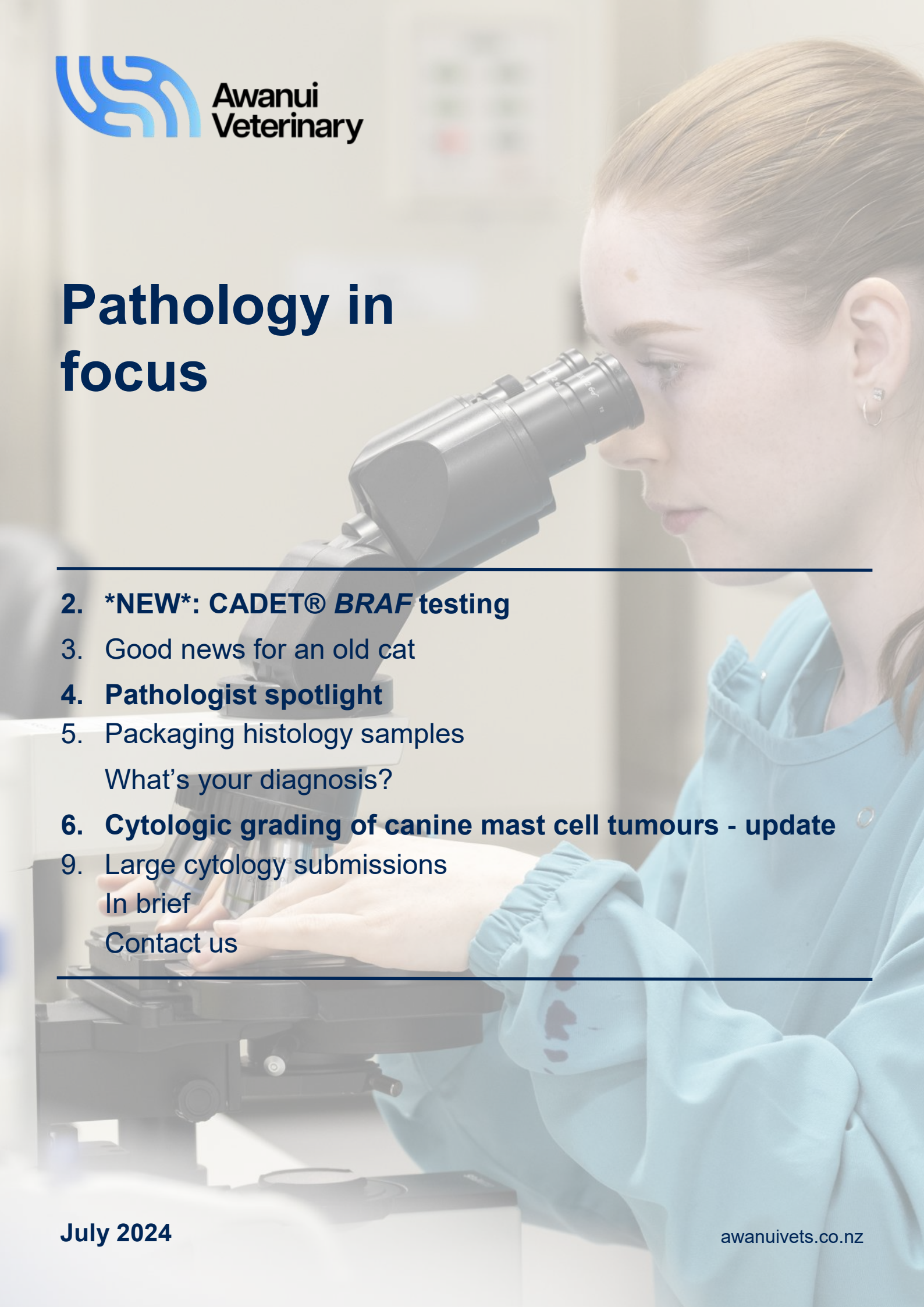
Awanui Veterinary

Pathology in focus



July 2024

awanuivets.co.nz

CADET® *BRAF* testing now available

- accurate canine bladder and prostate cancer test

If you visited our stand at the NZVA conference you will know that we are very excited to be able to now offer CADET® *BRAF* testing. Samples will be referred overseas for testing.

CADET® *BRAF* evaluates urine samples from dogs for the presence of cells containing a mutation for canine bladder/prostate cancer (TCC/UC). It's cutting-edge technology that is accurate and convenient for both veterinarian and pet owner.

Achieve accurate and early diagnosis of TCC/UC

CADET® *BRAF* is a highly sensitive test designed to monitor the b-raf mutation in TCC/UC cases during the course of their treatment, for therapeutic response and relapse. CADET® *BRAF* testing can be used for both the rapid, non-invasive assessment of dogs displaying clinical signs consistent with TCC/UC and for confirmed cases undergoing treatment.

How CADET® BRAF is used in a clinical setting

CADET® BRAF evaluates free-catch urine samples from dogs for the presence of cells harbouring the BRAF mutation or specific copy number variations associated with TCC/UC. The assays identify 95% of TCC/UC cases. The extremely low limit of detection of 10 mutation-bearing cells in a urine sample allows early diagnosis of a developing TCC/UC, often several months before any advanced clinical signs associated with the cancer become evident.

Clinical indications - when to use the CADET® BRAF and BRAF-PLUS

- Clinical cases presenting with haematuria, stranguria, and/or urinary incontinence with diagnostic imaging evidence of a mass in the bladder.
- During chemotherapy to monitor treatment success by decreased levels of b-raf mutation detection, or to monitor cancer relapse by re-occurrence of b-raf mutation tumour-bearing cells.
- Early diagnosis in clinical cases with recurrent, complicated or antibiotic-resistant urinary tract infections presenting with haematuria without ultrasonographic evidence of a bladder mass.
- Confirmation of the TCC/UC diagnosis of a bladder mass from a stained cytology slide following ultrasonography and cytological examination of a fine -needle aspirate from tumour-bearing cells.
- Early detection in high-risk dog breeds such as Terriers, Shetland and Australian sheep dogs, cattle dogs, Beagles and Border collies that are six years and older.

