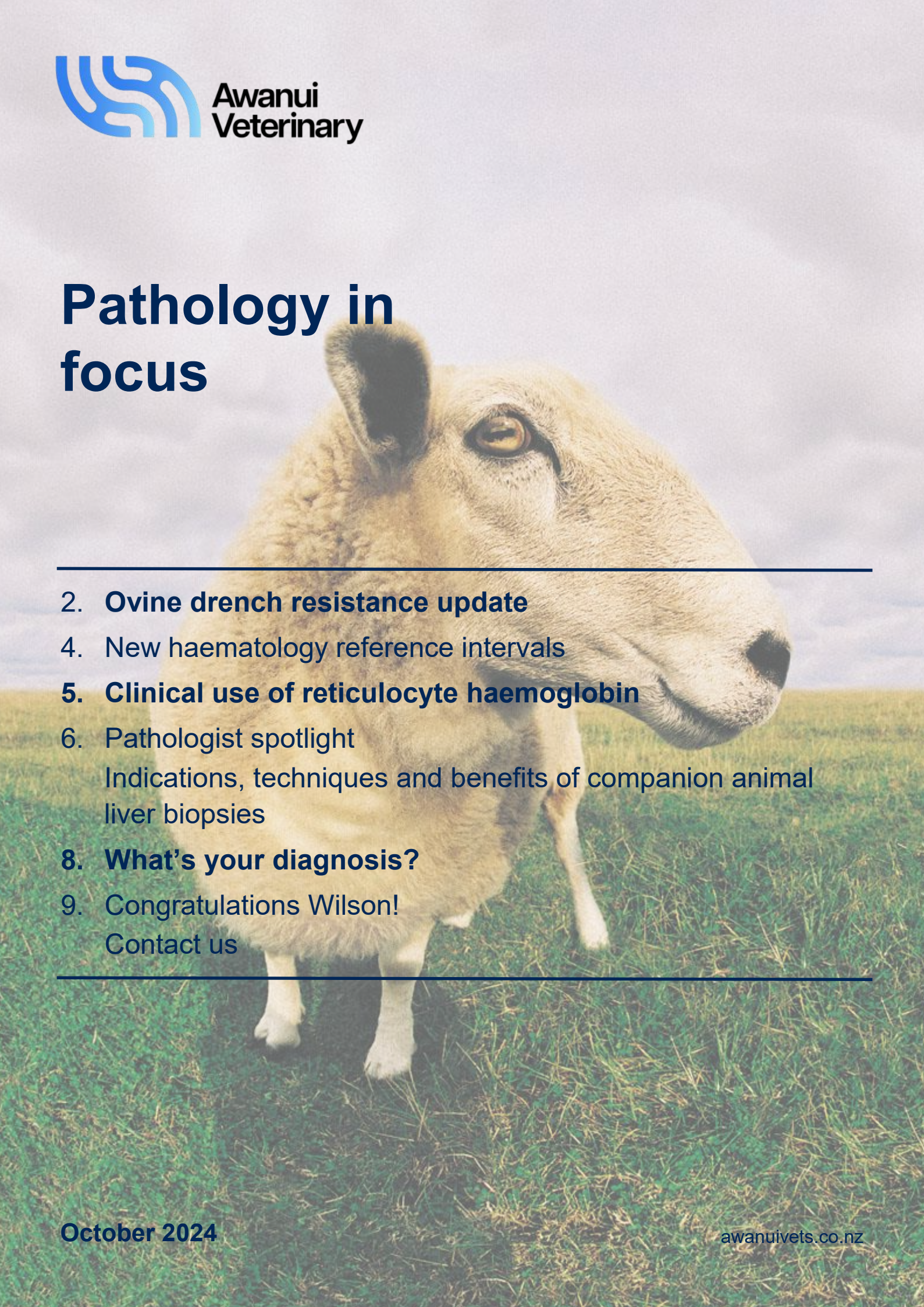
Awanui Veterinary

Pathology in focus



October 2024

awanuivets.co.nz

Ovine drench resistance update

Sarah Riddy

This report outlines the analysis of data submitted to the Awanui Veterinary network from June 2023 to May 2024 for fully differentiated faecal egg count reduction tests (FECRT) from sheep. The data was collected from cases where the test anthelmintic and sample groups were clearly identified, with a minimum of 10 animals per treatment group. To be considered valid, data at the genus level required a pre-treatment count of at least 50 eggs per gram (epg) per genus. All other test procedures and methodologies were as described by McKenna (2018).

Data was excluded if there were fewer than 50 epg of a genus in the pre-FECRT count or if the test anthelmintic was not clearly specified on the submission form. This season, only a small number of submissions were excluded due to incorrect identification of drench groups (using colours instead of anthelmintic names or active ingredients). This improvement reflects the ongoing educational efforts about FECRT across various sectors in New Zealand, resulting in a more accurate assessment of resistance nationwide. Submissions not clearly identified as FECRT were also excluded from the study. Additionally, submissions where part of the FECRT was conducted in-clinic, with only the larval culture analysed at Awanui Veterinary, were excluded due to non-compliance with the laboratory's accreditation and quality standards.

The final dataset included information from 173 farms over this period. The results of the analyses, which cover a total of 115 cases from the North Island and 58 cases from the South Island, are detailed in Table 1.

