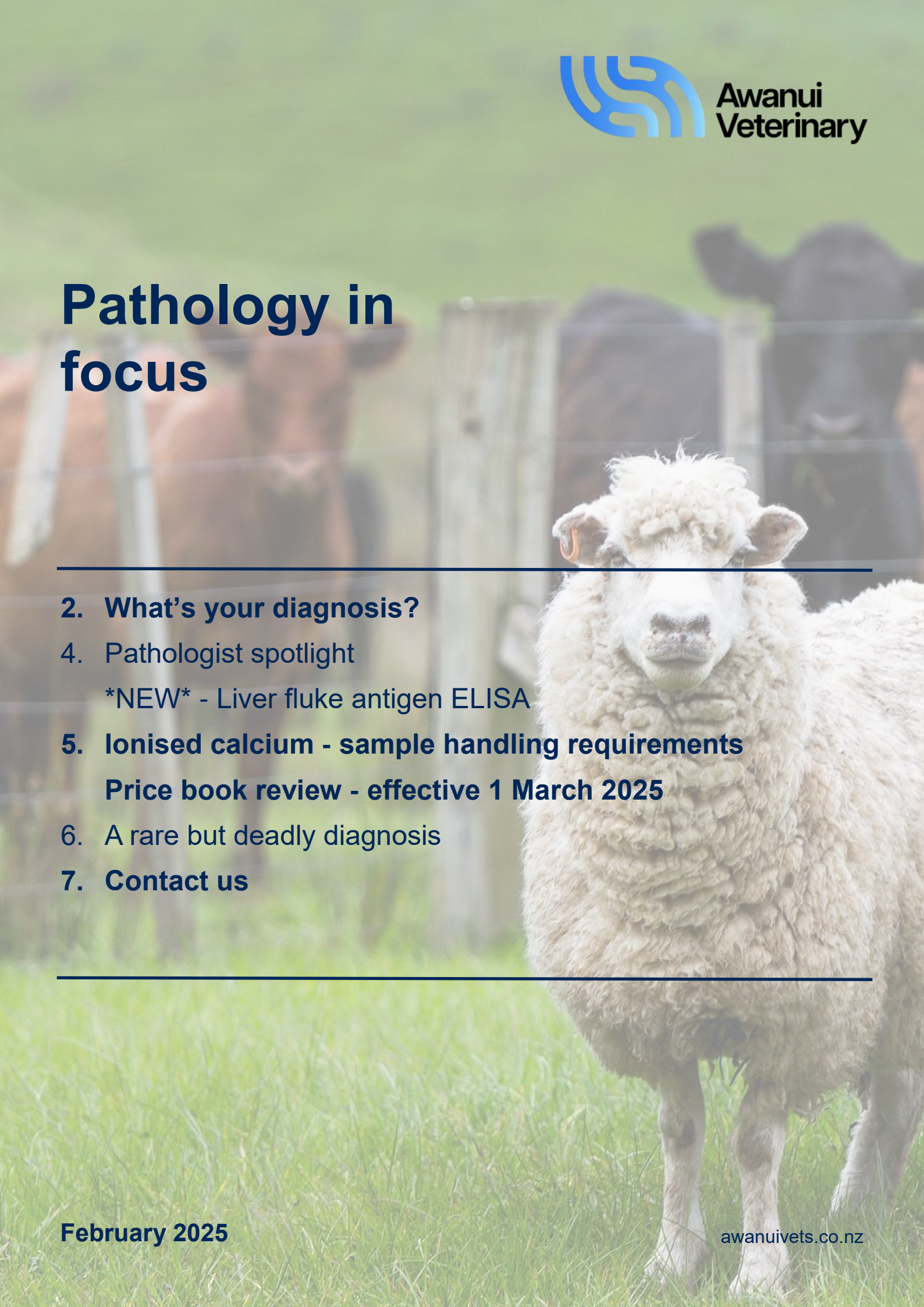
Awanui Veterinary

Pathology in focus



February 2025

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What's your diagnosis?

