Canine BRAF mutation detection

By Wilson Karalus

A non-invasive tool for diagnosing urinary transitional cell carcinoma

Introduction and case presentation

A nine-year-old female spay Shih Tzu mix presented to her veterinarian for haematuria and pollakiuria in the preceding seven days. Physical examination was unremarkable. However, urinalysis revealed the presence of pyuria and bacteria, and five days of amoxycillin-clavulonate was started. Clinical signs resolved after treatment; however, clinical signs returned 10 days after the cessation of antibiotics. Urine culture and sensitivity returned negative at this time, prompting further investigation. Abdominal ultrasonography revealed the presence of a large mass (2.57cm x 0.93cm) within the trigone region of the bladder, which extended distally into the urethra. Additionally, a 0.96cm-long urolith was noted within the bladder (Figure 1). Urinalysis was submitted from a sample collected via traumatic catheterisation and submitted for cytological examination with Awanui Veterinary.

Cytology findings

Cytological examination revealed the presence of epithelial cells, which displayed multiple criteria for malignancy. These included abnormally large nuclei, with marked variations in nuclear size (anisokaryosis), marked anisocytosis, multiple nucleoli and macronucleoli, and multinucleation. Occasionally there were also large amounts of clear to lightly eosinophilic material within the cytoplasm of cells (Figure 2).

Diagnosis and discussion

The history, clinical presentation, abdominal ultrasound and urine cytology findings were strongly suggestive of an epithelial carcinoma. Given the location, and morphology of the cells, a urinary transitional cell carcinoma (TCC) was considered the most likely diagnosis.

Canine TCC (also known as urothelial carcinoma [UC]) is the most common tumour of the urinary bladder in dogs. As in this case, dogs typically present with lower-urinary-tract signs such as pollakiuria, stranguria and haematuria. The trigone is the most classic location

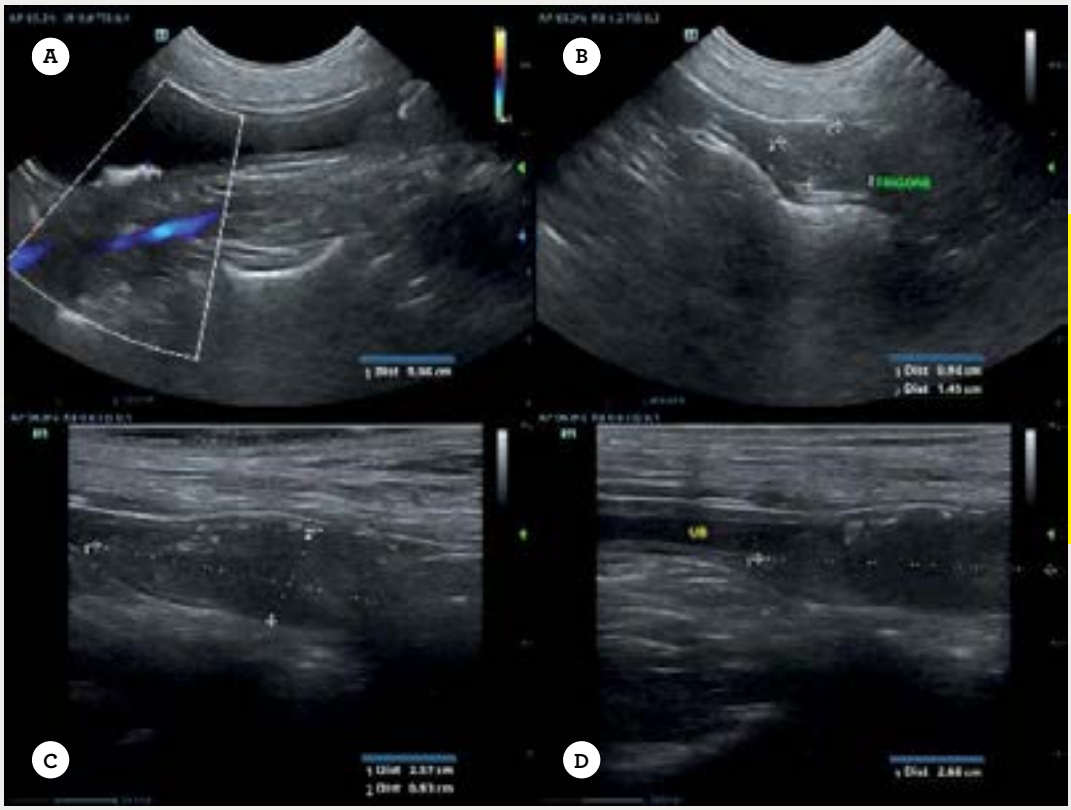

FIGURE 1: Ultrasound images of the bladder displaying the urolith (A) and mass present at the trigone, extending into the urethra (B, C and D)

