

NWL NEWS

The Newsletter of NationWide Laboratories

August 2007

Welcome to the NationWide Laboratories quarterly newsletter.

The second article in the "Infectious Diseases" section follows previous newsletters with discussion on another clinically important organism. Also included in this edition is an update on current practice and future developments in antibiotic susceptibility testing.

Infectious Diseases...

Part 2 – Babesiosis

Canine babesiosis (piroplasmosis) is an important worldwide, tickborne disease caused by haemoprotzoan parasites of the genus *Babesia*.

Babesia canis and *Babesia gibsoni* have been the two predominant species capable of natural infecting dogs. Several strains of these organisms and other species of *Babesia* exist. Natural infection occurs through the bite of infected **ixodid ticks** (*Rhipicephalus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*) which need to feed for two to three days for transmission to occur. In addition, fighting may play a role in transmission through bite wounds and intermingling of blood, through saliva, or through ingested blood. Transplacental transmission of *Babesia* is likely and may result in weak or fading puppies. After the organism enters the blood, they **infect RBCs** and multiply within them by repeated binary fission. A significant host immune response is generated, but fails to completely eliminate the infection, and animals that recover usually become chronic carriers.

Haemolytic anaemia is the hallmark of infection, but numerous variations and complications (**Multiple-Organ Dysfunction Syndrome – MODS**) may develop. Infected erythrocytes incorporate parasite antigens in the membrane and induce the production of antibodies and immune-mediated haemolytic anaemia. The presence of the parasite also results in osmotically fragile erythrocytes, haemolysis, and subsequent anaemia, haemoglobinaemia, haemoglobinuria, hyperbilirubinaemia, bilirubinuria, and jaundice. The release of endogenous pyrogens during haemolysis induces fever. Oxidative stress increases rigidity of erythrocytes which slows their passage through capillary beds and promotes vascular stasis contributing to **disseminated intravascular coagulation (DIC)**, **central nervous system disease**, **rhabdomyolysis** and **acute renal failure**. Progressive anaemia and vascular stasis contribute to inadequate tissue oxygenation and hypoxic cell damage. If irreversible, shock will induce death.

Many atypical signs or complications appear to be the result of the host inflammatory response. The resultant tissue damage causes release of cytokines which support widespread inflammation and additional damage to multiple organs. **MODS** complications resulting from the so-called systemic inflammatory response syndrome have been acute renal failure, hepatopathy, pulmonary oedema, rhabdomyolysis and cerebral dysfunction. **Thrombocytopenia** is observed in many cases of babesiosis and may be due to DIC, immune-mediated destruction or vascular injury.

Diagnosis:

A mild, normocytic, normochromic anaemia is generally noted in the first days after infection, and then becomes macrocytic, hypochromic, and regenerative as anaemia progresses. Most dogs are Coombs' positive and some may show autoagglutination of the erythrocytes in saline. Leukocyte abnormalities are inconsistent (neutrophilia, neutropenia, lymphocytosis, leukemoid reaction). Thrombocytopenia is a common finding. Serum chemistry values are usually normal. Hypokalaemia or hyperkalaemia, hypoglycaemia, low total proteins, low albumin, azotaemia, metabolic acidosis, high ALT and ALP activities and hyperbilirubinaemia may be seen. Urinalysis may reveal bilirubinuria, haemoglobinuria, proteinuria and granular casts.

Microscopic examination of stained blood smears may reveal the presence of intra erythrocytic trophozoites of Babesia (see photo). These may be piriform-shaped, amoeboid or round, single or in pairs. The detection of **anti-Babesia antibodies in serum may be done by indirect immunofluorescence or ELISA.** They demonstrate previous exposure to the parasite, so don't always correlate well with acute disease. Cross-reactivity among *Babesia* species occur. False negative results have been reported. **PCR is the most sensitive and specific means of confirming infection.**

Therapy:

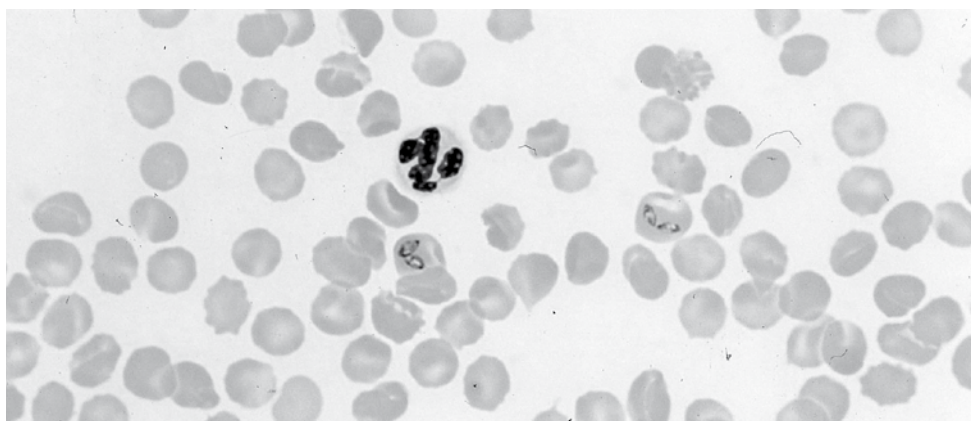
Dogs usually show clinical improvement within 24 hours of treatment with the most commonly used drugs: diminazene aceturate, phenamidine isethionate, imidocarb diprionate. Blood transfusion and glucocorticoids may also be needed.

Prevention:

The primary means of prevention is control of the vector ticks. Vaccination protocols have produced variable rates of protection.

Public Health Consideration:

Babesiosis is a significant tickborne zoonosis of people found throughout Europe. People serve as accidental hosts for *Babesia* of animals when they are bitten by infected ticks.



Developments in Microbiology...

When microorganisms are recovered from clinical samples the antibiotics that are used in susceptibility testing, and subsequently reported, are chosen following consideration of the species, site sampled and organism(s) recovered.

Every effort is made to ensure that the antibiotics reported are appropriate; however, we cannot guarantee efficacy *in-vivo*, or safety, in any particular species. Should you wish to know the suitability of a particular antibiotic please request specific testing on your laboratory submission form, this will be performed if the isolate is appropriate.

Antibiotics are tested for suitability by measuring the zone sizes around antibiotic impregnated disks which are placed onto an agar plate inoculated with the test isolate.

Susceptibility or resistance is determined by measuring the diameter of the zone of inhibition which develops around the disk. This varies according to the antibiotic/ isolate. Susceptibility results should only be determined when published zone size data is available (for example CLSI Guidelines).

When antibiotics are known to be ineffective (in-vivo) against a particular isolate they will not be included in the report, for example aminoglycosides are not effective against Streptococci, clindamycin and fusidic acid are not effective against Gram-negative organisms.

Reference guidelines are frequently updated, and we may, on your behalf, amend our antibiotic panels and methods of reporting in accordance with the most recent information. Recently it has been recognised that certain "class representatives" will reliably predict the susceptibility of other antibiotics in the same or a closely related class, therefore it is not necessary to test all class members individually.

Antibiotics whose susceptibility can be predicted based on the behaviour of their "class representatives", for various organisms, are listed below:

Gram Negative Organisms

Class representative	Predicts the susceptibility of:
Ampicillin	Amoxycillin
Cephalothin	Cephalexin, Cefuroxime
Tetracycline	Oxytetracycline, Chlortetracycline, *Doxycycline
Kanamycin	Framycetin

**Some organisms which are resistant to tetracycline may be susceptible to doxycycline; this can be tested separately when appropriate.*

Streptococci

Class representative	Predicts the susceptibility of:
Penicillin	Ampicillin, Amoxycillin, Amoxycillin/ Clavulanic acid, Cephalexin, Ceftiofur
Tetracycline	Oxytetracycline, Chlortetracycline, *Doxycycline
Clindamycin	Lincomycin

**Some organisms which are resistant to tetracycline may be susceptible to doxycycline; this can be tested separately when appropriate.*

It is worth noting that in-vitro testing of Streptococcal organisms can be unreliable and there is little published zone size data. In-vivo, however, Streptococci are considered reliably sensitive to penicillins.