The percentage of resistance to double and triple active anthelmintics against *Trichostrongylus* and *Teladorsagia* spp. has shown a slight increase compared to previous updates (Riddy, 2022, 2023). However, it's worth noting that the use of double and triple anthelmintics has risen as single anthelmintics have become harder to obtain commercially. Some farms even included two different triple anthelmintic combinations in their FECRTs. Among the 173 farms tested with a triple combination anthelmintic, 51% (99 out of 196) of Trichostrongylus cases exhibited resistance, up from 36% (43 out of 120) reported the previous year (Riddy, 2023).

Classification of cases by geographical location for both anthelmintic and genus resistance percentages is illustrated in Figures 1 and 2. Figure 1 indicates a rise in resistance for triple anthelmintics in both the North and South Islands, as noted previously. Specifically, resistance in the North Island has increased from 20% and 23% to 45% in this report. Similarly, resistance in the South Island has risen from 8% and 12% to 34%, which is concerning (Riddy 2022 and Riddy 2023).

While passive data collection can introduce challenges in accurately reflecting the true situation on farms across New Zealand, this upward trend should be closely examined in discussions about farm management.

Trichostrongylus and *Teladorsagia* spp. remain the primary species of concern regarding anthelmintic resistance in New Zealand. However, this year's warmer weather has led to the appearance of Haemonchus in larval cultures further into the season than in previous years.

The data analysed above represents a snapshot of a subset of FECRTs conducted throughout New Zealand, as we can only evaluate the FECRTs submitted to Awanui Veterinary for comprehensive analysis. The results may be influenced by factors such as the location of the submitters, collection methods, and the reasons for conducting the FECRT on each farm.

Table 1: *The prevalence of anthelmintic resistance identified in sheep nematodes by fully differentiated faecal egg count reduction tests (FECRTs) undertaken on case submissions to Awanui Veterinary laboratory during 2023-2024 (n=173 farms).*

PARASITE	BZ	LEV	ABA	MOX	BZ/LEV	LEV/ABA	DERQ/ABA	ABA/MONE	ABA/CLO	TRIPLE
Cooperia	0/21 0%	0/35 0%	12/60 20%	16/76 21%	1/108 1%	1/95 1%	1/21 5%	0/97 0%	5/9 55%	7/158 4%
Haemonchus	0/4 0%	0/13 0%	0/42 0%	2/64 3%	0/84 0%	0/34 0%	0/10 0%	0/82 0%	0/9 0%	4/136 3%
Nematodirus	4/10 40%	3/22 14%	6/34 18%	9/27 33%	8/47 17%	3/33 9%	2/6 33%	3/50 6%	0/1 0%	11/76 15%
Oesoph/Chabertia	0/18 0%	1/30 3%	0/52 0%	0/44 0%	2/80 3%	0/34 0%	0/13 0%	1/54 2%	0/2 0%	2/109 2%
Teladorsagia	13/25 52%	16/38 42%	29/67 43%	41/78 53%	46/112 41%	25/63 40%	6/27 22%	14/101 14%	5/9 56%	56/176 32%
Trichostrongylus	14/26 54%	18/37 49%	29/69 60%	53/88 60%	79/128 62%	35/70 50%	9/28 32%	6/116 5%	3/9 33%	99/196 51%
TOTAL	31/104 30%	38/175 22%	76/324 23%	121/377 32%	136/559 24%	64/329 20%	18/105 17%	24/500 5%	13/39 33%	179/851 21%

Benzimidazole BZ, Levamisole LEV, Abamectin ABA, Moxidectin MOX, Derquantel DERQ, Monepantel MONE, TRIPLE includes several brands with 3 actives in combination.

Continued overleaf

A higher volume of FECRTs submitted to our laboratories would provide a more accurate picture of the situation across the country.

In conclusion, as we adapt our approach to parasite control in New Zealand, Awanui Veterinary will continue to monitor resistance through this passive surveillance method. The combination of education, development of control plans with minimal anthelmintic use, and current data on anthelmintic resistance equips us with effective strategies to address these challenges.

References

- McKenna PB. Update on the prevalence of anthelmintic resistance. VetScript 31 (5), 46–47, 2018
- Riddy SF. Update on the prevalence of anthelmintic resistance 2021–2022
- Riddy SF. Anthelminthic resistance update 2022-2023