Lisa Hulme-Moir

Figure 1 is a photomicrograph of a blood film from a four year-old male castrated Australian Kelpie. The dog had been in an induced coma with constant rate infusion (CRI) of propofol to manage hepatic encephalopathy due to acute liver failure. Haematocrit was 0.23 L/L.

What's your diagnosis?

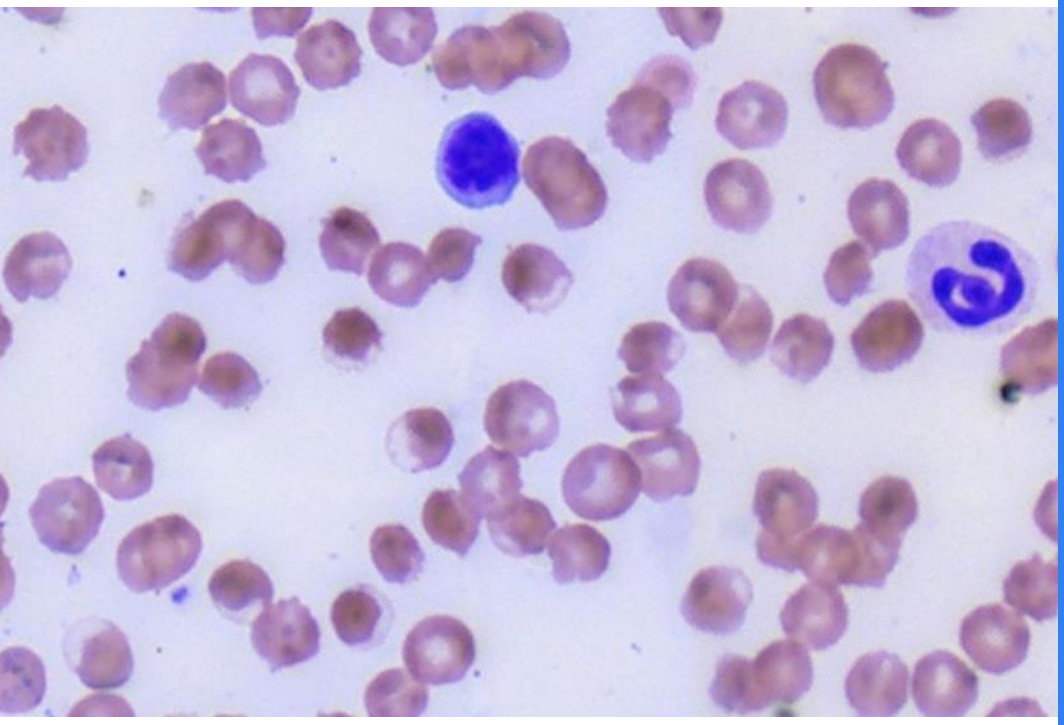


Figure 1 - Blood film from a four year-old male castrated Australian Kelpie, HCT 0.23 L/L.

Answer:

Eccentrocytosis indicating oxidative injury and a likely haemolytic anaemia.

Discussion:

Multiple eccentrocytes are present in the blood film (Figures 1& 2) indicating oxidative injury to the red cells. The anaemia was non/pre-regenerative at this point in time. Over the next two days the HCT fell to 0.14 L/L as the eccentrocytes were removed from the circulation. A strong regenerative response was evident five days later.

Prolonged or repeated exposure to propofol is a less frequently encountered cause of oxidative haemolytic anaemia in cats and dogs (Romans et al. 2020). In this case, oxidative stress due to the liver failure may also have been a contributory factor increasing the susceptibility of the red cells to oxidative injury. Interestingly, dogs have a tendency to develop eccentrocytes following oxidative injury whereas Heinz bodies are more typical in other animal species (Figure 3). Eccentrocytes are a result of direct oxidative injury to the red blood cell membrane and cytoskeleton. The opposing faces of the damaged cytoplasmic membrane stick to each other sequestering the cytoplasmic contents to one half of the cell. In contrast, Heinz bodies reflect denatured haemoglobin, which forms as a clump on the inner surface of the red cell membrane. In both cases, the presence of eccentrocytosis or Heinz bodies impairs the deformability of red cells causing them to become trapped and removed in the narrow vascular spaces of the spleen. As removal and destruction of the red cells is not immediate, many animals may be non or only mildly anaemic after initial exposure to an oxidant. A progressive anaemia, as seen in this case, may then develop over the subsequent 5-10 days. Clinical signs may therefore lag by some days after oxidant exposure. In some cases though, the integrity of red cell membrane may be impaired and intravascular haemolysis can occur causing more rapid clinical signs.