Staphylococci

The susceptibility of Staphylococci to the beta-lactam antibiotics (penicillins and cephalosporins) is determined by the beta-lactamase producing capability of the organism.

This can be reliably predicted from the results of penicillin/oxacillin testing, see below:

Organism behaviour	Susceptible to:	Resistant to:
Penicillin susceptible	All Penicillins and appropriate Cephalosporins	None of the β -lactams
Oxacillin resistant	None of the β -lactams	All Penicillins and Cephalosporins
Penicillin resistant/oxacillin sensitive	Oxacillin, Cloxacillin, Amoxycillin/ clavulanic acid, Cephalexin	Penicillin, Ampicillin, Amoxycillin

Other antibiotic susceptibilities which can be predicted from class representatives for staphylococci are as follows:

Class representative	Predicts the susceptibility of:
Tetracycline	Oxytetracycline, Chlortetracycline, *Doxycycline
Clindamycin	Lincomycin

**Some organisms which are resistant to tetracycline may be susceptible to doxycycline; this can be tested separately when appropriate.*

The use of class representatives has already been introduced into human microbiology and is likely to be incorporated into veterinary microbiology as official guidelines and zone size data evolve to accommodate this, thus the susceptibility of some commonly used antibiotics will be predicted rather than obtained by direct testing.

If you require further information or clarification about any of this information please do not hesitate to contact the laboratory.

Case Report...

CLINICAL DETAILS:

Nine year old female neutered Yorkshire terrier with history of polydipsia, polyuria, polyphagia, hair loss, pendulous abdomen was diagnosed with hyperadrenocorticism using the ACTH stimulation test. A **high dose dexamethasone suppression test** was performed to differentiate between a pituitary and adrenal tumour. **The results are presented below:**

Baseline Cortisol (nmol/L)	116
3 hour sample	71
8 hour sample	131

Interpretation

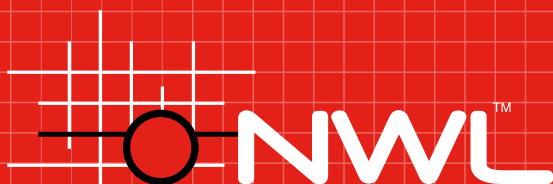
When interpreting high-dose dexamethasone tests, we look to see if there is evidence of suppression of cortisol values via the negative feedback effect of dexamethasone on the pituitary production of ACTH. So that we do not make incorrect judgments based on only subtle decreases in cortisol, we require there to be a 50% decrease in cortisol from the baseline value to be confident that there is indeed a significant suppression. On those occasions where there is greater than 50% suppression, we can be confident that the previously confirmed hyperadrenocorticism has a pituitary origin.

Because the chronic glucocorticoid excess produced by a functional adrenal tumour will have already suppressed pituitary ACTH production, the administration of exogenous dexamethasone is unlikely to be able to suppress it further and consequently there will not be a decrease in cortisol concentrations in adrenal

tumour cases. Unfortunately, not all pituitary based cases will show suppression of cortisol perhaps because their pituitary mass is large or because it has differentiated in such a way that it does not have mechanism to respond normally to exogenous steroid at this dose (0.1mg/kg). About 30% of pituitary cases will not suppress in a standard high-dose-dexamethasone suppression test. Consequently, when there is less than 50% suppression, we cannot be sure whether the origin is pituitary or adrenal.

In this case, the greatest suppression was at three hours but it was only 39% suppression ($100 \times (116 - 71) / 116$). Although this is suspicious for a pituitary origin, it is not sufficient for us to be confident that it is and we can't conclusively determine between pituitary and adrenal based disease.

Further testing: An endogenous ACTH assay or adrenal ultrasound examination would be required for confirmation of pituitary or adrenal tumour as the underlying cause.



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Coming up next issue...

- Monitoring Hyperadrenocorticism treatment
- Infectious Disease updates part 3
- Laboratory Service updates
- Case Report