocytosis*
- **Biochemistry** – *Increased AP, ALT, cholesterol, glucose*
- **Urinalysis** – *USG 1.013, +protein, +blood, WBCs 0-5, RBCs 0-5*

Differentials:

HAC was top of the list but other differentials included D. Mellitus, primary hepatopathy.

Further Diagnostic tests:

Low dose dexamethasone suppression test – results:

Endocrinology	Result	Reference interval
Cortisol basal (nmol/l)	123	28-250
Cortisol three hour post dex. (nmol/l)	40	<40
Cortisol eight hour post dex. (nmol/l)	89	<40

QUESTIONS:

How do you interpret these results?
Is this the test you would have chosen if HAC was suspected?
Are further test required?

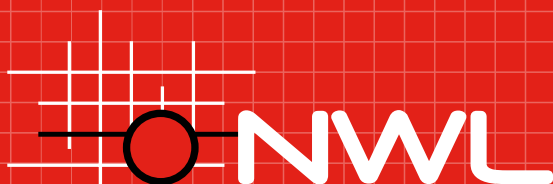
Answer:

These results are consistent with pituitary dependent hyperadrenocorticism given the appropriate clinical presentation. We interpret the results as follows: the eight hour result is greater than 40nmol/L, which can indicate the presence of hyperadrenocorticism. Additionally, there is greater than 50% suppression from baseline, which we would not expect from an autonomous functional adrenal tumour so the origin of the hyperadrenocorticism has to be pituitary.

Care should be taken in interpreting Low-dose dexamethasone suppression test results because false positives are common in situation of stress and non-adrenal illness. A diagnostic judgement in favour of hyperadrenocorticism should always take consideration of the endocrinology results in combination with the clinical findings and history of any other illness.

Either the Low-dose dexamethasone suppression test or ACTH stimulation tests would be appropriate in these circumstances. The advantage of the ACTH stimulation is that it is shorter in duration and its positive tests results are more believable. Unfortunately, we can't always believe negative ACTH stimulation test results. The low-dose-dexamethasone test gives more believable negative results but we can't always trust the positives.

Because we already have >50% suppression, we can be confident that we have pituitary based disease and no further differentiating tests (e.g. high-dose dexamethasone suppression test, endogenous ACTH) are required. To initiate Vetoryl® therapy it is recommended that a pre-treatment ACTH stimulation test be performed to serve as a baseline for therapeutic monitoring.



NationWide Laboratories

Lancefield House,
23 Mains Lane, Poulton le Fylde,
Lancashire FY6 7LJ
T: 01253 899215 F: 01253 891934
Email: nwlabs@nwlabs.co.uk
www.nwlabs.co.uk

Editor – Stacey Newton
BVSoc CertEM (Int Med) PhD MRCVS

Coming up next issue...

- **Infectious Disease updates part 2**
- **Laboratory Service updates**
- **Recent tests**
- **Case Report**