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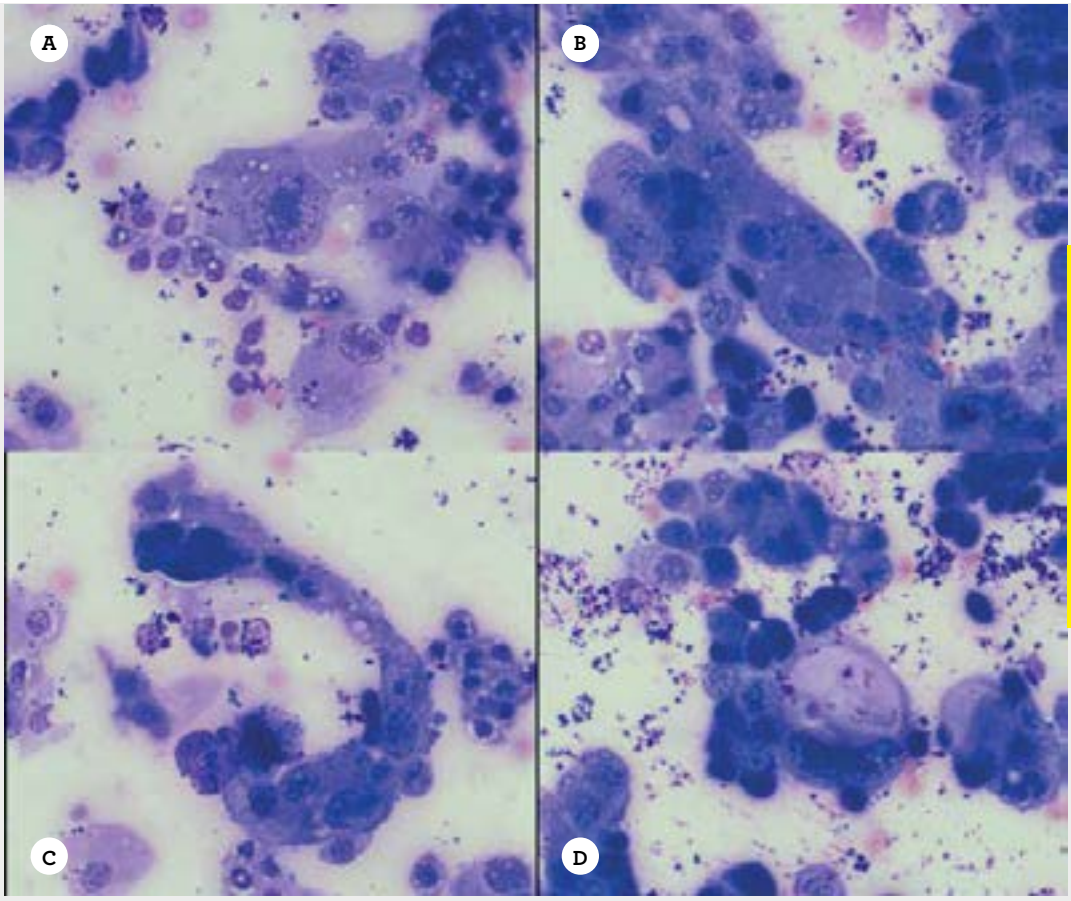

FIGURE 2: Urinary cytology images with an epithelial population displaying marked anisokaryosis, macronucleoli, marked anisocytosis, multinucleation and eosinophilic material within the cytoplasm

for TCC; however, the urethra is also commonly involved. Risk factors for TCC development include a strong breed association, with Scottish Terriers at a 21-fold increased risk when compared to mixed breed dogs (Fulkerson and Knapp, 2015). Additionally, a 3-6.5-fold increased risk in Eskimo dogs, Shetland Sheepdogs, West Highland White Terriers, Keeshonds, Samoyeds and Beagles is seen when compared to mixed-breed dogs (Fulkerson and Knapp, 2015). Females also tend to get TCC more often than males and repeated environmental exposures to carcinogens are speculated to play a role. However, exactly what type and how much of a role these environmental carcinogens play is still unknown (Glickman, et al., 1989; Glickman, et al., 2004; Raghavan, et al., 2004).

To make a definitive diagnosis of TCC, histopathological examination is required. Biopsy samples can be obtained via surgical cystotomy or transurethral cystoscopy. Surgical cystotomy is invasive and costly, while cystoscopy requires the use of specialised equipment. Cytological examinations of cells from samples acquired by various methods can also be considered and offer less invasive and cheaper alternatives in suspected cases of TCC. In this case, traumatic catheterisation was used to obtain samples for cytological examination.