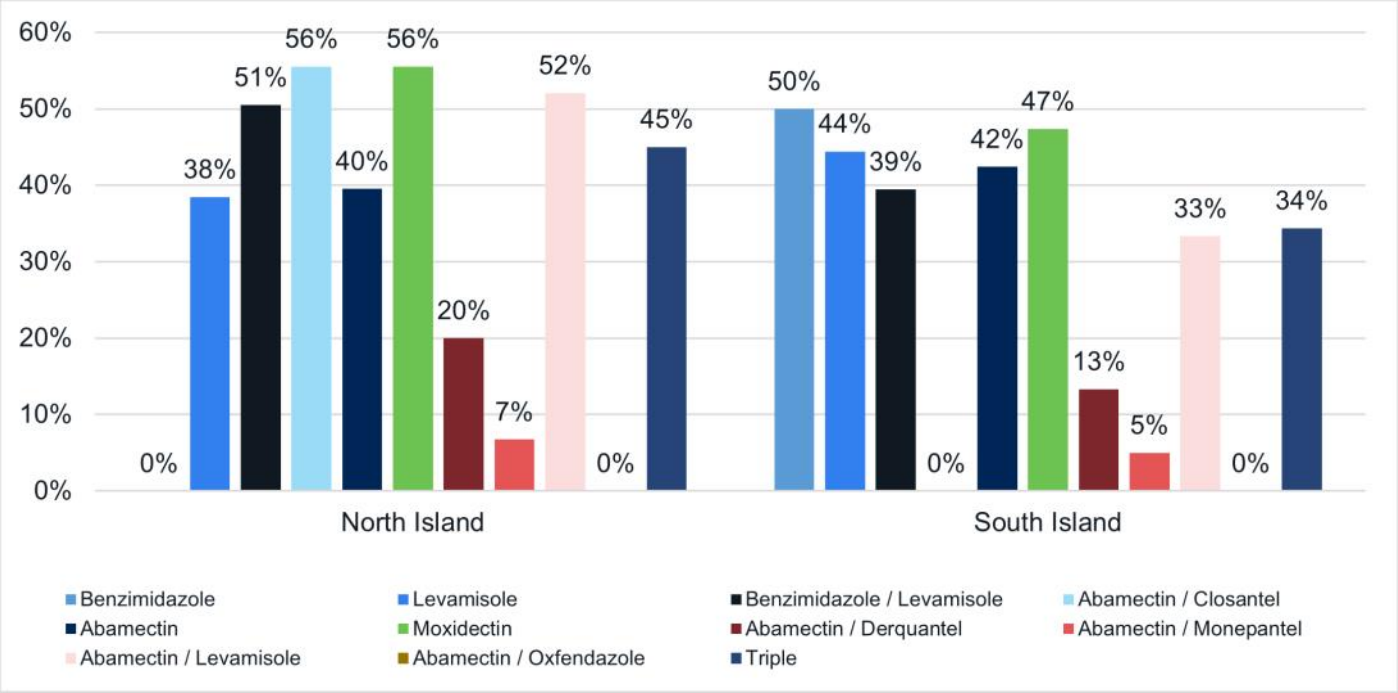


Figure 1: *Prevalence of resistance to anthelmintic recorded in sheep FECRTs submitted to Awanui Veterinary laboratories during 2023-2024 for the North Island and South Island (n=173 farms).*

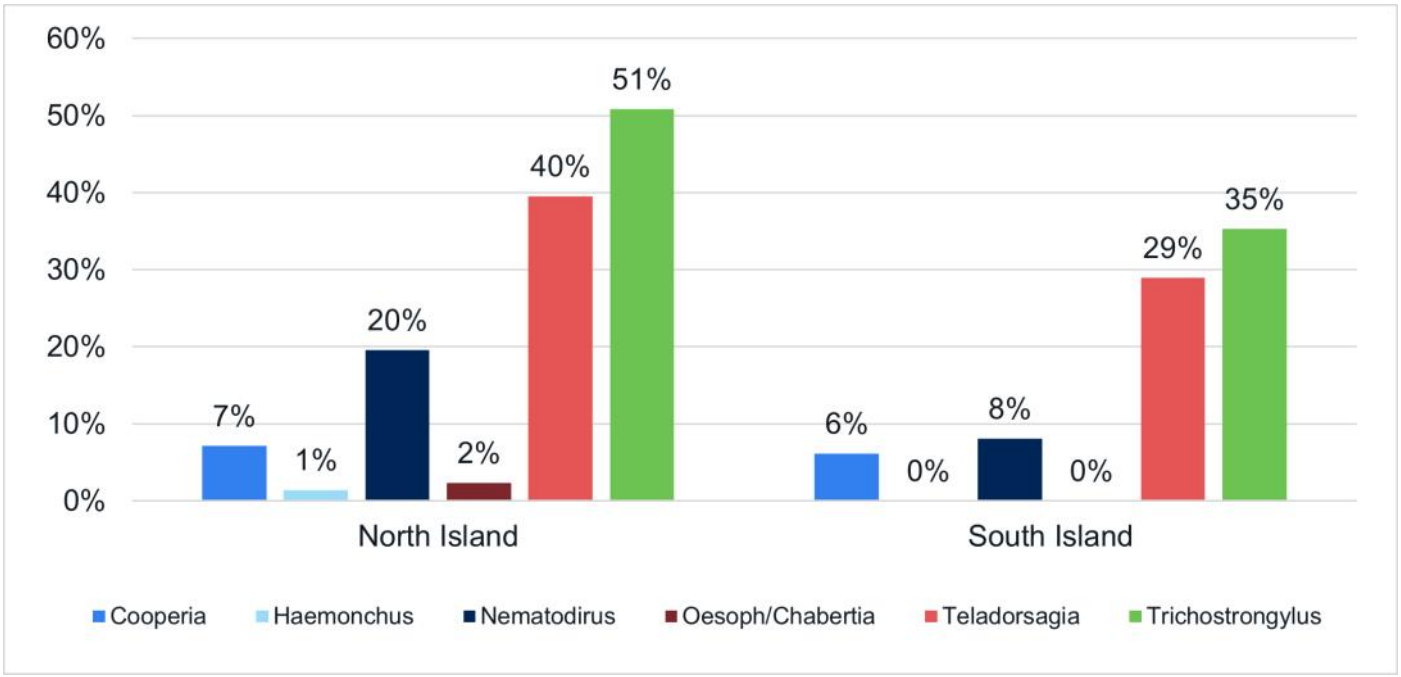


Figure 2: *Prevalence of resistance to anthelmintic to genus level recorded in sheep FECRTs submitted to Awanui Veterinary laboratories during 2023-2024 for the North Island and South Island (n=173 farms).*

New reference intervals

- canine and feline haematology

In case you missed our email in September, we are thrilled to announce our haematology reports now feature newly established reference intervals for cats and dogs (effective 30 September).