Aside from propofol, other causes of oxidative anaemia in dogs include onion or garlic (including foods that contain onion or onion powder such as baby food), zinc (e.g. swallowed objects or coins containing zinc), naphthalene (e.g. fumes from mothballs in cupboard/storage areas), paracetamol, vitamin K, and rodenticides. Eccentrocytes have also been reported in dogs with inflammatory disease, liver disease, diabetes and T cell lymphoma without exposure to an external oxidant (Caldin et al. 2005).

In cats, interpretation of Heinz bodies is a little more complicated. Feline haemoglobin contains more reactive sulfhydryl groups than other species making the haemoglobin more prone to denaturation. Additionally, the feline spleen has larger vascular spaces allowing red cells to pass through without deformation and making it less efficient at removing Heinz bodies (a process referred to as 'pitting'). Consequently, it can be normal for up to 5% of feline red cells to contain Heinz bodies and increased Heinz body formation can be commonly noted in a range of diseases without anaemia or oxidant exposure (Figure 4). This includes hyperthyroidism, diabetes (particularly with ketoacidosis),

liver disease and lymphoma.

In other animal species, Heinz bodies and eccentrocytes are always considered significant with the development of anaemia expected. Common causes of Heinz body haemolytic anaemia in cattle and sheep include brassicas, onions, zinc toxicity and copper toxicity (Figure 5). In the case of feed-related oxidants such as brassicas, anaemia may not develop until 1-2 weeks after ingesting the crop.

One final point about Heinz bodies aside from their pathological significance is their ability to interfere with tests on haematology analysers. This is particularly relevant to cats where Heinz bodies may occur more frequently. Red cells containing Heinz bodies are more resistant to the lytic agents used in some analysers and this can result in spuriously high leukocyte counts (Johnson et al. 2020). Heinz bodies also increase the turbidity of samples after lysis artifactually increasing the Haemoglobin concentration and it's related indices, MCH and MCHC. Artifactually high reticulocyte counts may also be produced by analysers as red cells containing Heinz bodies have high autofluorescence which make them indistinguishable from reticulocytes stained with the fluorescent dyes used on these analysers (Camus et al. 2017).

References:

- Caldin et al. A retrospective study of 60 cases of eccentrocytosis in the dog. *Vet Clin Path*, 34:224-231, 2005.
- Camus et al. The fluorescent foible of Heinz bodies. *Vet Clin Path*, 46:9-10, 2017.
- Johnson et al. Spurious marked leukocytosis in 2 cats with Heinz body haemolytic anaemia. *Vet Clin Path*, 49:232-239, 2020.
- Romans et al. Oxidative red blood cell damage associated with propofol and intravenous lipid emulsion therapy in a dog treated for 5-fluorouracil toxicosis. *J Vet Emerg Crit Care*, 30:481-486, 2020.