Sample collection and handling

Species: Canine

Specimen: 40 mL free-catch urine collected in CADET BRAF container

Container: CADET BRAF container (obtain from laboratory, no charge)

Collection protocol:

Urine must be placed in BRAF container within 15 minutes of collection and can be collected over multiple days. Containers can be obtained free of charge by contacting your local laboratory.

Special handling/shipping requirements:

Do not freeze, store urine in BRAF container at room temperature.

Turn-around time: 10-14 days

Pricing: Please contact your local laboratory.

This information is now on our website and you can download a flyer here. If you require any further information or would like to order a sample container, please contact your local laboratory.

Information and testing services provided by Asia Diagnostics, Hong Kong (Antech Asia Division).

Good news for an old cat

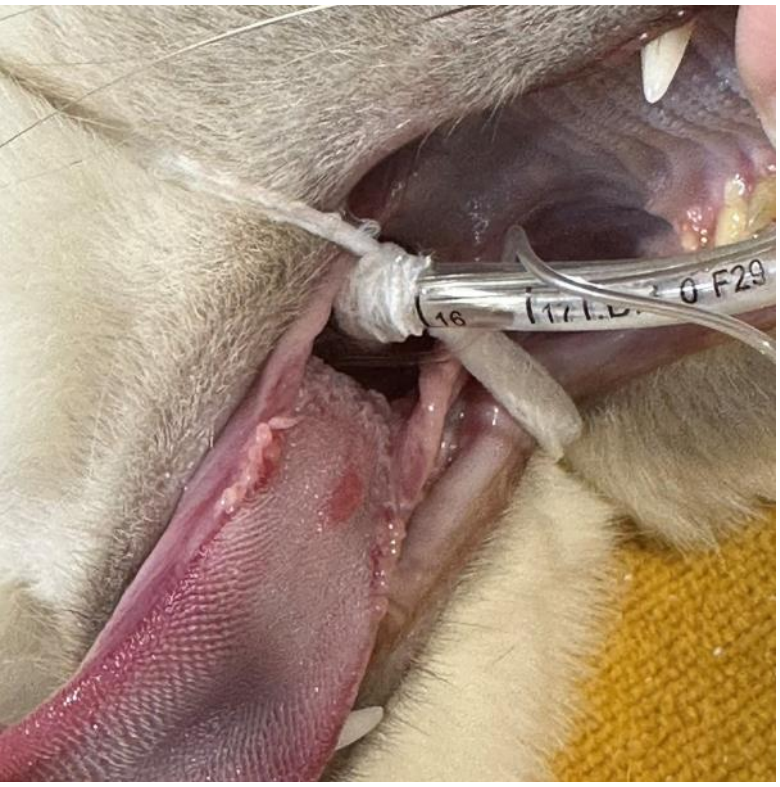
Emma Gulliver

This case explores a congenital cause of a mass lesion in the tongue of a senior feline.

Clinical history:

Jasper, a ten-year-old male neutered Tonkinese x Rex cat, presented for an ulcerative lesion on the dorsal surface of the tongue (Figure 1). He had a history of self-trauma and scratching at the face. The lesion was surgically excised and submitted for histopathological examination.

Figure 1: A small ulcerative lesion on the dorsal midline at the base of the tongue. Photo credit: Zachary Hon.



Laboratory findings:

The submitted tissue consisted of a 2 x 2 x 4 mm fragment of glossal mucosa (Figure 2A, arrow) and submucosa, with some deep skeletal muscle (Figure 2A, asterisk). In the superficial portion of the submucosa, there was a duct-like structure, lined by epithelium that was variably stratified squamous and parakeratinised, to pseudostratified columnar and ciliated (Figure 2B).

Expanding the submucosa adjacent to this was a multilobular aggregate of well differentiated epithelial tissue resembling thyroid gland. This was characterised by follicular structures filled with smooth, eosinophilic material resembling colloid and lined by a single layer of low cuboidal epithelial cells (Figure 2C).

Diagnosis:

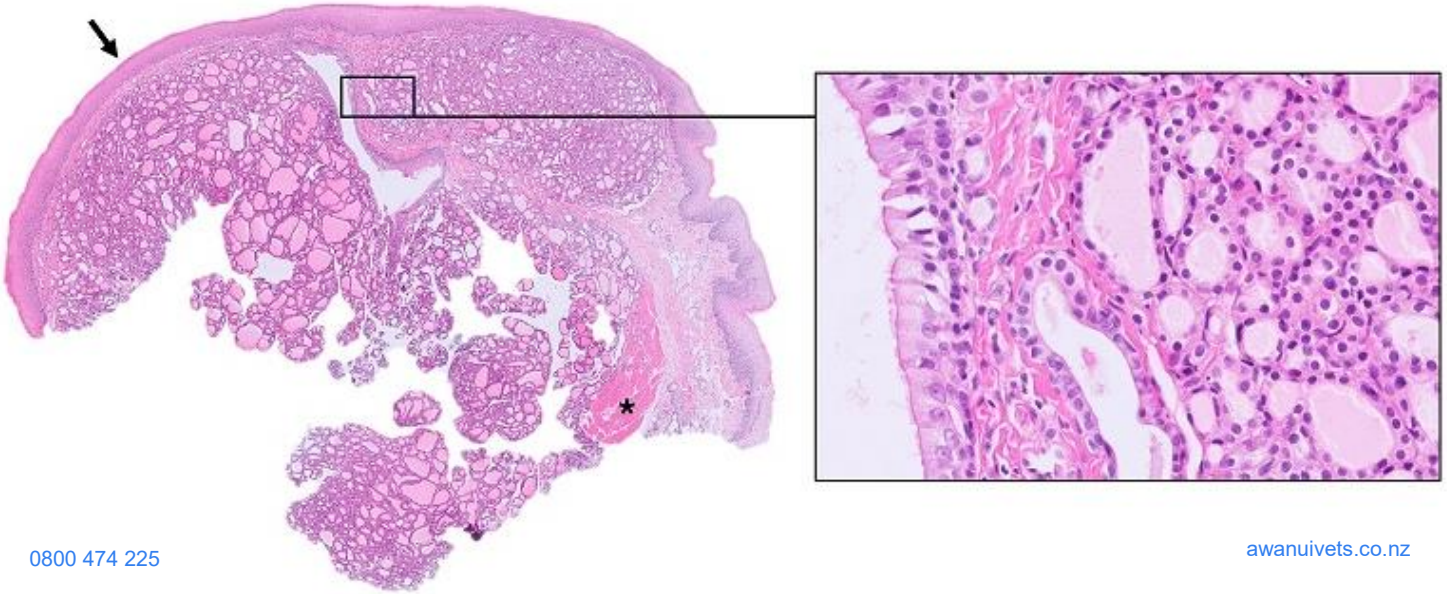
Thyroglossal duct remnant and ectopic thyroid tissue.

Discussion:

This is an uncommon mass lesion of the tongue in an older cat and is a result of anomalous embryonic development. The thyroid gland first develops around 17 -18 days gestation in the feline foetus and is derived from the pharyngeal pouches of the embryonic endoderm. As the thyroid bud migrates caudally from the base of the oropharynx, its path is marked by the thyroglossal duct. This duct usually atrophies in postnatal life and the site of origin of these structures is denoted the caecum linguae. The pharyngeal pouches also give rise to the ultimobranchial bodies, from which the parathyroid tissue is derived and fuses with the thyroid tissue as it migrates, around 19-21 days gestation[1].

Continued overleaf

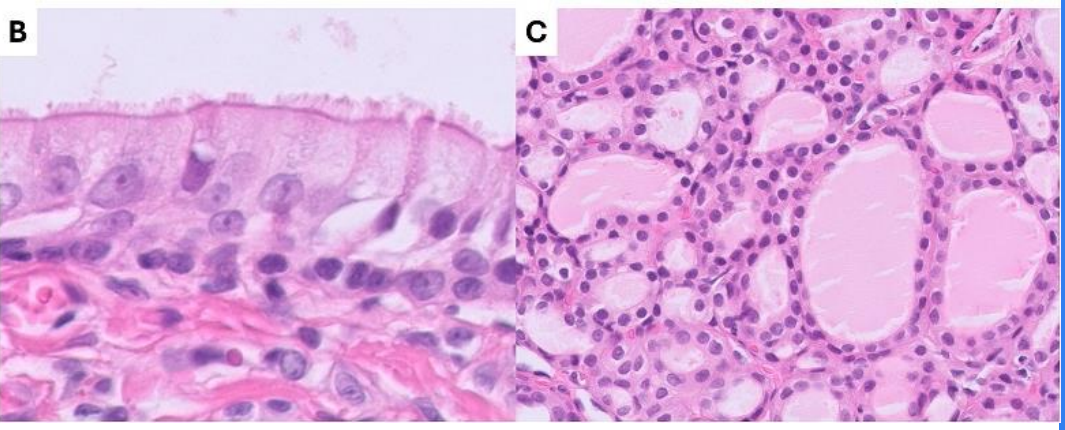
Figure 2A: Histology of the lesion. The submucosa is expanded with a multilobular proliferation of epithelial tissue and there is a duct in the superficial portion (inset). Arrow = glossal mucosa, asterisk = skeletal muscle.



Aberrant migration of the embryonic thyroid gland may lead to develop of ectopic ('accessory') thyroid tissue and failure of the thyroglossal duct to regress postnatally may lead to a persistent duct, which can form a cystic lesion. Ectopic thyroid tissue has been reported rarely in cats including formation of nodular to cystic lesions on the midline of the base of the tongue[2,3]. Ectopic thyroid tissue is relatively more common in the dog and can be found anywhere between the base of the tongue and the base of the heart[4]. From the limited reports in the literature, these lesions are usually amenable to surgical excision. It is reported widely in humans and affected people are usually euthyroid, with ectopic tissue generally present only at one site.

Considering Jasper's signalment and the gross appearance of the lesion, other differential diagnoses include eosinophilic granuloma, squamous cell carcinoma, plasmacytic stomatitis/glossitis, proliferative granulation tissue secondary to trauma, viral papilloma and foreign body. Histopathology allowed these other differential diagnoses to be excluded. A six-month follow up on Jasper indicated that there was no clinical concern for hyperthyroidism. The ulcerative lesion had healed, and he had stopped pawing at his face following the surgery.

Figure 2A & B: Histology of the lesion. (B) Part of the duct lining is pseudostratified columnar epithelium with a ciliated surface. (C) The epithelial structures resemble thyroid follicles filled with colloid.



References:

1. Knospe C. Periods and stages of the prenatal development of the domestic cat. *Anatomia, histologia, embryologia*. 31:37-51, 2002.

2. Patnaik AK, Peterson ME, Hidgon A. Ectopic lingual thyroid tissue in a cat. *Journal of feline medicine and surgery*. 2:143-146, 2000.

