

Welcome to the NationWide Laboratories quarterly newsletter.

In the last issue we covered an update on Feline Infectious Peritonitis and Reticulocytes. There was also the second part of the "picture" quiz to which the answers were included. In this Newsletter we continue with Part 5 of the Infectious Diseases series, a Laboratory update and another Case Report.

Infectious Diseases...

Part 5 – Lyme Borreliosis

General

The genus *Borrelia* contains tens of species and hundreds of serovars of spirochetal bacteria. Pathogenic and non-pathogenic species have been identified in both mammals and birds. *Borrelia burgdorferi* is the causative agent of Lyme Borreliosis, the most commonly diagnosed vector-borne disease in people.

Borreliae cannot survive as free-living organisms in the environment. They are host associated, being transmitted between vertebrate reservoir hosts (more than 200 species in Europe, eg. people, rabbits, mice, rats, voles, squirrels, hedgehogs, deer, birds) and haematophagous arthropod vectors (eg. ticks of *Ixodes* species). These ticks usually feed on more than one host during their two year life cycle.

Ixodes become infected when larvae feed on infected hosts. Infection in nature is maintained by over-wintering in infected nymphs. In the spring, nymphs transmit *Borreliae* to competent reservoir hosts. Nymphs are thought to be primarily responsible for the transmission of infection to domestic animals and people. Nymphs molt and over-winter as adults for the next tick season. *Ixodes* ticks can be simultaneously infected with additional animal and human pathogens, including *Rickettsia*, *Anaplasma/Ehrlichia*, *Babesia* species and multiple species of *Borrelia*.

NB. No evidence proves that infected pet dogs or cats pose a direct risk to people other than that they introduce ticks into a household. They increase the human risk of exposure by serving as reservoirs, but they are not the preferred hosts for those ticks.

Pathogenesis

Spirochete transmission requires that the tick be attached for at least fifty hours. Despite the numerous people and animals that are bitten, only a few develop clinical disease. Host immune reactions are likely involved in preventing many of these infections.

The percentage of dogs developing clinical disease is very low (5-10%) compared with the frequency of exposure based on seropositivity (75% in endemic areas) and the rate of infection demonstrated in ticks. *Borreliae* proliferate locally in skin at the site of tick attachment. From there, they replicate and migrate through many tissues, including connective tissue, muscles, joints, and nervous system. Dissemination via the blood is less common.

In some hosts, *Borrelia* evades immune clearance from the infected tissues for extended periods and clinical signs develop. Clinical illness results from the host's own inflammatory response against Borrelial antigens.

Clinical Signs

- A small, reddish lesion develops in the skin at the site of tick attachment and disappears within the first week.
- Systemic signs: fever, shifting lameness, articular swelling, lymphadomegaly, anorexia, vomiting, weight loss, lethargy and general malaise.
- Spread of the organism in connective tissue, muscle and joint is responsible for the lameness. The first limbs affected are usually closest to the site of tick attachment. Lameness in a particular limb lasts for a few days and then may shift to another limb or disappear. Despite the transient arthritis, pathologic changes are progressive.
- Chronic nonerosive polyarthritis may persist despite antimicrobial therapy.
- Protein-losing glomerulopathy has been described.
- Acute progressive renal failure associated with proteinuria, peripheral oedema and effusions has also been reported.
- Experimental infected dogs developed mild focal meningitis, encephalitis, and perineuritis, but neurological signs were not observed.
- Other syndromes reported in a few dogs include rheumatoid arthritis and myocarditis-induced cardiac arrhythmia.
- Cats may be more resistant than dogs to the development of clinical signs of Lyme Borreliosis. They may develop multiple-limb lameness, and joint, pulmonary, lymphoid and central nervous system inflammation. Arthritis or meningitis seems to be the predominant manifestation that would warrant investigation of Lyme Borreliosis in cats.

Diagnosis

No specific hematological or biochemical changes are pathognomonic of Borreliosis, although CSF, joint fluid, and urine may show evidence of inflammation. Consistent CSF values have not been found in dogs with suspected neurological dysfunction. The synovial fluid has increased cell counts of 5000 to 100,000 cell/ul (5-100 x 10⁹/l), mainly neutrophils (up to 95%).

