

NWL NEWS

The Newsletter of NationWide Laboratories

March 2008

Welcome to the NationWide Laboratories quarterly newsletter.

In the last issue we covered an update on Feline Leukaemia virus and very interesting "picture" quiz to which the answers were included. We begin the New Year with Feline Infectious Peritonitis, an Update on Reticulocytes and a second "picture" quiz.

Infectious Diseases...

Part 4 – Feline Leukaemia Virus

General

FIP develops in a small proportion of cats infected with FCoV. The disease is associated with viral mutation which allows replication of the virus in macrophages and monocytes. After infection, most cats shed virus for a variable period of time but the majority have stopped shedding by nine months post infection. In at least 25% of these cases, virus can also be detected in the blood. Over 90% of cats exposed to FCoV seroconvert, but the antibody titre eventually declines to 0 when they stop shedding virus. Thirteen percent of cats become lifelong carriers and permanently shed virus (faecal). Only 10% of seropositive cats go on to develop FIP. A small proportion of cats seem to be naturally resistant to infection. They do not develop an antibody response and do not shed virus.

Diagnosis of FIP

A definitive diagnosis is difficult and requires histological examination and possibly, immunohistochemistry of affected tissue. A tentative diagnosis may be made based upon the weight of evidence from the following tests:

- Multicat household. The seroprevalence of FCoV in single cat households is reported as 25% but can be up to 100% in multicat households.
- Signalment: most affected cats are three months to two years, with a second peak at > ten years.
- History: the clinical signs are often noted just after a stressful event e.g. re-homing, surgery.
- Clinical signs: anterior uveitis, pyrexia, lethargy, anorexia, weight loss, palpable mesenteric lymph nodes, ascites (25% of these also have pleural effusion), neurological signs (seizures, nystagmus, hyperaesthesia).
- Hyperglobulinaemia, polyclonal gammopathy and A:G < 0.7 (< 0.45 carries more weight). Raised globulins are present in more than 50% of cats with wet FIP and 75% of cats with the dry form. Other profile changes: normocytic normochromic anaemia, neutrophilia, lymphopenia, proteinuria (if the kidneys are involved).
- Abdominal effusion. Total protein > 35g/l and A:G ratio < 0.7 (< 0.45 carries more weight).
- FCoV antibodies (IF). Serology can be performed on serum or effusion. Cats which have come from a multicat household or rescue centre within the previous 6-12 months are likely to have antibodies to FCoV. Approximately nine out of ten of these cats will not develop FIP.
- Dry FIP: The antibody titre in dry FIP is high (often > 1280). Wet FIP: titres vary from 0 to high. PCR on the abdominal/pleural fluid is useful for cats with a titre of 0 and suspected FIP.
- PCR: detects viral genetic material in effusions and is useful in seronegative cats with suspected wet FIP. A negative result does not exclude the diagnosis.

Serological testing in other circumstances

1. Testing when there is suspected exposure of a healthy cat

FCoV is highly infectious and most in-contact cats will be seropositive. Only 10% of infected cats will develop FIP. The following may be useful:

If titre= 0: the cat is unlikely to develop FIP and is not shedding virus. However, a small number of cats with wet FIP have a titre of 0. It is probably safe to get another cat, but the new cat should also have a titre of 0.

Titre>0: There is a one in ten chance of the cat developing FIP and a one in three chance that the cat is shedding virus. It is probably unwise to bring in a new cat. Retest at 3-6 month intervals. The titres eventually decline in most unaffected cats.

2 Screening before mating

Seronegative: the cat is not excreting virus and can be mated to another seronegative cat

Seropositive: it would be best to find a mate who is also seropositive.

3 Screening a cattery

Since FCoV is highly infectious, sampling 3-4 individuals gives an overview of the status. Cats may eventually become seronegative if they are housed in small groups (<3) or the total number of cats is relatively small (<10). Test every 6-12 months to determine if the proportion of seronegative cats is increasing.

4 Screening a cat for introduction into a new household

Seronegative cats may be introduced. Seropositive cats may be shedding virus.