3. Reed TP, Brisson BA, Schutt LK. Cystic ectopic lingual thyroid tissue in a male cat. *Journal of the American Veterinary Medical Association*. 239:981-984, 2011.

4. Maxie G. Jubb, Kennedy & Palmer's Pathology of Domestic Animals. 6th ed. Elsevier Health Sciences. 2015.

Acknowledgements to Dr Simeon Pollock and Dr Zachary Hon at Glenfield Veterinary Clinic for their submission and clinical follow up.

Pathologist spotlight

Emma Gulliver joined our team at the beginning of 2023.

Emma completed her undergraduate veterinary degree at the University of Sydney in 2013. She took a gap year to travel Europe followed by over 5 years working as a small animal clinician in various locations throughout Australia and the UK. She decided to become a pathologist early in her veterinary career and subsequently completed her masterate and residency training at Massey University. She became a Diplomate of the American College of Veterinary Pathologists (Anatomic Pathology) in 2023.

Emma's areas of interest include neoplasia, immunopathology and companion animal disease. She has completed research projects on ovine Johne's disease, causes of mortality in kiwi and avian malaria in kiwi.

She lives in Auckland with her family and dynamic duo moggy and terrier cross.

Packaging histology samples

We've had some 'interesting' histology submissions lately, so we thought it was a good time to remind you of how samples should be packaged to avoid disasters and to best preserve the samples for examination.

Please do:

> Submit histology samples separately from any specimens for cytology. Don't put sample containers in the same specimen bag as cytology slides, as formalin fumes adversely affect the staining of smears impacting diagnosis.

> Put tissue samples in wide mouth jars, as fixed tissue becomes quite firm and warps in fixation; whilst you may have been able to squeeze it into a container, it can then conform to the shape of the container and may be impossible for us to retrieve it without cutting it or breaking the container.

> Use appropriate leak-proof specimen containers, to ensure the samples pose no biological or chemical risk during transport or on receipt at the laboratory. And make sure the lids are on tight. **Please no** food containers or repurposed pill bottles or teat seal containers, and definitely no gloves (photo 1).

> Use an adequately sized container - the rule of thumb for tissue:formalin volume is 1:10.

> Place some absorbent material in the specimen bag just in case the sample leaks.

> Provide a detailed clinical history and include some drawings or send some photos if you think it will help.

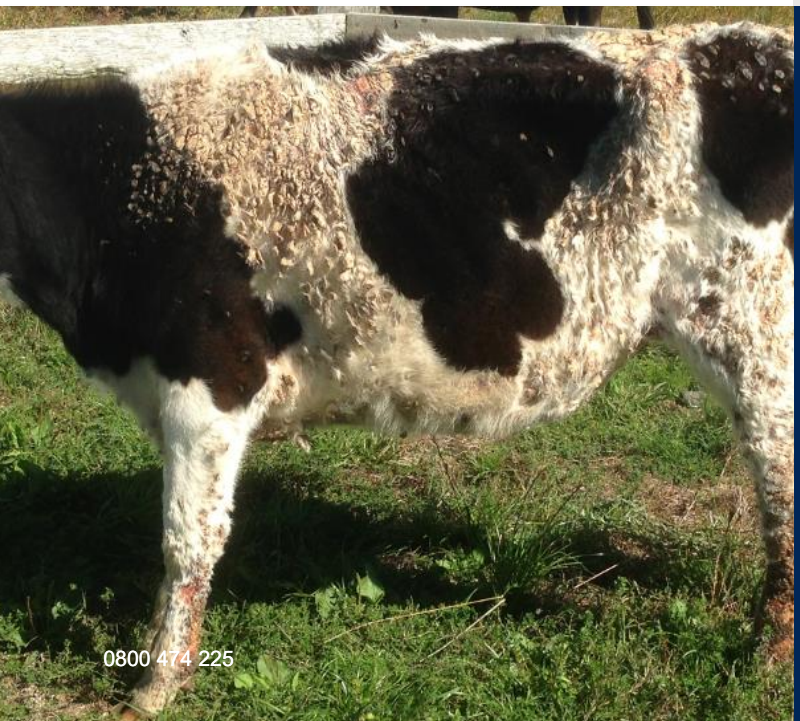
> Put the submission form in the pocket on the outside of the sample bag so it stays nice and dry and legible.

> Give us a call to discuss any tricky samples, we're happy to help anyway we can.

More information is available on our website:

- Vet Handbook - General information histology
- How to package a sample for transport

Photo 1: Formalin fixed sample received in a plastic glove and cardboard box.



What's your diagnosis?

A monthly spot quiz

Test your skills with this gross photo:

This 18-month-old heifer had conical "horn" like plaques over the majority of her body. She was pyrexic.

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

Cytologic grading of canine mast cell tumours

Sandra Bulla

An update:

Mast cell tumours (MCT) are the most common skin neoplasia in dogs, shown to occur with a frequency of approximately 20% of all canine skin tumours. Although it can occur in any breed, the prevalence is consistently reported in many studies from different countries to be higher in some breeds. Some examples of most affected dogs are Boxers, Labradors, Golden Retrievers and Bull Terriers (White et al., 2011, Warland and Dobson, 2013, Smiech et al., 2018).