This milestone comes after three years of meticulous research and collaboration. Our large-scale reference interval study involved the dedicated efforts of numerous individuals, and we extend our deepest gratitude to all the clinics, their staff, and clients who participated. Your contributions have been invaluable in developing these world-class, local reference intervals.

Traditionally, reference intervals in veterinary laboratories or published literature have often been derived from less representative populations, such as young animals undergoing desexing or samples from research colonies. Our new intervals, based on a diverse and extensive sample of pet animals from New Zealand, represent a significant advancement and provide a more accurate benchmark for veterinary diagnostics.

Thank you once again to everyone involved for making this achievement possible. We are excited to offer this enhancement to our services and support better health outcomes for pets across the country.

Why have we updated the reference intervals?

Periodic re-evaluation of reference intervals is a crucial aspect of maintaining high standards in laboratory practice. Over time, reference intervals may no longer accurately reflect the animal populations they are intended to represent. Factors such as changes in breed popularity, de-sexing practices, diet, disease control, and genetics can all influence these intervals. Additionally, variations in sample collection and transportation methods, as well as advancements in analytical technologies, can impact pre-analytical and analytical processes.

Several years ago, we upgraded our haematology analysers to the latest state-of-the-art technology. This provided an excellent opportunity to review and update our New Zealand-based reference intervals. By doing so, we ensure that our intervals remain relevant and accurate, reflecting the current population of pets and the latest advancements in diagnostic technology.

What animals were included in the study?

To be included in the study, animals had to be fully fasted, clinically healthy, not on any medication or history of vaccination in the previous two weeks, not pregnant, not from an SPCA or pound (i.e. the animals had to have a known clinical history), aged between 1 and 10 years and for dogs, not sighthounds.

Full biochemistry and haematology panels were run on the samples and any samples with artifact (lipaemia, haemolysis) or signs of disease on the chemistry or haematology results were excluded from the study.

In total, samples from 151 dogs and 109 cats were included in the final reference sample group. The median age of the dogs was 4 years old (range 1-10 years old) with a near even number of males and females. Most dogs (61%) were desexed and over 41 breeds were represented, the most common being Border collies (19), Staffordshire bull terriers (12), huntaways (13), Labrador retrievers (12), Golden retrievers (9), heading dogs (9), standard poodles (6), Cavalier King Charles spaniels (5), Border terriers (5) and Jack Russell terriers (5).

The majority of cats included in the reference sample group were desexed pet cats, with 10 animals from a research colony. There was a near even number of males and females and the median age was 5 years (range 1-10yrs). Three quarters of the cats were DSH, DMH, DLH and 20 cats were pedigree cats (most commonly Burmese, Maine Coon and Ragdolls).

If you have any queries regarding these intervals or would like further information on the reference interval study, please feel free to contact us.

Clinical use of reticulocyte haemoglobin in CBCs

Kathryn Jenkins

Reticulocyte haemoglobin (RET-He) is a valuable diagnostic marker for iron-deficient erythropoiesis in both dogs and cats. RET-He reflects current iron availability and enables earlier identification of iron-limited states.

Reticulocytes are immature red blood cells (erythrocytes), which develop in the bone marrow and are released into circulation prior to maturation. Maturation time in dogs takes around two days, whilst in cats aggregate reticulocytes mature within one day (leaving maturing punctate reticulocytes to circulate for a further 2 weeks). In routinely stained blood films, most reticulocytes appear as polychromatophils due to the RNA staining a diffuse blue. Special stains (e.g. New Methylene Blue, or analyser fluorescent stains) are used to accurately count reticulocytes, see Figure 1.

Iron deficiency can be either absolute or functional. Absolute iron deficiency occurs with decreased iron stores such as chronic blood loss (most common cause in dogs), or nutritional deficiency (uncommon in veterinary medicine). Functional iron deficiency occurs with normal iron body stores, but reduced availability of iron for erythropoiesis. This can occur with either anaemia of inflammation/chronic disease (most common cause in cats), or with portosystemic shunts (due to inadequate protein synthesis required for iron metabolism).

Iron deficiency anaemia secondary to chronic blood loss occurs as a consequence of both haemorrhage and reduced life span of fragile red blood cells. The characteristic morphologic changes of iron deficiency anaemia occur with chronicity, due to the relatively long lifespan of erythrocytes (around 100 days in dogs, and 80 days in cats). Initially, iron deficiency anaemia appears regenerative, and with time presents as small (microcytic) erythrocytes with increased central pallor (hypochromic, target cells) and morphologic changes associated with increased fragility (e.g. schistocytes, keratocytes) see Figure 2. Decreased RBC indices (MCV, MCH and MCHC) are seen in advanced cases, but are often within reference in early stages as they are insensitive to changes in small proportions of erythrocytes.