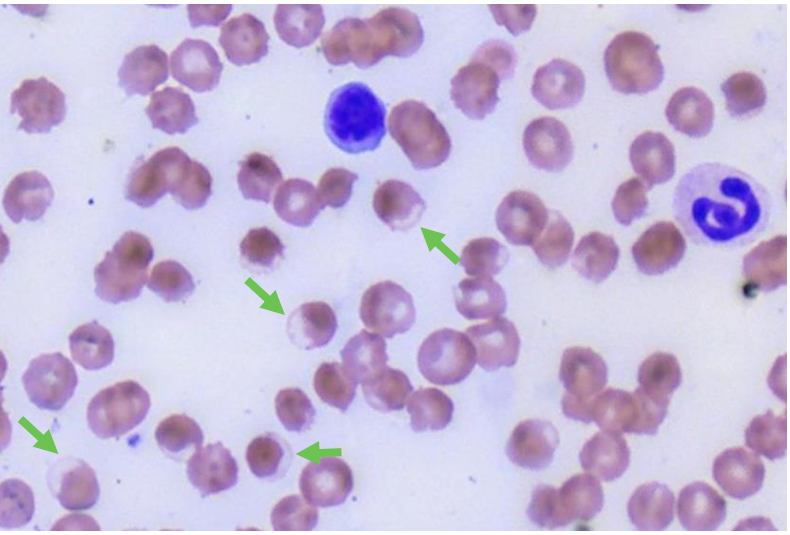


Figure 2 - Multiple eccentrocytes (arrows) in a blood film of a dog following constant rate infusion of propofol (Wright's stain). No Heinz bodies were detected on subsequent new methylene blue staining.

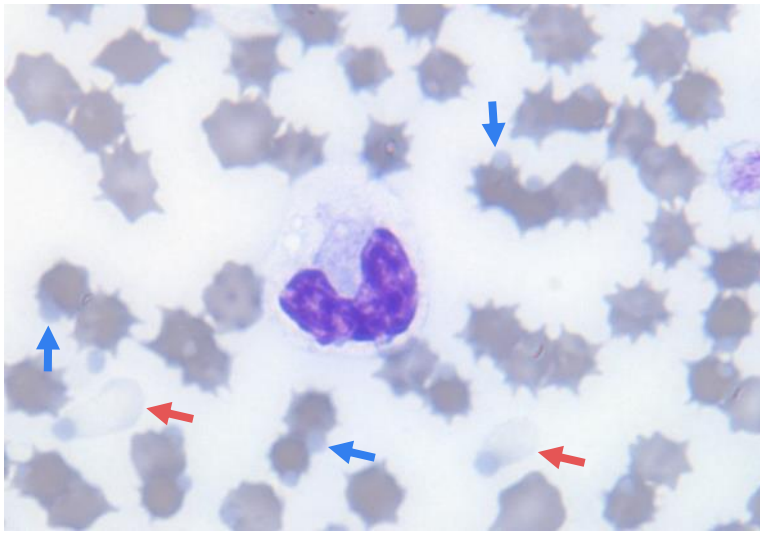


Figure 3 - Heinz body haemolytic anaemia in a cat. Numerous large Heinz bodies (blue arrows) can be seen projecting from the surface of the red cells. Some lysed ghost red cells containing Heinz bodies (red arrows) are also present. The red cells are crenated (echinocytes) due to sample aging/ prolonged contact to EDTA (spent several days in transit before arriving at the laboratory).

Figure 4 - (A) Heinz bodies (blue arrows) in a cat with hyperthyroidism that was not associated with anaemia. Compare these to the larger, more obvious Heinz bodies seen in Figure 3 above. (B) New methylene blue stain is used to perform manual reticulocyte counts but is also useful for confirming the presence of Heinz bodies (blue arrows), which stain a pale to medium blue colour. One reticulocyte (dark red arrow) and a NRBC are also present.