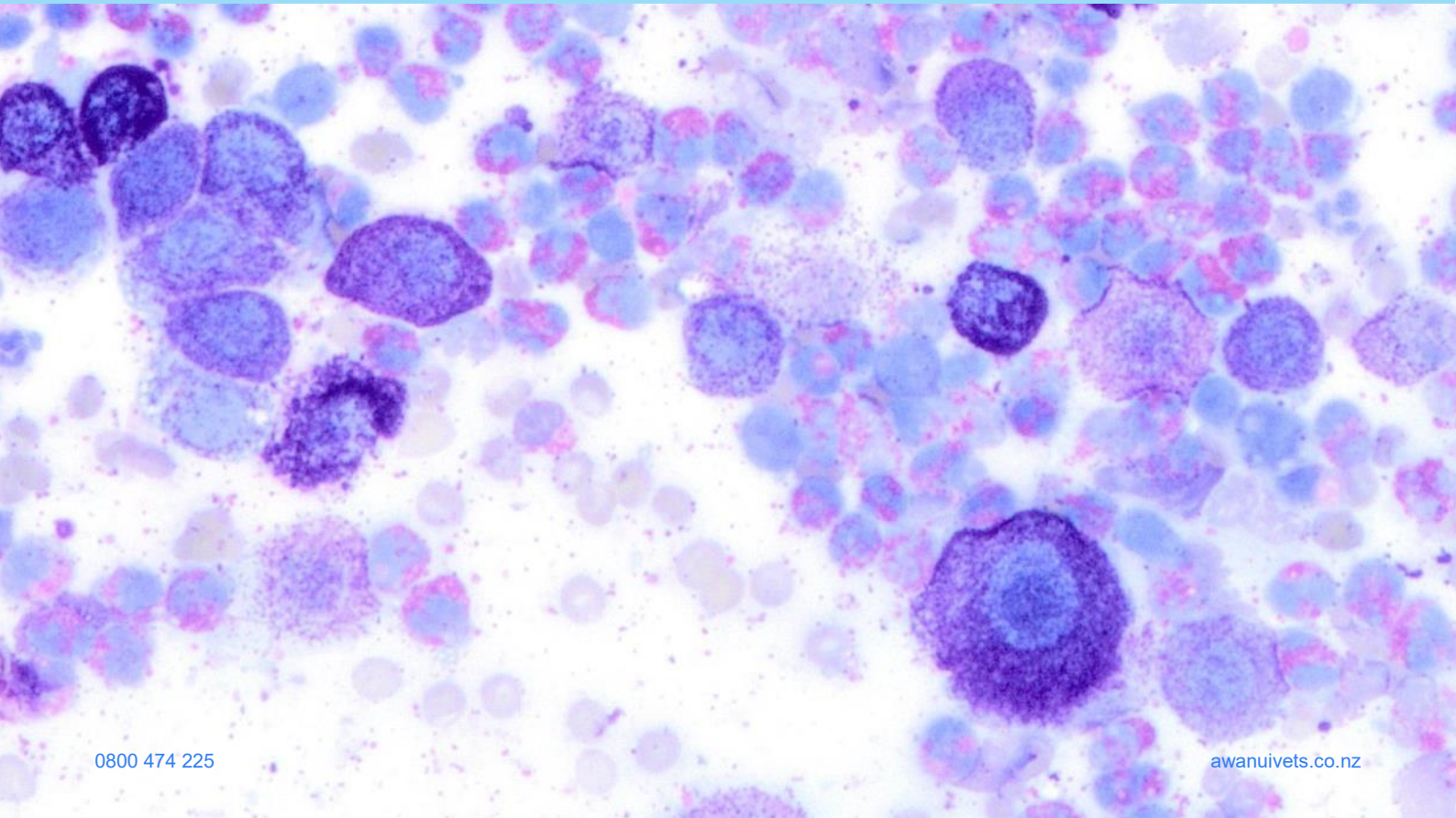
MCTs are more often cutaneous, although the clinical presentation of the lesions can vary widely. They commonly present as a hairless, singular, raised, and erythematous solid lesion. However, they can also be haired, vary from firm to soft, and be pruritic. Some cases may present as irregular, raised masses. They can sometimes invade the subcutaneous tissue and be ulcerated.

A mast cell neoplasia can also originate from the subcutaneous tissue. In these cases, the disease was shown to have mostly a favourable prognosis, with high survival times and low rates of reoccurrence. Metastasis and reoccurrence were strongly predicted by mitotic index in histopathology. Presence of multinucleation and tissue infiltration by the neoplasia rather than well circumscribed masses also predicted survival (Thompson et al., 2011). Although the location of MCT can frequently be correctly assessed by palpation and observation of macroscopic features, cutaneous and subcutaneous lesions can sometimes be similar and difficult to differentiate clinically. Thus, the location can only be definitively determined by histopathological examination of an excised mass (de Nardi et al., 2022).

Diagnosis of MCTs is commonly achieved by routine cytology. The typical cytological features of these neoplastic cells are easily recognised in aspirates from these masses. They usually exfoliate readily, yielding highly cellular slides containing numerous round, individualised cells with round nucleus and moderate amounts of pale cytoplasm. The cells in well granulated MCT have numerous cytoplasmic thick purple granules. In poorly granulated ones, the granules are smaller and present in low numbers to almost non-existent. Variable numbers of eosinophils and reactive fibroblasts and variable amounts of collagen fibrils are also often present.

The prognosis of canine cutaneous MCT is highly variable and depends greatly on histopathologic grading. Currently, there are two systems that are used for predicting prognosis in the evaluation of biopsy samples. The Patnaik three-tier system (Patnaik et al, 1984) classifies the tumour in three grades, depending on tissue invasion, presence of cellular atypia, granularity, nuclear features, mitotic count, and multinucleation. Dogs with grade I and III MCTs present the highest and lowest life expectancy, respectively. The grade II is more unpredictable, and prognosis can vary from good to poor.

Figure 1: Poorly granulated cells in a high-grade cutaneous mast cell tumour.