Anaemia of inflammatory disease occurs as a consequence of suppression of erythropoiesis (via inflammatory cytokines), reduced iron availability (via IL-6 upregulation of hepcidin), and extravascular haemolysis. These cases often present with unremarkable appearing erythrocytes on blood film review (i.e. normochromic, normocytic). However, with protracted cases, anaemia of inflammatory disease may also present with microcytic hypochromic erythrocytes. PCV in these cases is usually not lower than 20%, and poikilocytosis (such as schistocytes, keratocytes) are not typical. There may be an inflammatory leukogram and protein changes may include decreased albumin with increased globulin.

It is also possible to have cases of combined blood loss and inflammation.

RET-He content is decreased with both absolute and functional iron deficiency, and allows earlier detection of clinical cases prior to morphologic changes being detectable on the blood film or notable decreases in MCV, MCH and MCHC.

RET-He is measured by reference laboratory analysers (including the Sysmex XN used at Awanui Veterinary), and the ProCyte Dx in-house analyser. In cases of iron-deficient erythropoiesis, RET-He level is expected to be decreased. As reticulocytes circulate for a short time in peripheral blood, decreased RET-He is an early indicator of iron deficient erythropoiesis.

RET-He may appear falsely increased with macrocytosis, or rare cases presenting with microcytosis without hypochromasia (including breed related

Figure 2: Iron deficiency anaemia in a puppy. The erythrocytes appear small (microcytic), with increased central pallor (hypochromic), and increased target cells (bulls eye morphology, black arrow). There are also frequent schistocytes (blue arrow) indicating erythrocyte fragility. RET-He for this case was 14.4 pg.

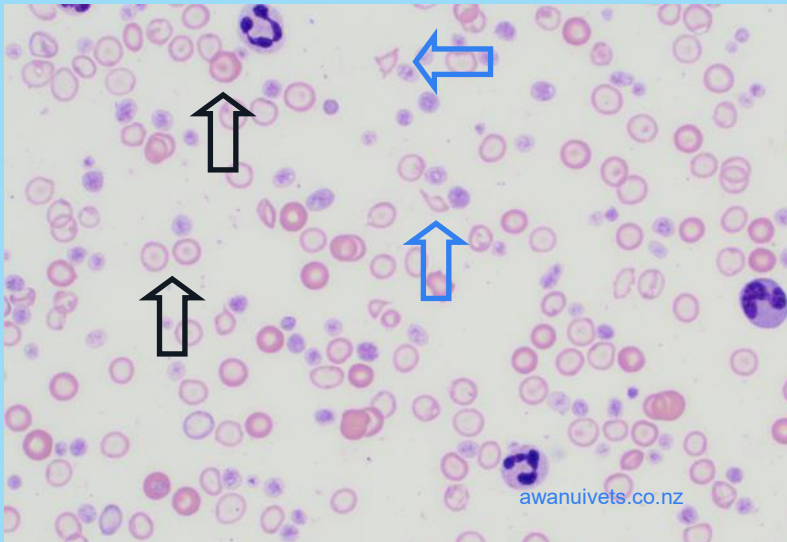
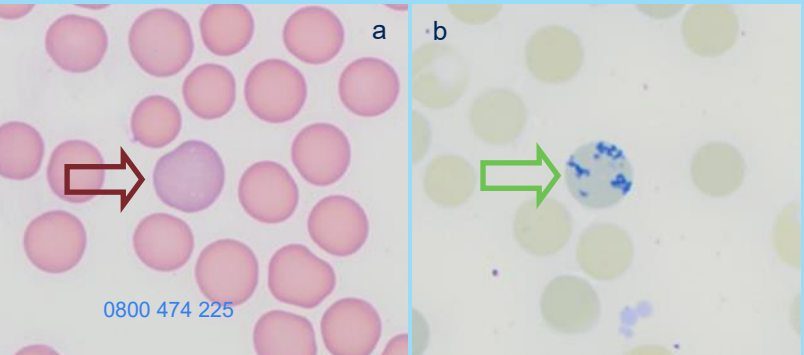


Figure1: Immature erythrocytes. a) A polychromatophil (red arrow) in a routine stain (appears larger and more basophilic than mature erythrocytes). b) This same cell would be called a reticulocyte if we use either a NMB stain (green arrow) which causes the RNA to clump in a reticular pattern, or with fluorescent stains that are used in some haematology analysers.



microcytosis in Akita and Shiba Inu, Chow Chow or Shar Pei). RET-He appears stable over 48 hours, however analysis of fresh blood is recommended regardless due to effects of storage artefact on other parameters.

Published studies suggest a RET-He below 20.9 pg supports iron deficient erythropoiesis in dogs, and below 12.5 pg supports iron deficient erythropoiesis in cats. Further research is required to see if RET-He levels can help differentiate absolute vs functional iron deficiency.

In cases of anaemia in clinical practice, a blood film review is an essential component of a full CBC work-up. Your local Awanui Veterinary pathologists are always here to help.

References:

- Keiner et al. Evaluation of reticulocyte hemoglobin content (RETIC-HGB) for the diagnosis of iron-limited erythropoiesis in cats. *Veterinary Clinical Pathology.* 49, 2020.
- Fuchs et al. Canine reticulocyte hemoglobin content (RET-He) in different types of iron-deficient erythropoiesis. *Veterinary Clinical Pathology.* 46/3, 2017.
- Fuchs et al. Evaluation of reticulocyte hemoglobin content (RET-He) in the diagnosis of iron-deficient erythropoiesis in dogs. *Veterinary Clinical Pathology.* 46/4, 2017.