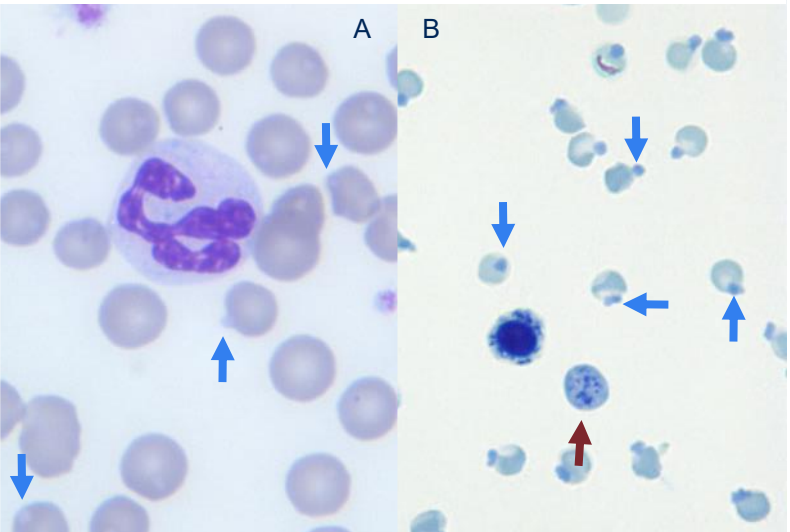
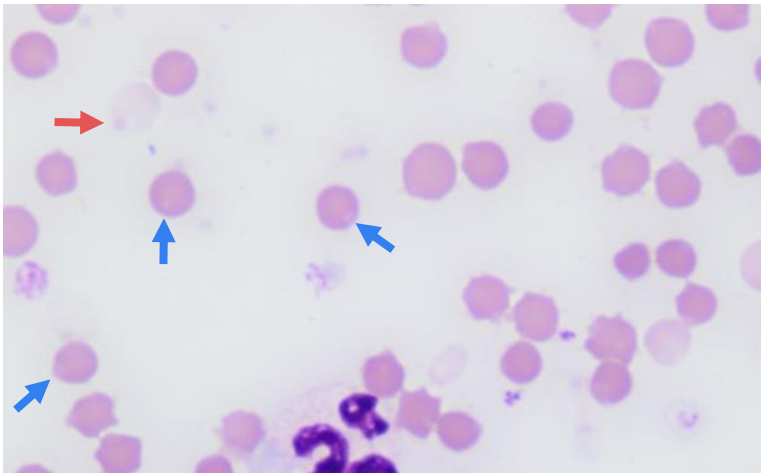


Figure 5 - Heinz body haemolytic anaemia in a sheep with chronic copper toxicity. Heinz bodies (blue arrows) can sometimes be very subtle and difficult to detect. In such cases, staining with new methylene blue may be required to confirm their presence/absence. A ghost red cell containing a Heinz body (red arrow) can be seen faintly in the background.



Pathologist spotlight

Lisa Hulme-Moir earned her BVSc from Massey University in 2000, then spent several years working in small and mixed animal practices both in New Zealand and the UK. In 2004, she transitioned into veterinary pathology, pursuing a PhD in leukocyte biology at Murdoch University in Perth, Western Australia. After completing her PhD, Lisa returned to the northern hemisphere and spent five years in a clinical pathology role at the University of Glasgow. She has been a valued member of our team since 2012.

Lisa has a broad interest in clinical pathology, with a particular passion for continuing professional development (CPD) and helping vets and vet nurses optimize their use of both external and in-clinic laboratory testing. Her husband, Brent, a large animal veterinarian, has nurtured her interest in production animal diagnostics, and she has since developed a keen focus on equine endocrinology.

Outside of work, Lisa enjoys gardening and keeping up with her children. Her personal hobbies, though mostly unfulfilled, include reading, listening to music, horse riding, and golf.

NEW

Liver fluke antigen ELISA test

Liver fluke (*Fasciola hepatica*) is endemic in certain areas of New Zealand, affecting all grazing ruminants. However, despite its prevalence, diagnosing liver fluke infection in farmed livestock before death remains challenging.

Liver fluke establish in the bile ducts of ruminants and interfere with liver function. Sheep do not establish immunity and infection can build up over several seasons. Weight loss, anaemia and ewe deaths can occur. For cattle, in high challenge areas, milk production and liveweight gain can show a response after treatment.

We currently provide various tests for liver fluke, including conventional faecal microscopy, serum and milk antibody testing, and gross pathology/liver dissection. All of these tests have their limitations - conventional microscopy has a relatively low sensitivity; antibody testing will continue to test positive even after anthelmintic treatment; and gross pathology requires the animal to be dead and is complicated by other conditions that also produce pathological changes to the liver, e.g. facial eczema.

We are very excited to now offer a liver fluke-specific test that detects infection through an ELISA on faecal samples. This test can yield a positive result even outside the fluke's egg-laying period, and unlike the serum antibody ELISA test, this faecal antigen ELISA will only test positive if flukes are present in the bile duct.

Sample type:	Faeces
Species:	Cattle, sheep
Turn around time:	2-3 days
Price:	$45.98 ex. GST

Note: Testing can be carried out on individual samples only, and cannot be done on pooled/composite samples.

