

NWL NEWS

The Newsletter of NationWide Laboratories

October 2007

Welcome to the NationWide Laboratories quarterly newsletter.

The previous issue presented the second part in the Infectious Disease Article series. There was also an update on our current Antibiotic susceptibility testing in the Microbiology Department. This is now up and running and you will have noticed an alteration in the presentation of the microbiology reports. We now have part three of the Infectious Disease Article Series, updates from the Endocrinology and Histology departments and a case report refresher.

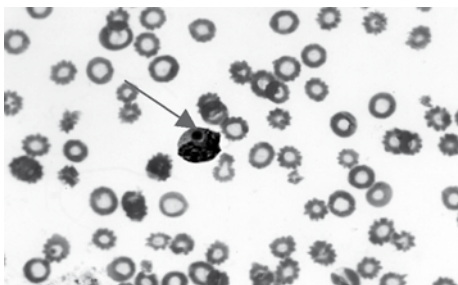
Infectious Diseases...

Part 3 – Ehrlichiosis

Ehrlichia are Gram-negative obligate intracellular bacteria. For many years, *E. canis* was the only species to cause disease in dogs. Additional Ehrlichia species that infect dogs and other hosts are now recognised. They recently underwent reclassification based on genetic analysis resulting in some species of the genus Ehrlichia being moved to a different family (Anaplasmataceae) and genus (Anaplasma) explaining the change in name of some of these agents. Genetic analysis produced three geno/serogroups, with the members of any geno/serogroup commonly producing cross-reactive antibody titres. **These organisms infect blood cells** and are characterised as being monocytotropic (residing in monocytes and macrophages), granulocytotropic (neutrophils and eosinophils) or thrombocytotropic (inside platelets).

Ehrlichiosis is a bacterial disease transmitted through the bite of infected ticks. Ehrlichia may use one or several vectors. The same tick vector may harbour different species of Ehrlichia. In addition, the same tick may serve as vector for several genera and species of infectious agents: Ehrlichia, Babesia canis, Babesia gibsoni, Borrelia burgdorferi and Bartonella spp., resulting in co-infections. Other known vectors of Ehrlichia are snails and flukes.

Canine Ehrlichiosis is a multisystemic disorder. The course of clinical infection results in a variety of acute and chronic syndromes of varying length in time, but can also be subclinical or fatal. The classical presentation is characterised by depression, lethargy, mild weight loss, and anorexia, with or without haemorrhagic tendencies (due to thrombocytopenia and thrombopathy). If present, bleeding usually is manifested by dermal petechiae, ecchymoses, or both. Epistaxis is common. In addition, uveitis, polymyositis, polyarthritis, glomerulonephritis, and central nervous system signs (seizures, ataxia, vestibular deficits, and cerebellar dysfunction). **Co-infection with other tick-borne diseases** can make it difficult to attribute clinical signs to a single specific agent.



Clinical Pathology:

The most consistent haemogram abnormalities are **thrombocytopenia** and **mild non-regenerative anaemia**. However, infected dogs may have normal platelet counts. **Pancytopenia** may be seen in the **chronic phase** of the disease, the result of hypoplasia of all bone marrow precursor cells. Granular lymphocytosis, which may be confused with well-differentiated lymphocytic leukaemia, also has been reported. **Polyclonal gammopathy** is most common, but monoclonal gammopathies have been reported.

Diagnosis:

- **Serology** – based on the detection of serum antibodies by indirect immunofluorescence (IFA). This test detects antibodies as early as seven days post-infection, but some dogs may not become seropositive until 28 days post-infection. Clinical signs can occur before the development of serum antibodies. There is variable cross-reactivity among several species of Ehrlichia. Because of latent infections, a positive antibody titre does not necessarily mean that the clinical manifestations are due to Ehrlichiosis at the time of presentation. This is especially true in endemic areas where many healthy dogs have positive titres. Some dogs may spontaneously resolve infection but remain seropositive.
- **PCR** – is a sensitive method for diagnosing acute Ehrlichiosis. There are limitations to this test: it is expensive, and both false-positive and false-negative results occur. It may yield positive results within 4-10 days of exposure.