Pathologist spotlight

Kathryn Jenkins joined our team in 2019 and is based in our Palmerston North laboratory. She earned her BVSc from Massey University and spent several years working in companion animal practice in New Zealand. She then returned to Massey to complete a residency in clinical pathology. After finishing her residency, Kathryn successfully passed both Membership exams in pathology and obtained American Board Certification in clinical pathology.

Kathryn especially enjoys all aspects of haematology and cytology of cats, dogs, small mammals, birds and exotic species.

Indications, techniques and benefits of companion animal liver biopsies

Bernie Vaatstra

Liver disease is very common in dogs and cats and frequently presents a diagnostic challenge. A survey of 1,725 cat veterinary visits found a prevalence of liver disease or about 7% diagnosed through laboratory and imaging studies (Melchert et al, 2016). A post-mortem survey of 200 unselected dogs from first opinion practice found a 12% prevalence of chronic hepatitis, suggesting overall liver disease prevalence is even higher (Watson et al, 2010).

Liver disease may be primary or develop secondary to systemic disorders (e.g. endocrinopathy, GI and pancreatic disease). Primary liver disorders are broadly classified by the WSAVA as vascular, biliary, parenchymal, or neoplastic (Rothuizen et al, 2006).

Some animals have multiple overlapping processes.

Given the multiplicity of metabolic and immunological functions performed by the liver, disease may induce a wide range of non-specific clinical manifestations. See Table 1.

Diagnosis of liver disease therefore relies on synthesis of a range of clinicopathological findings - clinical signs, serum biochemistry (liver enzymes, function tests, pancreatic lipase, and serum proteins), tests for infectious agents (e.g. Leptospira serology/PCR, *Toxoplasma* and *Neospora* serology, bacterial cultures), imaging, fine needle aspirate, and biopsy. While all these techniques have their advantages and limitations, biopsy remains the gold standard for accurate diagnosis.

However, information gleaned from a biopsy depends heavily on appropriate case selection, careful technique, and adequate sample handling and preparation.

Table 1. *Clinical signs occurring in decreasing order of probability in dogs with chronic hepatitis (Webster et al. 2019).*

Clinical signs of chronic hepatitis in dogs	% affected
Decreased appetite	61
Lethargy/depression	56
Icterus	34
Ascites	32
PU/PD	30
Vomiting	24
Diarrhoea	20
Hepatic encephalopathy	7.1
Melena	6.1
Abdominal pain	3.1

Liver biopsy indications

Indications for liver biopsies in companion animals include the following (Unterer 2017):

1. Persistent elevation in liver enzymes (serum ALT, AST, and ALP)
2. Abnormal liver function tests (serum bile acids, albumin, bilirubin, BUN, cholesterol, glucose)
3. Imaging evidence of structural liver disease including masses
4. Breed associated liver diseases

Prior to biopsy, non-hepatic causes of liver enzyme increases should be excluded. In addition, infections such as leptospirosis and FIP should be ruled out. Risk of haemorrhage also needs to be considered. A study of dogs undergoing percutaneous ultrasound guided liver biopsy reported a high incidence (42.5%) of clinically silent, major haemorrhage, but few complications (1.9%). While it has been historically advised to assess haemostasis parameters prior to liver biopsy, the same study showed no correlation between mild abnormalities in haemostasis as detectable by routine tests (PT, APTT, platelets) and risk of post-biopsy haemorrhage (Reece et al, 2020). Ultimately, the decision to biopsy is a clinical one weighing up the risks and benefits.