Serologic Testing

The presence of an elevated antibody titer to *B. burgdorferi* demonstrates exposure to the spirochete; it does not prove that the clinical illness is caused by the organism. In endemic areas, asymptomatic animals are often seropositive as a result of exposure to non-

pathogenic forms of *B. burgdorferi* or other spirochetes. The patient should have a history of tick exposure, compatible clinical signs and a rapid response to antibiotic therapy for a positive diagnosis to be achieved. Quantitative serial measurements of antibody titers help determine the specificity of the infection. ELISA and immunofluorescence tests are available. Both techniques demonstrate some degree of cross-reactivity between *B. burgdorferi* and other bacteria, including *Leptospira*.

False-negative antibody tests occur probably related to the time course of the disease. Dogs seroconvert at four to six weeks after exposure.

Titers are at their highest levels at three months and last for two years.

Intrathecal production of antibodies to *B. burgdorferi* can be demonstrated in the CSF of dogs with neurological dysfunction.

Detection of Organism

Microscopic examination of body fluids or tissues has low sensitivity due to small numbers of spirochetes in the clinical specimens. Culture of the organism from samples of a diseased patient pre- or postmortem (eg. fascia, pericardium, peritoneum, meninges, synovium) is the most definitive means of diagnosis, but again has low sensitivity. PCR is highly specific, but has limited sensitivity and false-negative results may occur in CSF, synovial fluid and urine. Sensitivity increases if synovium or skin are taken. PCR cannot distinguish live from dead organisms.

Therapy

Antibiotics are often given empirically in an attempt to make a therapeutic diagnosis. Improvement commonly occurs 24 to 48 hours after therapy is initiated. Doxycycline (generally the drug of choice), amoxicillin, azithromycin and ceftriaxone may be used for a minimum of thirty days. This is associated with a reduction in antibody titers and organisms in tissues.

Prevention

Given the high exposure rate compared with the low incidence of clinical illness in endemic areas, prophylactic treatment with antibiotics at the time of identified tick attachment is expensive and impractical. Early removal of ticks is recommended. Vaccines are marketed to prevent Borreliosis in dogs. Vaccines given early in life before exposure offer the best means of protection, but do not replace adequate tick control (animals and environment). Besides, the multiple infecting agents may make protection more difficult.

PUBLIC HEALTH CONCERN

Dogs and cats are sentinel hosts but not reservoirs for human infection. In the same environment, dogs and cats have a greater risk of exposure than humans because of their greater likelihood of contacting the tick vector. Pets may bring infected ticks into the household, but ticks generally do not refeed after detachment. Even in areas where Borreliosis is endemic, the risk of infection from a tick bite is very low.

Laboratory Service Updates!

Urine Culture and use of Boric Acid as a Urine Preservative...

Urine is an excellent bacterial culture medium and free catch samples are often contaminated by low numbers of organisms (<1x10² organisms/ml) from the urethra, perineum or prepuce. Delay in processing samples maintained at room temperature can lead to significantly increased bacterial counts and, since semi-quantitative culture is required for laboratory diagnosis of UTI, lead to errors in diagnosis (1, 2).

To reliably culture plain unpreserved samples it would be necessary to process or refrigerate them within two hours of collection as refrigeration can be an effective means of preventing significant increases in bacterial counts for up to 48 hours.

Clearly, in a veterinary clinical environment the practical approach is to use preserved samples for urine microbiology. Boric acid (syn borate, borax) at 10-20 g/l (1-2%) is an effective means of preserving urine at room temperature for at least twenty-four hours. Some authors claim efficacy for three to four days but this is controversial (3, 4, 5, 6). It is bacteriostatic for almost all common urinary pathogens (4).

The commercial Boric Acid tubes used by NWL (red-topped tubes) are designed to produce a final concentration of 2% when maximally filled [5ml urine]. Under filling of tubes is a common occurrence, particularly if samples are submitted in Universal Boric Acid tubes designed for human specimens. Concentrations of Boric Acid >4% [<2.5 ml] can be toxic leading to false negative culture results.

