Vetnostics

Partners in Practice

Spring 2013

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Vetnostics

Welcome...

Spring is upon us and so is the Vetnostics Spring 2013 newsletter!

The focus in this edition is on histopathology, specifically with regard to bone and eyes, amongst the other usual instalments.

As always, please contact me (ph 02 9005 7272 or email doug.hayward@vetnostics.com.au) if you have any requests/ideas for future newsletters or any other queries.

Diagnoses of skeletal lesions by biopsies:
Technical difficulties and solutions

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Introduction

Nearly all veterinarians will be presented regularly with cases having an unusual skeletal lesion. Clinical signs seen in patients with skeletal lesions will generally include lameness, pain or swelling. Radiography can provide evidence of skeletal or joint disease and some experienced veterinarians will be confident to act upon pictorial information alone.

Increasingly we are using advanced imaging to better characterise musculoskeletal pathology. In some circumstances, signs of pathology are observed on MRI or CT while remaining invisible on standard radiographs.

There are three directions that owners and veterinarians may elect to take when they identify a skeletal lesion, particularly if it is judged to be osteoaggressive.

These are:

[1] Do nothing (often dictated by finances).

[2] Proceed on the basis of the radiological features that have been identified. While radiography generally provides a useful differential diagnostic list, it is rarely diagnostic by itself. Omission of collecting tissue by biopsy can occasionally lead to a disaster.

[3] The third and preferred option is to biopsy the lesion and obtain a microscopic diagnosis that will enable appropriate planned treatment. Establishing an unquestionable diagnosis is the shared responsibility of the clinician, radiologist, surgeon and pathologist.

Over many years we have discussed the vagaries and frustrations experienced where a biopsy taken from a lesion is not definitive, sometimes not even diagnostic. Obtaining biopsies from skeletal lesions requires careful planning to maximize harvest of interpretable tissue that will lead to a definitive diagnosis. This paper describes a process to maximize the likelihood of obtaining a definitive diagnosis, thereby preventing having to re-biopsy the lesion.

Difficulties and solutions

Careful planning prior to taking a skeletal biopsy is imperative to increase the likelihood of reaching a diagnosis. It is very frustrating to go to the trouble of anaesthesia and collection of a biopsy without getting a useful answer.

Therefore, use radiographs or even a CT scan to not only localize the skeletal lesion but also to pinpoint optimal sites through which the biopsy should be harvested. The use of fluoroscopy may prove useful in some cases.

A further point to remember is that the edge and/or the periosteal surfaces are often reactive while the centre may be necrotic or haemorrhagic. Consequently you must be reasonably aggressive to go beyond this reactive region to obtain the best chance of reaching a diagnosis.

Clinical history

A pertinent clinical history will assist in a final diagnosis as certain lesions are more common in different species and in different breeds at certain ages and locations within the skeleton.

Radiology

Radiographs allow for planning, defining and identifying the best approach to obtain the most potentially useful tissue to biopsy. Of equal importance, radiography may identify areas of the lesion that are unlikely to provide useful diagnostic information or from which considerable amounts of reactive osteogenesis will be sampled.

Radiographs taken after a biopsy will allow visualization of whether the lesion has been entered or not; penetration of the overlying reactive osteogenic cuff and through to the main lesion is essential. Sadly, we often receive a biopsy of superficial reactive tissue, minus the principal lesion. After the biopsy procedure, re-radiography of the lesion with radiopaque markers will allow identification of each biopsy site. Such information allows the surgeon to determine whether further biopsies need to be taken. Additionally, the radiographs (films or digital images) should accompany the biopsies being submitted to the pathologist.

Instrumentation and methodology

In smaller patients, a Jamshidi needle (Figure 1) is useful. However, in most larger patients, a Michelle trephine (Figure 2) can be used giving larger samples.

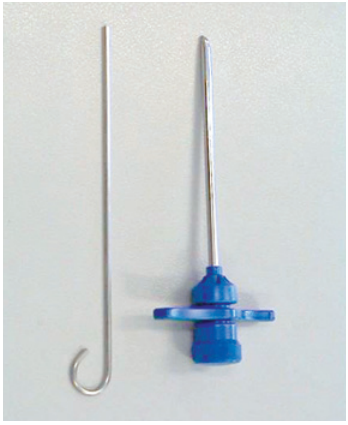


Figure 1: Jamshidi needle

Figure 2: Michelle trephine

The Jamshidi needle has a tapered tip so as it is thrust with an oscillating motion into the bone a core is pushed into the shaft. When the needle has been pushed in deep enough it is twisted through 360 degrees to disconnect the core. A "plunger" is provided to place into the tip to push the core out through the hub of the needle. Trying to push the sample out the tapered tip would crush it. The sample collected is quite small and several should always be collected.