Liver biopsy technique

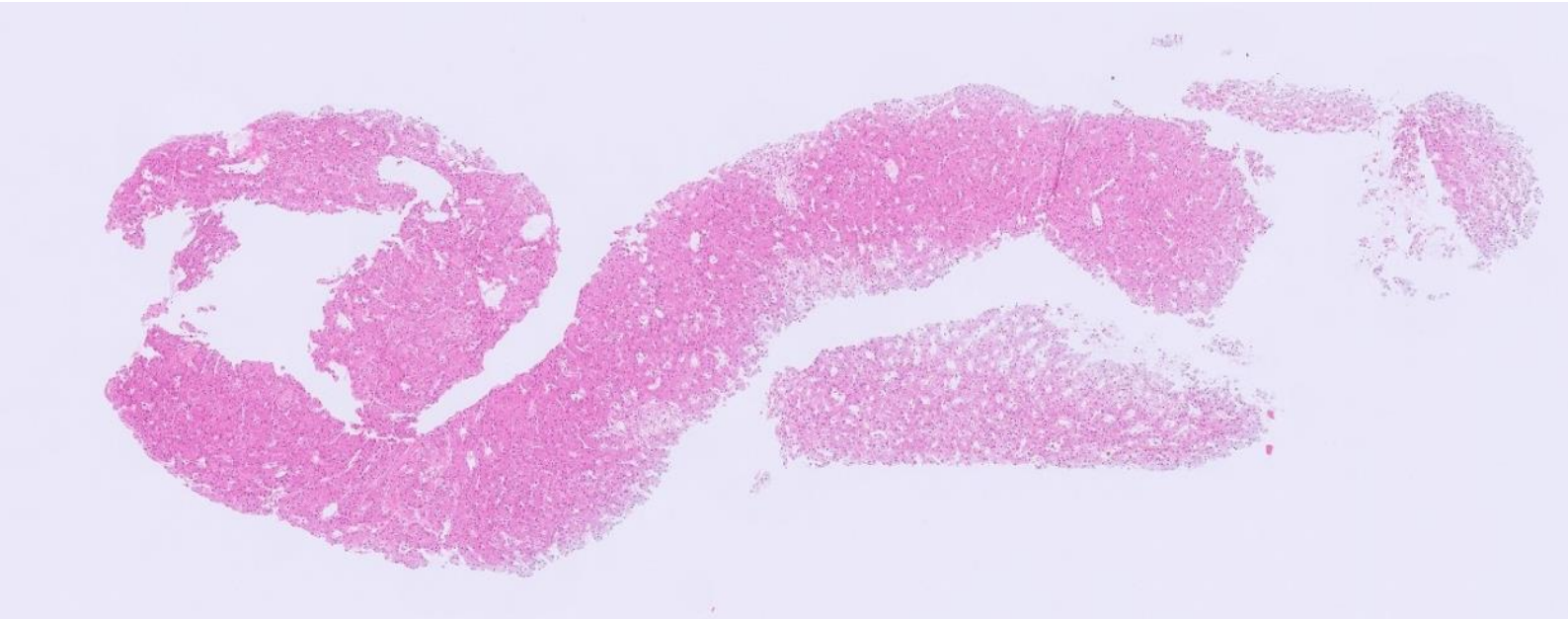
In order to accurately evaluate all compartments of the liver (hepatocellular, biliary, vascular, interstitium), samples need to include enough architecture. Ideally, 12 -15 portal tracts should be examined to have confidence in diagnosing or excluding lesions of the biliary tree and vasculature (see Figure 1). Practically, this usually means submitting three biopsies from different lobes. Avoid the quadrate lobe (adjacent the gallbladder) due to the presence of artefact. Avoid the caudate lobes if assessing for vascular anomalies such as portal vein hypoperfusion. This leaves the left and right medial and lateral lobes as the best locations to sample, unless targeting a focal lesion.

Studies have shown that surgical wedge biopsies are the best for diagnosis of liver diseases. However, given the morbidity involved in this procedure, ultrasound guided percutaneous needle/trucut biopsies (14g for larger dogs, 16g or 18g for smaller dogs and cats), punch biopsies (8mm) and laparoscopic cup (5mm) biopsies can be used as alternatives. It is important to avoid crush trauma with the cup and punch biopsies in particular. Take into account that the diagnostic accuracy of biopsies obtained by needle, cup or punch ranges from 60-70% when compared with large wedge biopsies (Kemp et al, 2015).

Liver biopsy uses

Biopsy helps to categorise the pathology, estimate chronicity, guide management, evaluate progress, and inform prognosis. Targeted histochemical stains can be used for identifying and quantifying fibrosis, assessing

Figure 1. *Tru-cut liver biopsy from a dog with persistently increased liver enzymes. A single needle biopsy was submitted which lacked portal tracts, limiting interpretation. H&E 20x.*



architectural collapse or regeneration, estimating copper stores (Figure 2), visualising glycogen or lipid, and identifying microorganisms. In some cases, liver biopsy may only provide a morphological diagnosis and a list of suggested differentials, whereas in others, a more specific aetiological diagnosis is possible.

Scenarios where biopsy is particularly useful, and often essential for diagnosis, include:

- Canine chronic hepatitis vs non-specific reactive hepatitis
- Feline lymphocytic cholangitis vs hepatic lymphoma
- Feline neutrophilic cholangiohepatitis vs lymphocytic cholangitis
- Benign vs malignant neoplasia
- Primary vs metastatic neoplasia
- Glycogen vs lipid vacuolation
- Portal vein hypoperfusion
- Ductal plate malformation
- Copper hepatopathy (in conjunction with liver copper analysis)
- Identification of infectious agents (protozoa, bacteria, mycobacteria, fungi, viral inclusions)
- Toxic hepatopathies
- Chronic passive congestion
- Cirrhosis
- Storage disorders

Limitations

The value of liver biopsy may be limited by factors such as non-representation of multifocal or regional lesions, handling artefact, sampling from inappropriate sites, and the presence of chronic end stage or resolved lesions. Interpretation is always made in light of the clinical picture and, ideally, serial biochemistry, imaging, and function tests.