More recently, a more objective two-tier system was proposed (Kiupel et al., 2011), based on mitotic count, presence of multinucleation, bizarre nuclei, and karyomegaly. The high-grade MCTs have a worse prognosis than low-grade MCT. The current criteria for most pathologists and the recommendation by consensus of pathologists and oncologists are that both grading systems are applied concurrently to better classify the disease (Berlato et al., 2021, De Nardi et al., 2022). Additionally, in certain cases the use of other prognostic markers might be useful, especially for grade II or in subcutaneous MCT. The most used are mitotic index and staining for AgNOR, Ki67, and KIT. The presence of AgNOR and Ki67 are associated with significant decreased survival whereas the pattern of KIT expression can help determine if biologically aggressive behaviour is to be expected. Increased cytoplasmic KIT expression, opposed to membranous expression, is associated with an increased risk of local recurrence and lower overall survival (Kiupel et al., 2004).

In the last decade, a few studies attempted to describe a system for cytologic preparations of MCTs samples that could predict clinical behaviour of the tumour. In 2014 and 2016, two studies applied the Kiupel grading system on cytology slides. Both focused on evaluating the presence of mitotic figures, multinucleated cells, bizarre nuclei, and karyomegaly. Histopathology was used as the gold standard for grading, and overall, both studies found good correlation between MCT grades determined cytologically versus histologically. Cytologic grading matched histologic grading in 94% of cases in both studies. However, there were important cytologic limitations, including fewer numbers of cells for evaluation and lower occurrence of mitotic figures on cytologic preparations compared with histologic preparations. (Scarpa et al 2014, Hergt et al 2016).

Then in 2016, a group of researchers from the University of Georgia, USA developed a new system that compared mast cell granulation as well as some nuclear features to the Kiupel histopathologic grade. The tumour was classified as high grade if it was (a) poorly granular (Figure 1) or (b) had at least 2 of the following 4 features: presence of any mitotic figures, anisokaryosis (>50%), bi/multinucleation, or nuclear pleomorphism (Figure 2). The cytologic grading scheme had 88% sensitivity and 94% specificity relative to histologic grading. Although there is a tendency to overestimate tumour grade compared with histopathology, as a screening test, this is preferable to underestimating tumour grade, which might result in aggressive tumours being undertreated (Camus et al., 2016). This system is currently the most widely used system by pathologist when trying to estimate MCT grade in cytology.

In an effort to improve the cytological grading published by Camus, a group from Brazil proposed the simultaneous assessment of the tumour microenvironment, with the incorporation of fibroblasts and/or collagen fibrils in cytologic grading scheme