A study to evaluate the effect of sample volume on bacterial toxicity of Boric Acid (5) found that, after twenty-four hours at room temperature, tubes filled to

20% capacity [1ml] or less had significantly reduced counts for several bacteria including strains of *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. Counts of *E.coli* and *K. pneumoniae* were also significantly reduced in tubes filled to 40% capacity [2ml]. Bacterial counts were not significantly reduced in any of the tubes filled to >60% capacity [>3ml].

Paradoxically, in a separate study, lower concentration of Boric Acid (10g/l or 1%) were found to be weakly bactericidal for some strains of *Pseudomonas aeruginosa* (4). However, overfilling of NWL tubes is not possible therefore this could only occur if Boric Acid crystals were emptied out or spilled.

In a retrospective study of 100 samples received at NWL only 45% of Boric Acid tubes were fully-filled according to the manufacturer's recommendations.

- 17% were filled to <40% capacity
- 14% were filled to >40% but <60% of capacity
- 69% of tubes were filled to >60% capacity

Negative cultures were obtained in 71% of the tubes filled to <60% capacity but a lower culture negative rate of 59% was observed in the samples filled between 60 and 100% of capacity.

Of the 55% of urine samples submitted in Boric Acid tubes that were incorrectly filled, in 31% the concentration of Boric Acid was sufficiently high to be potentially toxic for certain organisms including some significant urinary pathogens.

When samples are submitted for urinalysis and culture and sensitivity testing we require a minimum of 0.5ml of urine in a plain tube for urinalysis/sediment examination.

It may help to adequately fill the Boric Acid tube first before transferring the remaining urine into the plain tube. If there is insufficient volume to submit both tubes it is possible to perform limited urinalysis on the "borax" sample. Boric Acid has no known effect on urine protein, glucose, cell counts and casts. It may affect the pH, SG, type and number of crystals observed (3).

References

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Case Report...

Signalment: Fourteen year old male Golden retriever

History: Lethargy, anorexia, enlarged spleen and liver on palpation

Haematology Report:

Red Blood Cells	*2.56	10 ¹² /l	5.4-8.5
Haemoglobin	*5.7	g/dl	12-18
Haematocrit / PCV	*0.18	l/l	0.37-0.56
MCV	70.5	fl	65-75
MCHC	32	g/dl	31-35

WBC and Differential:

White Blood Cells	*565.1	10 ⁹ /l	5-18
Neutrophils	1%	5.65	10 ⁹ /l 3.7-13.32
Band Forms	0%	0.00	10 ⁹ /l 0-0.54
Lymphocytes	*99%	559.45	10 ⁹ /l 1.00-3.60
Monocytes	0%	0.00	10 ⁹ /l 0.20-0.72
Eosinophils	0%	0.00	10 ⁹ /l 0.10-1.25
Basophils	0%	0.00	10 ⁹ /l 0.05-0.18
Platelet Count	*97	10 ⁹ /l	200-900
Platelet Comment	Numbers appear reduced on the smear		
Film Comment	No evidence of regenerative response		

Description:

Severe anaemia and thrombocytopenia were confirmed by slide exam. There is no evidence of erythroid regeneration.

The most striking abnormality in this haemogram is the markedly elevated lymphocyte count. Most of them are small well differentiated lymphocytes, but medium-sized lymphoid cells are also present in circulation. The latter do not clearly show a nucleolus.

Interpretation:

- Chronic lymphocytic leukaemia.
- Bicytopenia suggesting bone marrow pathology eg myelophthisis.

Further investigation:

Aspirate or biopsy of liver, spleen to check for systemic involvement, Bone marrow aspirate and biopsy, Flow cytometry.

Coming up next issue...

- Infectious Diseases Part 6
- Services Update – Allvervet Changes
- Case Report/Quiz

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.

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



No. 1733
No. 2529



NationWide Laboratories

NationWide Laboratories Poulton
23 Mains Lane, Poulton-le-Fylde, Lancashire FY6 7LJ
Tel: 01253 899215 Email: nwlabs@nwlabs.co.uk

NationWide Laboratories Leeds
Gate Way Drive, Yeadon, Leeds LS19 7XY
Tel: 0113 250 7556 Email: nwl.leeds@nwlabs.co.uk

NationWide Laboratories Swanscombe
Unit 5, Galley Hill Industrial Estate, London Rd,
Swanscombe, Kent DA10 0AA