(References: Beef + Lamb NZ, BioX Diagnostics kit insert)

Ionised calcium

- sample handling requirements

Samples for free calcium (ionised) testing must be handled appropriately prior to submission in order to ensure they remain suitable for testing on receipt in the laboratory. If the samples do not meet all the criteria stipulated below, they will be unsuitable for analysis.

Specimen: Serum

Container: Plain red top tube

Minimum sample volume: 1.3mL mini tube, must be full.

Collection protocol: Vacutainer tubes must be filled to the very top. Preferably fill vacutainers using a syringe and needle. If a tube lid is removed, this must be replaced as soon as possible to reduce exposure to air (samples must be kept anaerobic).

Special handling/shipping requirements: Allow sample to clot at room temperature for minimum of 30 minutes before refrigerating. Do not centrifuge sample. Transport to laboratory chilled, on the same day as collection (chilled sample only, no direct ice contact with sample) .

Note: If samples are not clotted prior to refrigerating, free calcium becomes sequestered in the clot and will result in lowered values.

Notes for interpretation of results: Free ionised calcium testing requires strict adherence to collection and storage requirements for accuracy. If not tested immediately after collection, storage time and temperature, and any exposure to air can affect the pH of the sample which significantly affects the result. Storage at room temperature or refrigerated usually results in falling pH over time which increases ionized calcium values. Freezing and exposure to air results in increased pH and reduced ionised calcium values.

If you have any questions regarding sample collection for ionised calcium, please just give your local laboratory a call and they will be happy to help you out.

Price book review - effective 1 March

In order to maintain the high quality of our service and invest in its continuous improvement, we will be implementing a price increase effective 1 March 2025. This adjustment reflects factors such as inflation, labour costs, and the rising costs of courier and supplies.

A PDF version for downloading and use in clinic, plus a CSV version for uploading to your practice management system (if required) are available (contact us here or speak to your local laboratory) and request the format you require.

- Clients using ezyVet with our SDI integration will have their PMS updated centrally, so you will not need to update in clinic. All other ezyVet users will need to update details manually. If you wish to change to the SDI integration please contact ezyVet directly.
- Custom biochemistry panel prices will increase by 3% (where applicable).
- Updated pricing for consumables will be available via our online store from 1 March 2025 (a user account is required to place online orders).
- Awanui is Toitū carbonreduce Certified, and accordingly only a limited number of hard copy price books will be available on request from your local laboratory.

Please note: This review does not effect any contracted pricing. Quotes that have been provided before this date will be honoured until the stated expiry date – please reference the quote number when submitting samples.

We greatly appreciate your ongoing support and the trust you place in Awanui Veterinary for your diagnostic testing needs. Your business is invaluable to us, and we are committed to meeting your needs and enhancing the services we provide.

For more information, please contact your local laboratory manager - we are more than happy to answer any queries you may have.

A rare but deadly diagnosis

Cristina Gans

Clinical history

A one-year-old DLH cat presented to the veterinarian for acute -onset anorexia and lethargy. Additional findings on clinical examination were a reduced body condition and moderate pyrexia. After treatment with antibiotics and non-steroidal anti-inflammatories, the cat displayed a marked improvement in appetite and demeanour and was discharged from the hospital. Shortly afterwards, the cat developed neurological signs, including ataxia and nystagmus. The cat was rushed to an emergency veterinary hospital but died just prior to arrival.

Gross findings

A full necropsy was performed by the referring veterinarian, and lesions were identified in the brain, kidney and liver. A single 10 mm diameter mass was identified in the cerebral cortex, from an area associated with the right temporal and frontal lobes. Two well-demarcated masses (measuring 7 and 11 mm) protruded from the cortex of the right kidney (figure 1). These masses were all described as green and gelatinous by the referring veterinarian, but upon fixation, the colour changed to a dark brown-black pigmentation. A small focal cream-coloured lesion was identified in the right lateral lobe of the liver.