A Michelle trephine is essentially a tube with cutting teeth on the end and a handle on the other. Various diameters are available, with about 4-6 mm being the most useful diameter. A stab incision should be made into the soft tissue and then using the trephine, with the trochar in, bluntly dissect to the surface of the bone. The trephine is thrust into the bone with an oscillating motion. Using the depth markings on the side of the trephine a full 360 twist is made when the desired depth is reached. The core biopsy is pushed out with a "pusher" from either end.

To obtain several samples, stab incisions can be made in a row along the length of the lesion, or core samples can be collected from one incision by using the trephine or Jamshidi needle in several radiating directions (Figure 3).

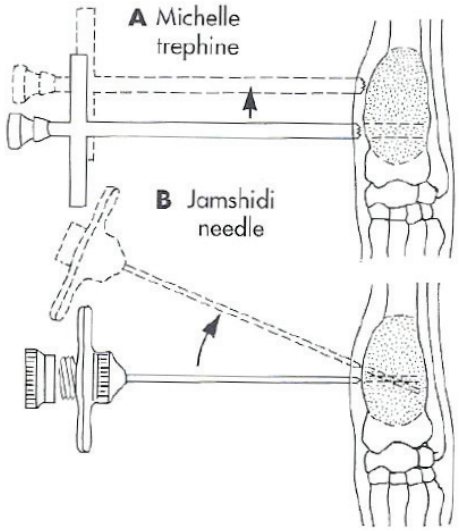


Figure 3: Techniques for multiple biopsies with Michelle and Jamshidi biopsy instruments.

In over 90% of cases, Jamshidi needle biopsies have a relatively high accuracy in separating neoplastic lesions from non-neoplastic lesions. Provided at least 3 adequate biopsies are harvested, identification of tumour type is achieved in about 80% of cases (Picci et al 1985). Although the risk of pathological fractures using a Jamshidi needle is negligible, the narrow needles do induce considerable crush artifact; a confounding factor in reaching a diagnosis.

Accuracy of diagnosis is increased with Michelle trephine biopsies because more tissue is available with less crush artifact. The flip-side is a slightly increased risk of inducing pathological fractures (Wykes et al 1985).

The best chance of reaching a diagnosis is from collection of a large wedge but such a procedure does have all the risks of open surgery as well as a greater risk of fracture when compared to those collected with a Jamshidi needle or Michelle trephine.

We do not consider amputation following radiographic identification of neoplasia acceptable for the simple fact that such osteoaggressive lesions have also been found to be induced by fungal infection. Nevertheless, for the rare occasions that present with a pathological fracture and an underlying radiographic osteoaggressive lesion, a case could be made for amputation for relief of pain in some circumstances.

Fine needle aspirates can be collected and in some cases may provide reasonable results, but with a distinctly lower accuracy in determining the type of malignancy.

Open excision and wedge biopsy or even sampling bone using rongeurs can be useful, especially in the mouth. Bleeding can be a problem when collecting samples and electrocautery may be needed after excision.

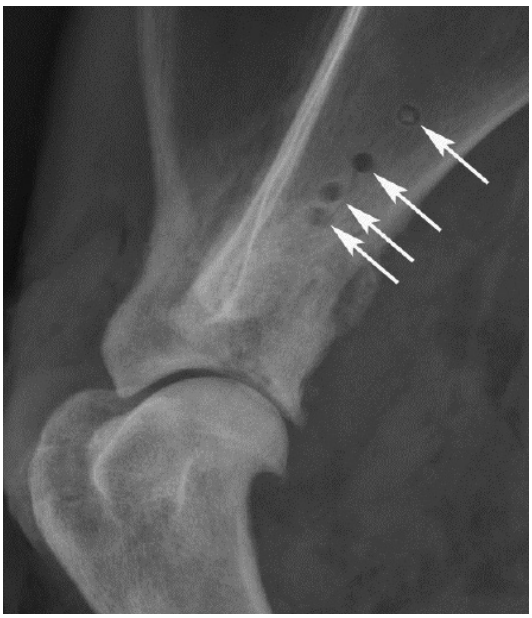


Figure 4: Lateral shoulder radiograph. Skeletal pathology within the neck of the scapula. Arrows indicate biopsy sites (Michelle trephine).

Regardless of the sampling technique chosen, multiple biopsies should be the aim. Take at least 3 biopsies in appropriate areas as more samples raise the probability of gaining a diagnosis; from about 80% if only one biopsy is taken, to 90 to 95% if three biopsies are collected (Picci et

Diagnoses of skeletal lesions by biopsies: Technical difficulties and solutions *continued*

al 1985). The three biopsies should be collected from the following radiographically identified regions:

a) centre of the lesion
b) an advancing zone
c) from either the interface of lesion / resident tissue or a lucent area within the trabecular bone.