In one study, samples collected by traumatic catheterisation were the most sensitive and specific, with 100% sensitivity and specificity reported from 11 cases across two institutions (McAloney, et al., 2021). However, this does not allow visualisation of the mass and may not always yield sufficient material to make a diagnosis. When traumatic catheterisation was compared to samples collected by fine needle aspiration and other methods (urinary sediment examination, tissue imprint, brush swabs, prostatic wash and urine collection from the renal pelvis), there was a similar high-sensitivity range (78.1–94.4%) but a lower specificity range (50–77.8%) (McAloney, et al., 2021).

A risk factor to consider with the above diagnostic options is surgical seeding of tumour cells throughout the abdomen. This can occur with surgical cystotomy, accidental aspiration when collecting a cystocentesis sample and transabdominal aspiration, which is not recommended (Childress, et al., 2011; Gilson and Stone, 1990; Nyland, et al., 2002). If the tumour develops within the abdominal wall, it is more likely to behave aggressively and will not respond well to medical therapy, with a median survival time of 57 days reported in one study of 24 dogs (Higuchi, et al., 2013).

Other complications include urinary tract infection, urinary tract obstruction and metastasis. In a study by Fulkerson and Knapp (2015), up to 20% of dogs had evidence of distant metastasis at the time of diagnosis, while another study of 137 dogs with TCC (Knapp, et al., 2014) found 58% had evidence of distant metastasis at the time of necropsy. The overall prognosis for TCC is guarded to poor, with approximately 85% of dogs surviving less than six months. With surgical treatment alone, the median survival time is 100 days; six months for single-agent chemotherapy; eight months for combinations of chemotherapy; and approximately one year for combinations of chemotherapy and COX inhibitors (Meuten, 2020).

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Introducing the Canine BRAF mutation test

More recently, a non-invasive test for TCC has been validated for dogs – the PCR assay for the detection of the BRAF mutation gene. This mutation can be detected in a 30ml, free-catch urine sample, sometimes before a detectable mass is present. It was discovered in studies in 2015 as a single nucleotide substitution of T to A at nucleotide 1784 (Mochizuki, et al., 2015; Mochizuki and Breen, 2015). Up to 85% of TCCs contain the BRAF mutation and it is absent in healthy control dogs, so its presence in urine is strongly suggestive of TCC (Mochizuki et al., 2015; Mochizuki and Breen, 2015). However, 15% of dogs with TCC do not express the BRAF mutation, or it is at a level too low to detect, so false negatives are a factor to consider. The BRAF mutation test is particularly useful in cases such as this one, where there is high suspicion for TCC and you want to confirm this without the use of more invasive or costly procedures. It also carries minimal risks and is easy for the client to collect. However, both tests are currently unavailable in New Zealand and must be submitted for overseas referral.

Awanui Veterinary has recently developed a duplex qPCR assay to detect the canine BRAF mutation. The test is based on detection methods developed by Okumura and Ohsato (2023). Since the mutation is a single-nucleotide polymorphism, PCR primers and locked nucleic acids probes were carefully designed to detect the target mutation with a very high sensitivity and specificity (80% and 99–100% respectively). So far, trial results have been promising and have shown that the duplex qPCR can detect and distinguish the mutant from wildtype DNA in urine samples (Figure 3). It is hoped that this test will launch in the near future, offering an affordable, non-invasive and convenient screening test for suspected TCC cases.

Urine was submitted from this case as part of its validation and returned positive for the single-base canine BRAF mutation, further confirming the diagnosis as a TCC (Figure 3). (VS)

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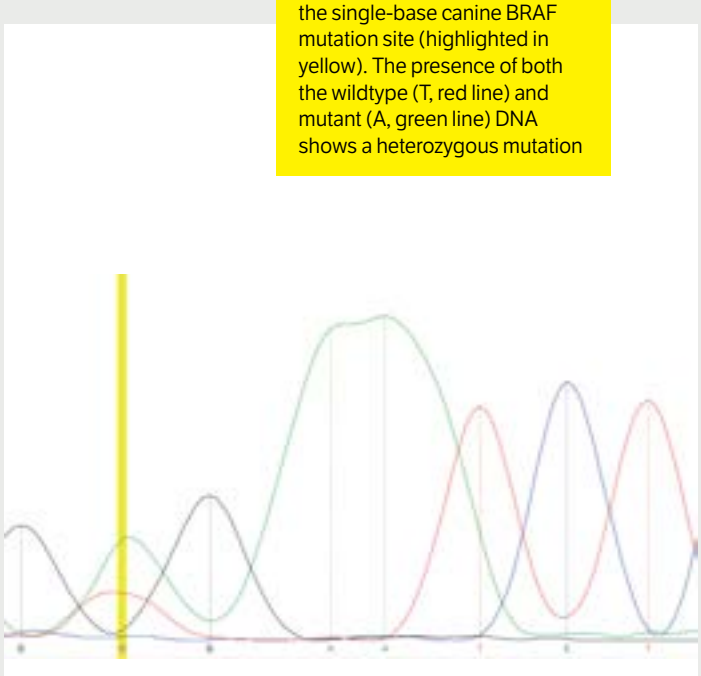
FIGURE 3: A DNA sequencing chromatogram representing the single-base canine BRAF mutation site (highlighted in yellow). The presence of both the wildtype (T, red line) and mutant (A, green line) DNA shows a heterozygous mutation



Awanui is requesting urine from cases diagnosed as TCC or highly suspected of TCC to continue the validation of this test (see next page). For more information, email palmerston.vetlab@awanuigroup.co.nz.