In untreated animals, positive PCR confirms infection by an Ehrlichial species, whereas positive serologic tests only confirm exposure.

It is unknown whether blood, bone marrow and splenic aspirates are optimal for testing. Joint fluid, CSF, and aqueous humour may also be tested.

PCR should be used in conjunction with serology, not instead of it, for the initial diagnosis of Ehrlichiosis in untreated patients.

Treatment:

- **Drugs** effective in canine Ehrlichiosis include doxycycline, (oxy)tetracycline, chloramphenicol, and imidocarb dipropionate. Enrofloxacin is ineffective against Ehrlichia.
- **Supportive treatments** may be needed, e.g. steroids, administration of parenteral crystalloids or colloids or blood transfusions.

Monitoring Response:

Resolution of thrombocytopenia is indicative of good response to therapy. They begin to increase 24-48 hours and are usually normal within 14 days. Gradual resolution of hyperglobulinaemia over six to nine months also suggests therapeutic elimination of the Ehrlichia. After successful treatment, antibody titres decline and generally become negative within six to nine months.

PCR proves useful in distinguishing successfully treated animals with persistently high antibody titres from unsuccessfully treated animals with persistent Ehrlichia infection.

PCR should be repeated after antimicrobial therapy has been discontinued for two weeks. It is difficult to determine if dogs can be cleared of the infection. Dogs may be re-infected as the antibodies are non-protective.

If a dog does not respond to treatment for Ehrlichiosis in the anticipated time frame, then another cause of the clinical signs should be considered (e.g. co-infection; testing for other tick-borne agents is warranted).

- **Prevention** of infection in endemic areas can be accomplished by maintaining strict tick control programs.
- No vaccine is currently available for the prevention of canine Ehrlichiosis.

Zoonosis:

Some Ehrlichial species that infect dogs may also infect humans through tick bites.

Laboratory Service updates...

Free T4 by equilibrium dialysis (FT4D): its use in the diagnosis of hypothyroidism in the dog:

Almost all (>99.9%) of circulating T4 is bound to carrier proteins leaving only a tiny fraction available to interact with tissues. This free fraction can be measured in an ultra sensitive radioimmunoassay following an equilibrium dialysis step. The analysis of FT4D is the most accurate way of assessing the physiologically important thyroid status of an animal. Samples are dialysed, separating FT4 from serum proteins and protein bound T4. In most cases, TT4 and FT4D will be highly correlated, but this is not always so and in the following circumstances it is advisable to measure FT4D instead of, or in addition to, TT4:

Non-thyroidal illness:

One of the contributing mechanisms to the low TT4 we see in non-thyroidal illness is an alteration in thyroid hormone-protein binding. Although TT4 concentrations may be greatly reduced, the lower protein affinity for T4 means a higher fraction is available as free hormone and the FT4D concentration usually remains within the reference range. This makes FT4D a good test for distinguishing non-thyroidal illness from true hypothyroidism as the cause of a low TT4. Less than 10% of dogs with non-thyroidal illness have a FT4D below the reference interval.

Concurrent therapies:

Part of the effect of certain therapies on TT4 is mediated through thyroid hormone-protein binding meaning that FT4D is less commonly and less dramatically affected by concurrent therapy. FT4D is the analysis of choice when glucocorticoid or barbiturate therapies cannot be withdrawn prior to embarking on a thyroid diagnostic investigation.

Free T4 by equilibrium dialysis in the diagnosis of Hyperthyroidism in the cat:

This is the most accurate way to measure free T4 and is preferable to older analog methods, which have little, if any, diagnostic advantage over total T4. FT4D is generally less affected by non-thyroidal illness and altered protein levels.

Occasionally cats with severe non-thyroidal disease will have elevated FT4D concentrations, therefore results must always be interpreted along with Total T4. In cases where the results of basal thyroxine estimation are equivocal it is possible to perform dynamic tests to evaluate thyroid function.

The ability to accurately measure free T4 (FT4D) has, to a large extent, removed the need to rely on dynamic testing.

Histology Samples:

Fixed tissue is retained for about four weeks. If you have any queries about histology reports please phone and discuss the case with the pathologists. If necessary we will go back to the fixed tissue and take more blocks (no extra charge). Slides and tissue embedded in paraffin wax are retained for several years.