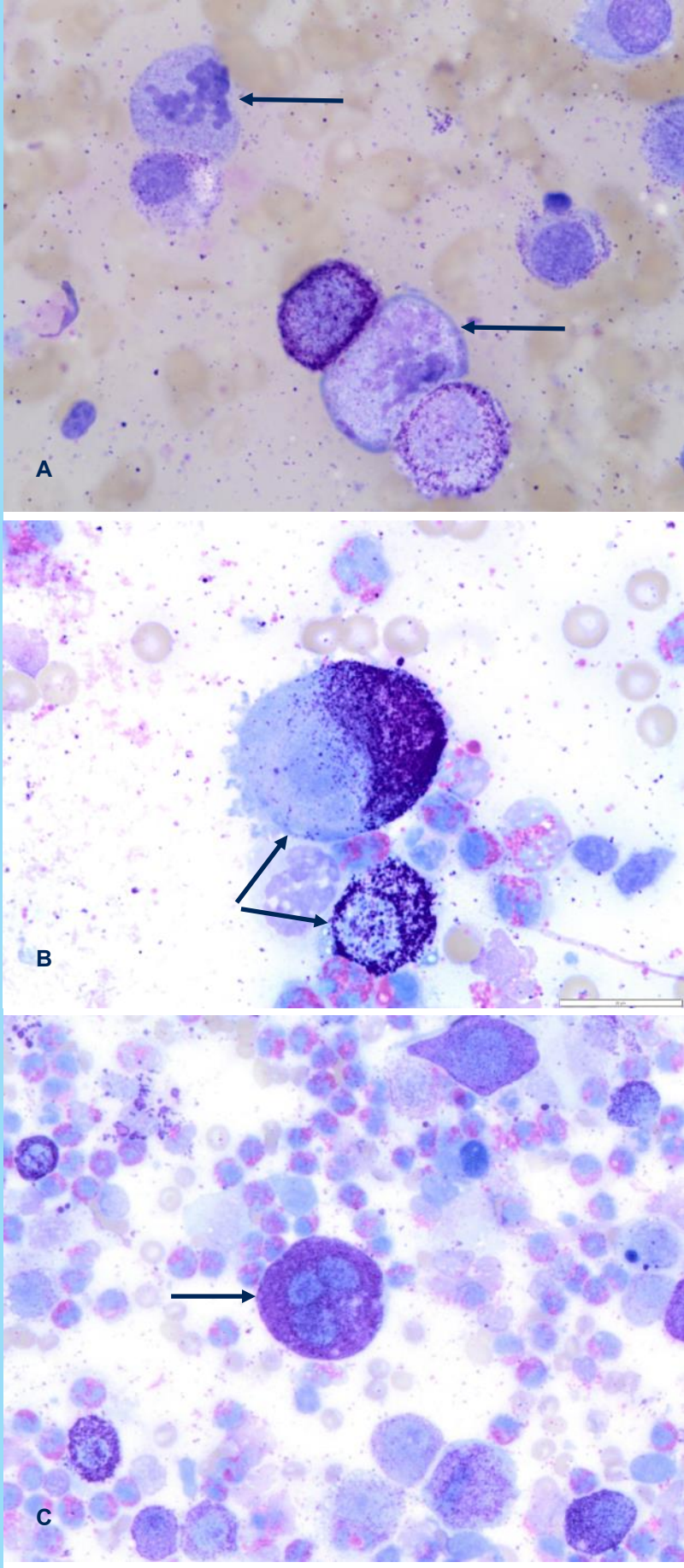


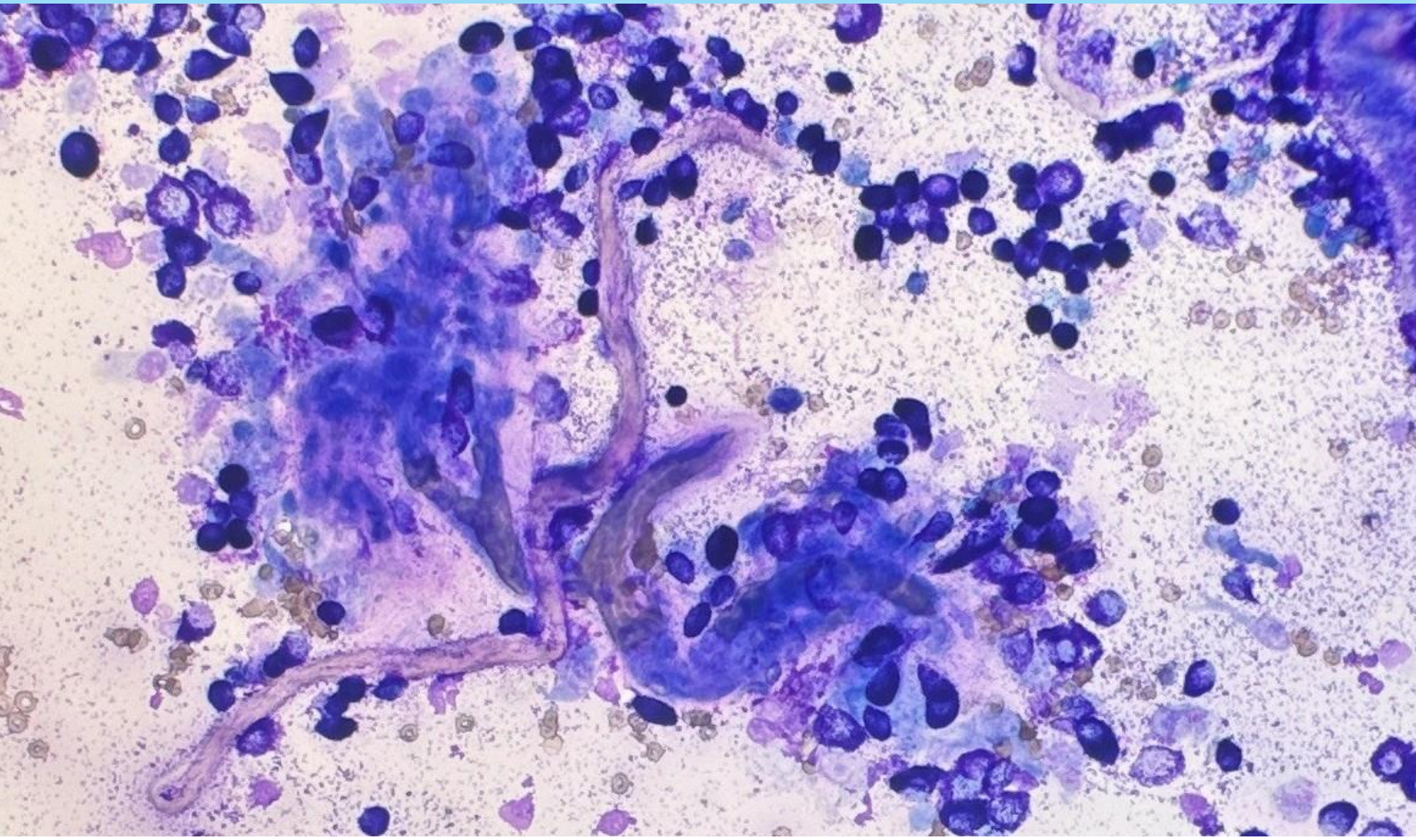
Figure 2. A high-grade cutaneous mast cell tumour in a dog. Presence of mitotic figures (A), anisokaryosis (B) and multinucleation (C).

(Paes et al., 2022). They compared the Camus cytologic grading scheme with their modified system, patient one year survival rates, and the Kiupel histopathologic grade. Their work supported the results obtained by Camus and showed that fibroblasts and/or

collagen fibrils are associated with lower grades of malignancy. The presence of granulated cells and high concentrations of fibroblast and/or collagen were what most correlated with high survival (Figure 3).

Due to the intrinsic features of cytology compared to histopathology evaluation, there are some limitations in cytologic grading of cutaneous MCTs. For examples, MCTs are known to be heterogeneous in the presence of mitosis and other nuclear features. Thus, the evaluation of nuclear morphology might be difficult since these features might not be easily found on the cytology slides. However, it is important to notice that cytologic grading is not supposed to replace histopathology, but rather be used as an initial screening test. This cytologic information might help the clinician formulate a patient care plan, guiding on how aggressive should be in the patient's workup. Additionally, a combination of cytology and histopathology grading might be what will provide the best prognostic information (Berlato et al., 2021, Krimer, 2002).

Figure 3. A low-grade cutaneous mast cell tumour. Well granulated cells and high amounts of collagen and fibroblasts.