REFERENCES

Childress MO, Adams LG, Ramos-Vara JA, Freeman LJ, He S, Constable PD, Knapp DW. Results of biopsy via transurethral cystoscopy and cystotomy for diagnosis of transitional cell carcinoma of the urinary bladder and urethra in dogs: 92 cases (2003–2008). *Journal of the American Veterinary Medical Association* 239(3), 350–6, 2011

Fulkerson CM, Knapp DW. Management of transitional cell carcinoma of the urinary bladder in dogs: a review. *The Veterinary Journal* 205(2), 217–25, 2015

Gilson SD, Stone EA. Surgically induced tumor seeding in eight dogs and two cats. *Journal of the American Veterinary Medical Association* 196(11), 1811–5, 1990

Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. *Journal of the American Veterinary Medical Association* 224(8), 1290–7, 2004

Glickman LT, Schofer FS, McKee LJ, Reif JS, Goldschmidt MH. Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. *Journal of Toxicology and Environmental Health*, Part A Current Issues 28(4), 407–14, 1989

Higuchi T, Burcham GN, Childress MO, Rohleder JJ, Bonney PL, Ramos-Vara JA, Knapp DW. Characterization and treatment of transitional cell carcinoma of the abdominal wall in dogs: 24 cases (1985–2010). *Journal of the American Veterinary Medical Association* 242(4), 499–506, 2013

Knapp DW, Ramos-Vara JA, Moore GE, Dhawan D, Bonney PL, Young KE. Urinary bladder cancer in dogs, a naturally occurring model for cancer biology and drug development. *ILAR Journal* 55(1), 100–18, 2014

McAloney CA, Evans SJ, Hokamp JA, Wellman ML, White ME. Comparison of pathologist review protocols for cytologic detection of prostatic and urothelial carcinomas in canines: A bi-institutional retrospective study of 298 cases. *Veterinary and Comparative Oncology* 19(2), 374–80, 2021

Meuten DJ. (Ed.) *Tumors in Domestic Animals*. John Wiley & Sons, 2020

Mochizuki H, Breen M. Comparative aspects of BRAF mutations in canine cancers. *Veterinary Sciences* 2(3), 231–45, 2015

Mochizuki H, Kennedy K, Shapiro SG, Breen M. BRAF mutations in canine cancers. *PloS One* 10(6), 1–9, 2015

Nyland TG, Wallack ST, Wisner ER. Needle-tract implantation following US-guided fine-needle aspiration biopsy of transitional cell carcinoma of the bladder, urethra, and prostate. *Veterinary Radiology & Ultrasound* 43(1), 50–3, 2002

Okumura S, Ohsato YA. Novel rapid detection method for a single-nucleotide substitution mutation derived from canine urothelial and prostatic carcinoma cells present in small amounts in urine sediments. *Plos One* 18(9), 1–13, 2023

Raghavan M, Knapp DW, Dawson MH, Bonney PL, Glickman LT. Topical flea and tick pesticides and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. *Journal of the American Veterinary Medical Association* 225(3), 389–94, 2004

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REQUEST FOR SAMPLE COLLECTION

Awanui Veterinary aims to develop a molecular test targeting a specific genetic marker (the BRAF mutation), which has an established association with canine bladder and prostate cancers TCC/ UC) and PC. Previous studies have found the mutation in ~80% of related cancers while absent in healthy control dogs.

We are looking for urine samples of cases diagnosed with (or suspected of having) TCC/UC or PC and would appreciate veterinarians arranging the collection of ~30ml of urine and sending it to one of our labs.

Sampling instructions:

1. The sampling container can be obtained free of charge by contacting your local Awanui Veterinary laboratory. It contains a preservative solution; please don't discard it.

2. Add ~30ml of fresh (not more than 15 minutes after collection) free-catch urine to the container (top-up to the red line) and mix well. Urine can be collected over multiple days.

3. The sample can be stored at room temperature. Do not freeze it.

4. Include the following information:

- Animal ID
- Age
- Sex
- Spayed/neutered
- Diagnosis
- Veterinary practice
- Date
- Clinical history and comments.

5. Return the information along with the urine container (and any biopsy sample of the case, if it is available) to your local Awanui Veterinary laboratory. ■