Histological findings

Within the renal cortex of both kidneys and within the cerebral cortex of the brain were multiple foci comprised of central necrosis surrounded by neutrophils and macrophages and further surrounded by fibrosis and granulation tissue. Within necrotic foci were abundant pale brown, septate, branching hyphae with slightly bulbous 3 to 6 µm diameter walls (figure 2). Also present were moderate numbers of 6 to 10 µm diameter ovoid to spherical conidia. Within the brain, neutrophils occasionally infiltrated the walls of blood vessels within the parenchyma, consistent with a vasculitis. Rare fibrin thrombi and rare fungal hyphae were sometimes associated with these vessels. The lesion in the liver corresponded to an area of mixed inflammation, but fungal hyphae were not identified in this lesion.

Diagnosis

Pyogranulomatous and necrotising encephalitis and nephritis within intralesional pigmented fungi, consistent with disseminated phaeohyphomycosis.

Discussion

Fungal encephalitis due to phaeohyphomycosis is a rare presentation in cats, but it has been reported in multiple case reports worldwide, including in Australasia. Other differential diagnoses for infectious causes of encephalitis for NZ cats include feline infectious peritonitis, feline panleukopenia virus, other viral causes (FIV/FeLV/FHV-1), *Toxoplasma gondii*, *Cryptococcus*, and bacterial infections. Most infectious causes of CNS disease result in neurological signs that are acute in onset and progressive, with some cases becoming chronic (Gunn-Moore and Reed, 2011).

Phaeohyphomycosis is an umbrella term which includes a number of pigmented dematiaceous fungi including *Cladophialophora bantiana* (previously known as *Cladosporium bantiana*). Dematiaceous fungi are mostly soil organisms associated with plant material as plant pathogens. Although infection is uncommon, the most common presentation in cats is the presence of single or multiple cutaneous ulcerated plaques or nodules. Access through broken skin, either directly through trauma or indirectly through contamination of a pre-existing wound is suspected as the primary mode of infection; however oronasal infection has also been

Figure 1. Cross-section of formalin-fixed right kidney. Two brown-black masses protrude from the renal cortex (arrows).

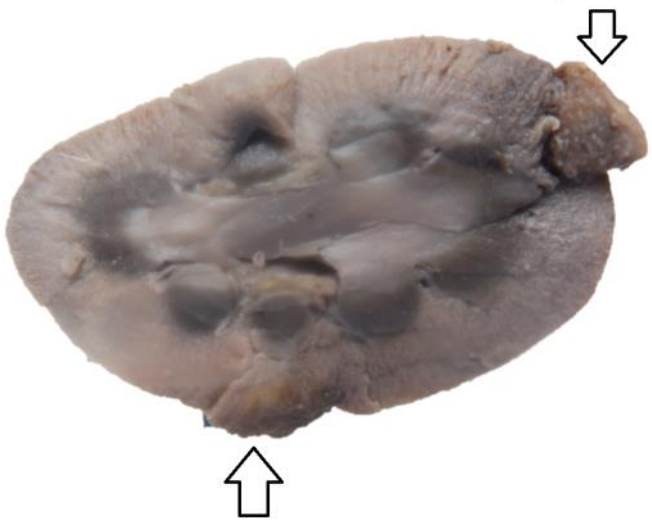
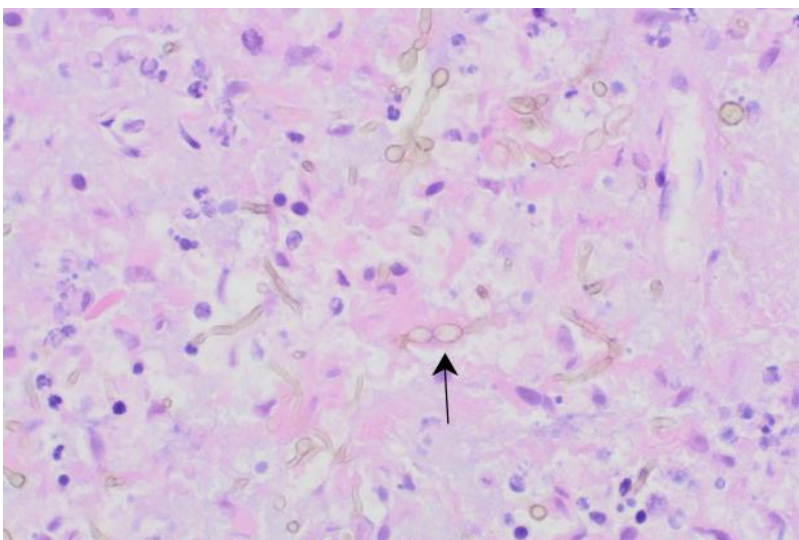