References:

- Berlato D, Bulman-Fleming J, Clifford CA, *et al*. Value, Limitations, and Recommendations for Grading of Canine Cutaneous Mast Cell Tumors: A Consensus of the Oncology-Pathology Working Group. *Vet Pathol.* 58:858-863, 2021.
- Camus MS, Priest HL, Koehler JW, *et al*. Cytologic Criteria for Mast Cell Tumor Grading in Dogs With Evaluation of Clinical Outcome. *Vet Pathol*. 53:1117-1123., 2016.
- Hergt F, von Bomhard W, Kent MS, Hirschberger J. Use of a 2-tier histologic grading system for canine cutaneous mast cell tumors on cytology specimens. *Vet Clin Pathol*. 45:477-483, 2016.
- Krimer PM. Inclusion of fibroblasts and collagen fibrils in the cytologic grading of canine cutaneous mast cell tumors. *Vet Clin Pathol*, 51:464-466, 2022.
- Kiupel M, Webster JD, Bailey S, *et al*. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Vet *Pathol.* 48:147-155, 2011
- Kiupel M, Webster JD, Kaneene JB, *et al*. The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. *Vet Pathol*. 41:371-7, 2004.
- de Nardi AB, dos Santos Horta R, Fonseca-Alves CE, *et al*. Diagnosis, Prognosis and Treatment of Canine Cutaneous and Subcutaneous Mast Cell Tumors. *Cells* 11:618, 2022.
- Paes PRO, Horta RS, Luza LC, *et al*. Inclusion of fibroblasts and collagen fibrils in the cytologic grading of canine cutaneous mast cell tumors. *Vet Clin Pathol*. 51:339-348, 2022.
- Patnaik AK, Ehler WJ, MacEwan EG. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. *Vet Pathol*. 21:469-474, 1984.
- Scarpa F, Sabattini S, Bettini G. Cytologic grading of canine cutaneous mast cell tumors. *Vet Comp Oncol*. 14:245-251, 2014.
- Śmiech A, Ślaska B, Łopuszyński W, *et al*. Epidemiological assessment of the risk of canine mast cell tumours based on the Kiupel two-grade malignancy classification. *Acta Vet Scand*. 60:70, 2018.
- Thompson JJ, Pearl DL, Yager JA, Best SJ, Coomber BL, Foster RA. Canine subcutaneous mast cell tumor: characterization and prognostic indices. *Vet Pathol*. 48:156-68, 2011.
- Warland J, Dobson J. Breed predispositions in canine mast cell tumour: a single centre experience in the United Kingdom. *Vet J.* 197:496-8, 2013.
- White CR, Hohenhaus AE, Kelsey J, Procter-Gray E. Cutaneous MCTs: associations with spay/neuter status, breed, body size, and phylogenetic cluster. *J Am Anim Hosp Assoc.* 47:210-6, 2011.

Large cytology submissions

We occasionally receive cytology submissions with large numbers of smears. These cases take a huge amount of time to process, stain and examine.

In order to better reflect the cost involved in these cases (>10 smears) we have changed the descriptions and pricing for cytology testing. The options marked with an asterisk below have been changed. Your options for cytology on smears and bone marrow are now as follows:

1. Smear - single organ/site - up to 6 slides
2. Smear - single organ/site - up to 10 slides*
3. Smear - additional site or resample (same organ) or extra smears - up to 6 slides*
4. Bone marrow (including CBC) - up to 10 slides*

If more than 10 smears need to be examined from a single site (including bone marrow), further charges will be incurred (see 3. additional site pricing). For example, if you submit more than 10 slides on a case, you will be charged for “up to 10 slides” (2. or 4.) as well as an additional site fee (3.) for each group of 6 slides in excess of 10.

An updated price book with these changes was sent out to our mailing list in late June, but if you did not receive a copy please email us and we will send one through to you.

Contact your local laboratory and speak to a clinical pathologist if you have any questions or concerns.

In brief

- Promotional ACTH testing vouchers from Boehringer Ingelheim continue to be valid this month. They entitle you to **one free test** on a horse not previously diagnosed with PPID and must be used before the end of July 2024.
- Forgot your password for our website? No need to call, simply use the PASSWORD RESET button on the login screen to update it. Plus check out the FAQs provided for more helpful tips!

From page 3: What’s your diagnosis?

Dermatophilus. Histologically, the horizontal laminated sheets corresponded to keratin and crust, containing numerous bacterial colonies which were filamentous and occasionally appeared beaded. On examination of the scab material by Gram’s stain, revealed characteristic Gram-positive septate branching filaments which were longitudinally as well as transversely divided to form spherical or ovoid cocci in multiple rows, with the typical ‘tram-track’ appearance suggestive of D. congolensis.

Contact us

- contacting Awanui Veterinary couldn’t be easier.

EMAIL

auckland-vetlab@awanuigroup.co.nz
palmerston.vetlab@awanuigroup.co.nz
christchurch.vetlab@awanuigroup.co.nz
dunedin.vetlab@awanuigroup.co.nz

PHONE

0800 474 225

WEBSITE

awanuivets.co.nz

FACEBOOK

www.facebook.com/AwanuiVets

CATEGORY MANAGERS

- Rachel Howie - Rachel.howie@awanuigroup.co.nz - 027 604 8690
- Paul Fitzmaurice - Paul.fitzmaurice@awanuigroup.co.nz - 027 644 6892

LABORATORY MANAGERS

- Auckland - Miriam Reddy - Miriam.reddy@awanuigroup.co.nz - 09 574 4717
- Palmerston North - Tara Gowland - Tara.gowland@awanuigroup.co.nz - 06 350 2944
- Christchurch - Daniel Westlake - Daniel.westlake@awanuigroup.co.nz - 03 363 6717
- Dunedin - Denise Carian-Smith - Denise.carian-smith@awanuigroup.co.nz - 03 489 2632

WEBSITE ADMINISTRATOR

- Karen Cooper - Karen.cooper@awanuigroup.co.nz - 027 290 8778

Awanui Veterinary