Central biopsies are more likely to be helpful in diagnosis of neoplasms than those from interface zones (Wykes et al 1985).

Collection of other samples while taking a skeletal biopsy

A lesion near to a joint often induces effusion within the joint and sampling this fluid should be part of your routine work-up. Joint fluid needs to be labeled clearly as "Joint fluid" because if it mistaken for blood/serum a different technical procedure may be used which may negatively influence results. For cytological assessment, joint fluid should be submitted in an EDTA blood tube to improve cell preservation. Concurrent submission of air-dried smears of joint fluid is useful as well.

A subperiosteal tap is an excellent procedure to obtain a good microbiological specimen without actually entering the osseous lesion. It consequently has very little risk of spreading the infectious agent or causing a pathological fracture. Microbiology samples should be submitted in sterile containers and/or on transport swabs. Blood culture bottles may additionally be used for submission of joint fluid for culture, if required.

Complications from bone biopsies

Complications are typically few and include pathological fractures, infection and spread of malignant cells along the biopsy tract. These complications are extremely uncommon provided appropriate techniques are used and precautions undertaken.

SUMMARY

At least 3 biopsies from the lesion accompanied by radiographs or CT or MRI of the lesion.

If possible include radiographs taken after the biopsy with the needle still in position.

A summary is provided in the table below from ***Small Animal Surgery (1999) Publisher: Mosby. St Louis.***

TABLE 32-5

Important Considerations for Bone Biopsy

- Obtain samples from the radiologic center of tumors.
- Obtain multiple samples.
- Take radiographs after biopsy to confirm biopsy site.
- Using Jamshidi needles may reduce the risk of pathologic fracture.
- Have pathologists experienced in evaluating bone biopsies perform histology.

Case illustrating some of the frustrations of taking a bone biopsy

A dog presented with a foreleg lameness which was attributed to the shoulder joint. On radiography, the lesion involved the scapular neck adjacent to the Glenoid cavity (Figure 4). Multiple Michelle trephine biopsies were taken and only the biopsy closest to the Glenoid cavity had diagnostic tissue (Figure 5). A provisional diagnosis of undifferentiated sarcoma was made. Amputation revealed the limited extent of the neoplasm (Figure 6) and how the biopsies nearly all missed the lesion. After amputation, ample tissue was available for examination and a diagnosis of histiocytic sarcoma was confirmed with immunohistochemistry.

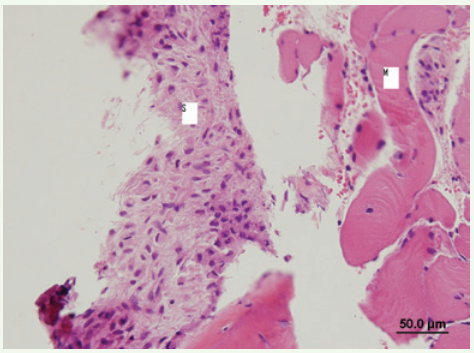


Figure 5: Biopsy from the neck of the scapula. M= muscle fibres. S = sarcoma cells

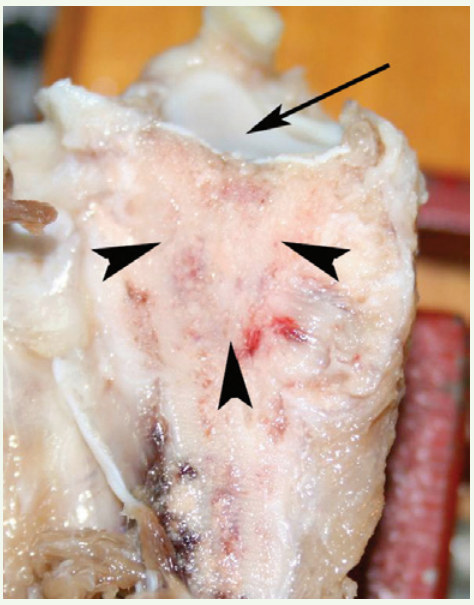


Figure 6: The scapular neck bone has been opened and the arrow heads delineate the extent of the sarcoma. The Glenoid cavity is identified by the arrow.