Laboratory Service Updates!

New "Non-interpreted" Profiles...

Recognising that some clients are familiar with the interpretation of laboratory results, NWL has introduced a new, competitively priced multi-parameter profile without veterinary interpretation and included two pre-anaesthetic screens in a new "**Profiles without interpretation**" section on page four of the new 2008 price list.

Of course, if after receiving the results, you wish to discuss the case and receive a further report from our pathologists, that service is available for an extra administrative fee.

Reticulocytes and their use in classifying anaemias... Regenerative or non-regenerative?

Classification of an anaemia as a regenerative (accompanied by a bone marrow response) or non-regenerative anaemia helps to direct further investigation and to identify the cause of the reduced red cell mass. Regenerative anaemia includes haemorrhage and haemolysis while a persistent non-regenerative anaemia often points towards systemic or renal disease or bone marrow suppression. The presence of increased MCV, polychromasia, anisocytosis and circulating nucleated RBCs may indicate a regenerative anaemia but the marrow response is best quantified using a reticulocyte count.

Immature, RNA-rich erythrocytes are recognised as polychromatophilic cells (which stain bluish) on Romanowsky stains. Their presence is confirmed in fresh blood using supravital stains, e.g. New Methylene Blue which causes precipitation of the intracellular ribosomal RNA protein, with the formation of dense aggregates (reticulin) (Fig 1). When identified using this staining technique, the immature cells are termed reticulocytes. The number of reticulocytes present may be counted by manual or automated methods and expressed as the percentage of the

total number of erythrocytes. This value can be used as a guide to the degree of regeneration but is affected by the total number of red cells present and the absolute reticulocyte count is the preferred method of recording reticulocyte data.

$$\text{absolute reticulocyte count (x109/l)} = \text{observed reticulocyte \%} \times \text{RBC}$$

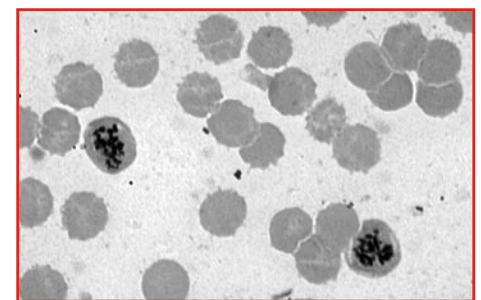
Interpretation of absolute reticulocyte count is outlined in Table 1. In the cat, two forms of reticulocytes are identified. Aggregate reticulocytes are present in the circulation for a short time before they mature into punctate reticulocytes, which may persist for one to two weeks. In general, counting methods are directed at quantifying the aggregate reticulocytes.

During a regenerative response, nucleated erythrocytes may also be observed in the peripheral blood. Identification of these cells without reticulocytes need not reflect increased erythropoiesis and can be associated with bone marrow disease (including FeLV infection), lead toxicity and poor splenic function.

Table 1: Interpretation of absolute reticulocyte counts in dogs and cats

Degree of regeneration	Canine reticulocytes x109/l	Feline aggregate reticulocytes x109/l
Slight	150	50
Moderate	300	100
Marked	> 500	> 200

Figure 1: Dense aggregates or ribosomal RNA in canine reticulocytes



Picture Quiz Part 2...

The following pictures demonstrate cell morphology, microorganisms, inclusion bodies and artefacts that may be seen on blood smears. Can you identify them? Answers below.

Answers...

- 1) Babesia canis [arrows] and a mature neutrophil [arrow; this is an artefact – not inclusion body]
- 2) Platelet superimposed on an erythrocyte [red arrow]
- 3) Babesia gibsoni [arrows]
- 4) Distemper inclusion bodies [arrows]
- 5) Microfilaria
- 6) Anisocytosis (variable erythrocyte size), polychromasia [red arrows] which are indications of erythrocyte regeneration, giant platelet [black arrow] which if in large numbers can result in spurious low automatic count and demonstrates the importance of looking at fresh smears. Note the mature neutrophil. Protein crescents in the background (high protein content) [arrows] and not to be confused with microfilaria or trypanosomes.
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