References

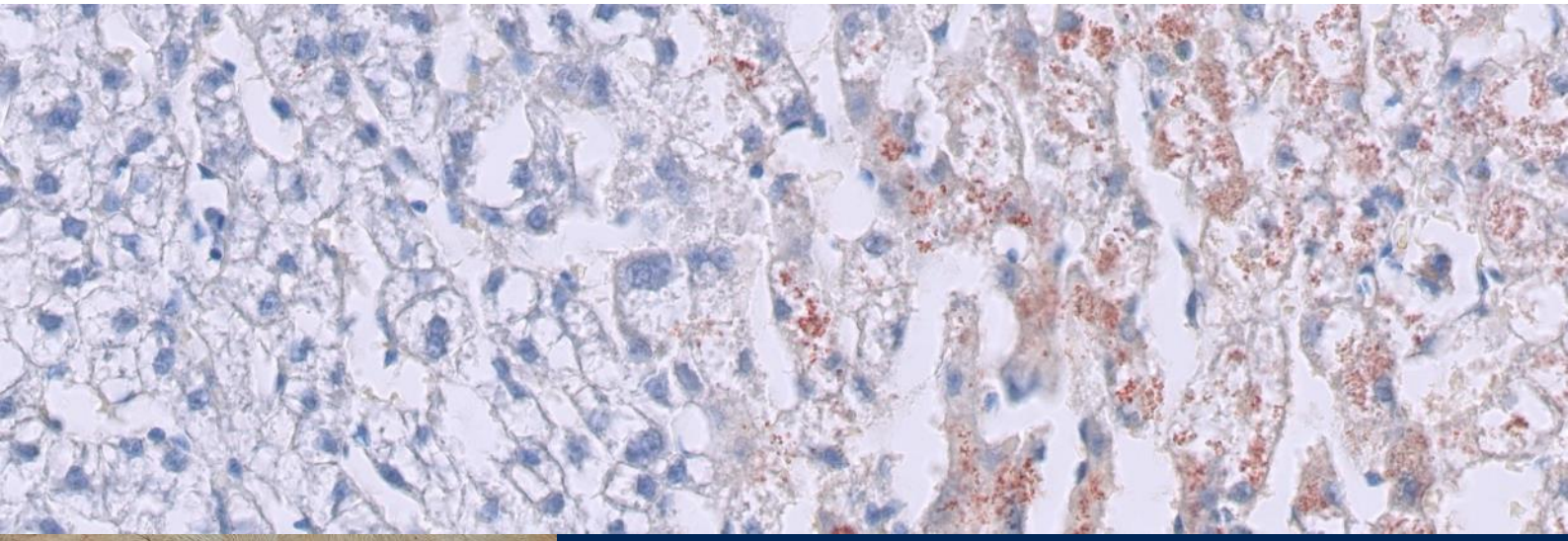
Kemp SD, Zimmerman KL, Panciera DL, Monroe WE, Leib MS, Lanz OI. A comparison of liver sampling techniques in dogs. *J Vet Intern Med*. 29:51-7, 2015

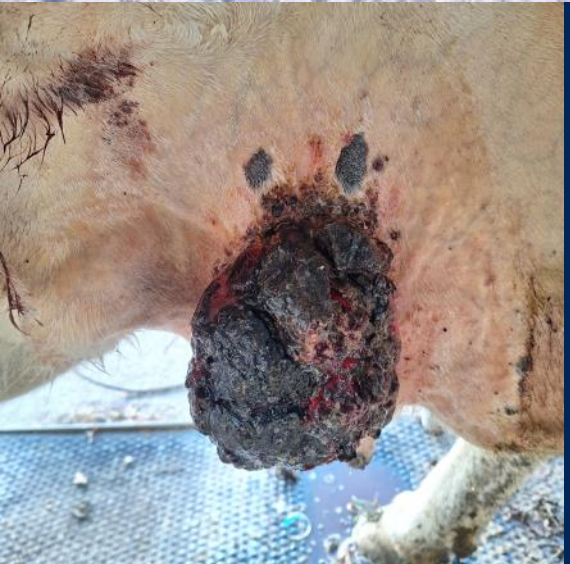
Melchert A, Souza FB, Guimaraes-Okamoto PTC. Prevalence of Liver Disease in Cats from Botucatu City, Sao Paulo State, Brazil. *World Small Animal Veterinary Association Congress Proceedings*, 2016.

Reece J, Pavlick M, Penninck DG, Webster CRL. Hemorrhage and complications associated with percutaneous ultrasound guided liver biopsy in dogs. *J Vet Intern Med.* 34:2398-2404, 2020.

Rothuizen J, Bunch SE, Charles JA. *WSAVA standards for clinical and histological diagnosis of canine and feline liver disease*: Elsevier Health Sciences, 2006.

Figure 2. *Liver biopsy from a dog with excessive copper accumulation. The hepatocytes on the right of image contain characteristic red-brown granules. Rhodanine copper stain 400x.*





What's your diagnosis?

A monthly spot quiz

Test your skills with this gross photo:

12 year-old Friesian cow with ulcerated slow-growing mass near the cranial aspect of udder. Does not seem to be affecting milk production. Not obviously painful during milking.

What's your diagnosis? *(Answer on last page).*

Congratulations Wilson!

Wilson Karalus is our latest Diplomate of the American College of Veterinary Pathologists (ACVP). We congratulate Wilson on successfully passing this esteemed examination!

Wilson joined our team at the start of this year. He graduated from Massey University in 2013 and spent his first three years in dairy practice in Canterbury. He then moved to Portland, Oregon, where he worked as a small animal general practitioner and emergency veterinarian for four years. Following that, he returned to Massey University to complete a residency in anatomic pathology. He has since earned an MVS from Massey and now holds the Dip. ACVP (Anatomic Pathology).

Wilson enjoys all aspects of diagnostic pathology and is particularly interested in production animal infectious diseases, and neoplasia of any species.

From page 4: What's your diagnosis? Squamous cell carcinoma. The udder is an uncommon location for this tumour, although it has previously been reported in cattle. Development on non-pigmented skin is suggestive of a UV radiation as a potential cause. SCCs are locally infiltrative and metastasis to the regional lymph nodes can occur late in the disease course. A check of the animal for evidence of lymphadenopathy involving local lymph nodes should be carried out.

All of our laboratories will be **closed on Monday October 28** for the Labour day public holiday.

Contact us

- contacting Awanui Veterinary couldn't be easier.

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