Most small lesions and punch biopsies will fit into the routine histology pots. To fix adequately there should always be an excess of formalin in the pot. If larger lesions are squashed into a small container they will not fix properly and will start to decompose. This may adversely affect histology.

So what can you do with larger lesions requiring histology?

There are two options:

- Take small wedges of representative parts of the large lesion and fix in routine histology pots, using more than one pot if necessary to make sure that the pieces are in adequate formalin.

This method is useful for a variety of lesions which appear well circumscribed. Even if the lesion has areas which appear different, multiple small pieces can be taken if necessary.

- Place the large lesion in a large container of formalin and fix for two to three days at least. When the lesion is fixed, wrap it in formalin soaked tissue or paper towel and seal in a leakproof plastic bag. Package this in a second leakproof plastic bag containing absorbent material and send to us. When we receive the sample we will put it back into a large quantity of formalin and will take representative blocks for histology.

This method is recommended for larger samples. For example tumours excised with large margins of apparently normal tissue. We can then take a transverse section through the centre of the lesion and two sections at 90° to this. This enables the margins to be fully assessed.

Splenic lesions in dogs can be very large and congested. These may need to be incised to help the formalin penetrate the tissue, but if we receive the whole spleen we can then take representative samples of lesions.

If the initial sections only include blood clots we can go back to the tissue and take more blocks for histology to rule out the presence of underlying lesions.

Lesions from different sites should ideally be put in different pots. Fixation can distort tissue and make some fresh lesions of variable size look similar following fixation.

Also fixation changes colour and texture, so descriptive terms such as the colour and "firmer" or "softer" can be unhelpful in identification of different lesions in the same pot when the fixed samples are received (although these terms are useful in the clinical history... see below!).

Signalment and clinical history are vitally important when submitting samples. Some tumours vary in malignant behaviour depending on species and some breeds are predisposed to certain conditions.

Also hormonal influences can be significant so it is important to note whether the animal is neutered and also when the animal was neutered.

Case Report...

CLINICAL DETAILS:

A 13 year old, male neutered Labrador presented to the Veterinary Surgeon with a history of 2.5 kg weight loss over the last two months. The dog is a diagnosed Diabetes Mellitus case and was previously stable. In house blood glucose 9.0 mmol/l.

Interpretation:

The following paragraph shows how to interpret the fructosamine result. The fructosamine is high and suggests poor current control of the Diabetes. The moderate increase in the AP and slight increase in the ALT are likely consistent with the secondary vacuolar hepatopathy that usually occurs with Diabetes Mellitus.

Serum fructosamine measures the glycation of serum proteins and is an accurate measure of the average serum glucose concentration over one

to two weeks in the dog and one to three weeks in the cat. High levels are indications of uncontrolled diabetes mellitus or poor metabolic control of diabetes mellitus.

To monitor the efficiency of insulin therapy the following ranges may be helpful:

Glycaemic Control:	Fructosamine umol/l:
Prolonged hypoglycaemia	<300 umol/L
Excellent control	350-400 umol/L
Good control	400-450 umol/L
Fair control	450-650 umol/L
Poor control	>650 umol/L

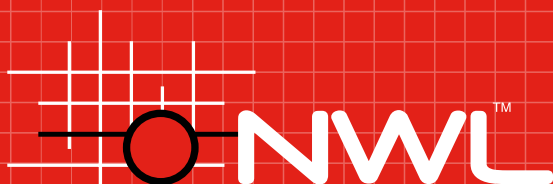
Total protein and albumin levels will affect the results.

Clinical Pathology:

Biochemistry:	Result	Reference interval
AP	599	(0-135 iu/l)
ALT	94	(0-40 iu/l)
Urea	7.1	(3.5 – 7.0 mmol/l)
Fructoseamine	727	(258-343 umol/l)

Haematology:	Within reference intervals
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Remaining Results:	Within reference intervals
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Coming up next issue...

- Infectious Diseases Part 4
- Services Update
- Case Report

Please feel free to contact the Editor if you have any queries or would like us to include articles or cases on a particular subject.