Figure 2. Within necrotic foci in the brain are abundant pale brown, septate, branching hyphae with slightly bulbous 3 to 6-um diameter walls. Haematoxylin and eosin 50x.



suspected. Direct transmission between hosts has not been reported (Lloret et al, 2013).

Systemic dissemination is uncommon, and encephalitis is infrequently reported. Encephalitis in cats is most commonly associated with *Cladophialophora bantiana*, due to the neurotropic nature of the fungus (Russell et al, 2016; Mariani et al, 2002). Other reported sites include the lungs, nasal cavity, liver and kidneys. CNS infections are thought to occur via the respiratory route, but ocular and aural routes of entry have also been suggested (Bouljihad et al, 2002).

In this case, no gross pulmonary disease or skin wounds were identified and the route of infection was undetermined. Fungal hyphae within rare blood vessels are suggestive of a haematogenous route for the dissemination.

Ancillary diagnostic tests may be helpful in the diagnosis of a fungal encephalitis, but are rarely specific for a particular infectious cause. Serum biochemistry and routine haematology may be supportive of systemic inflammation and lower the clinical suspicion for other causes such as feline infectious peritonitis. Reported findings on CSF analysis may be inconsistent with suppurative or granulomatous pleocytosis and variable increases in protein content present in some, but not all, cases. MRI and/or CT imaging may identify a mass lesion, although the cause of such lesions is rarely specific. Definitive antemortem diagnosis of fungal encephalitis (with the exception of *Cryptococcus*) is rare. However, in one case report of an adult cat with disseminated lesions in the liver and kidney, pigmented fungi were identified on cytology of masses in the liver and kidney (Cohen and Johnson, 2025).

Most feline cases have not identified any underlying predisposing factors; however, there have been cases associated with corticosteroid administration and lymphocytic leukaemia (Bouljihad et al, 2002). In this case no other systemic disease was identified, and there was no history of corticosteroid administration. The FIV/FeLV status was unknown for this patient.

Acknowledgements to Nick Page and Rolleston Veterinary Services for submission of this case.

References:

Bouljihad M, Lindeman CJ, Hayden DW. Pyogranulomatous meningoencephalitis associated with dematiaceous fungal (Cladophialophora bantiana) infection in a domestic cat. Journal *of Veterinary Diagnostic Investigation.* 14:70-72, 2002.

Cohen J, Johnson K. Disseminated phaeohyphomycosis caused by Cladophialophora in an immunosuppressed cat. *Veterinary Radiology & Ultrasound.* 66(1), 2025.

Gunn-Moore D A, Reed N. CNS disease in the cat: current knowledge of infectious causes. *Journal of Feline Medicine and Surgery.* 13:824-836, 2011.

Lloret A, Hartmann K, Pennisi MG, Ferrer L, Addie D, Belák S, Horzinek MC, et al. Rare opportunistic mycoses in cats: phaeohyphomycosis and hyalohyphomycosis: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery.* 15:628-630, 2013.

Mariani CL, Platt SR, Scase TJ, Howerth EW, Chrisman CL, Clemmons, RM. Cerebral phaeohyphomycosis caused by Cladosporium spp. in two domestic shorthair cats. *Journal of the American Animal Hospital Association.* 38:225-230, 2002.

Russell EB, Gunew MN, Dennis MM, Halliday CL. (2016). Cerebral pyogranulomatous encephalitis caused by Cladophialophora bantiana in a 15-week-old domestic shorthair kitten. *Journal of Feline Medicine and Surgery Open Reports.* 2 (2), 2016.

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