References

Wykes PM, Withrow SJ, Powers BE, Park RD: Closed biopsy for diagnosis of long bone tumours: accuracy and results J Am Anim Hosp Assoc 1985; 21:489-494.
Picci P, Bacci G, Campanacci M: Histological evaluation of necrosis in osteosarcoma induced by chemotherapy. Cancer 1985; 1515-1521.
Waters DJ: "Musculoskeletal System". In Textbook of Small Animal Surgery. Ed. Slatter D, 2nd ed Vol 2, W. B. Saunders, Philadelphia 1993, pp2213- 2230.

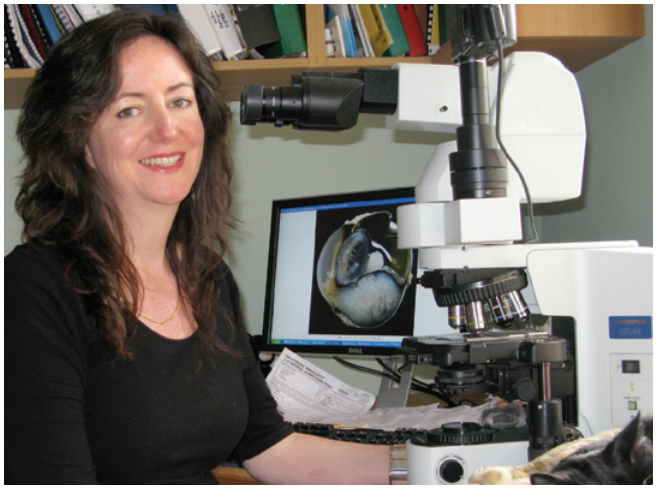
Welcome Dr Karen Dunn

Consultant Veterinary Ocular Pathologist

Vetnostics welcomes Dr Karen Dunn as our consultant veterinary ocular pathologist. Karen is a University of Queensland Veterinary School graduate with a long-standing interest in ocular pathology, beginning in 1992 with a 4 year anatomic pathology residency at the Animal Health Trust in the UK.

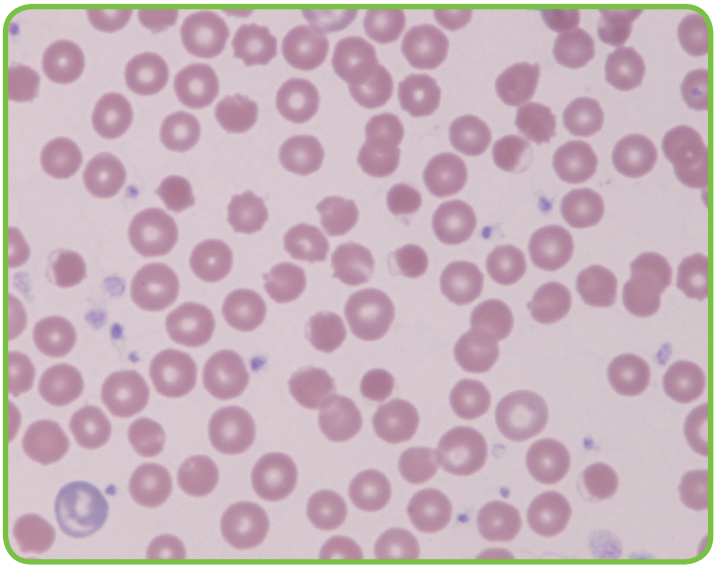
After leaving the Animal Health Trust, Karen worked for 5 years as a diagnostic histopathologist at Rest Associates in Suffolk, England. However, her passion for ocular pathology led Karen to set up FOCUS (later FOCUS-EyePathLab) in 2006, a dedicated ocular pathology service for specialist veterinary ophthalmologists and clinicians with a particular interest in ophthalmology. Karen worked with well known ocular pathologist Professor Richard (Dick) Dubielzig at the eye pathology service (COPLOW) at the University of Wisconsin, Madison, in the summer of 2006.

Karen is also a member of the British Association of Veterinary Ophthalmologists (BrAVO), and regularly attends (and occasionally presents at) British and European clinical Ophthalmology meetings, and more recently presented to the Ophthalmology Chapter at Science week in 2013. Karen is a regular reviewer for papers submitted to Veterinary Ophthalmology, and has collaborated on a number of scientific papers published in this field. Karen has also been closely involved in a number of research projects, including successful Veterinary Ophthalmology Diploma candidate research dissertations, and is in collaboration with the Molecular Biology Department at the Animal Health Trust exploring the role of genetic markers in prognostication for canine and feline uveal melanomas in-vivo.



Further information on Karen's ocular pathology service is available at http://www.FocusEyePathLab.com where Karen's dedicated ocular pathology submission form (PDF) can be downloaded from the Forms page. This FOCUS-EyePathLab submission form contains prompts for more detailed information necessary for interpretation of ocular pathology specimens, and **MUST** accompany your usual Vetnostics Laboratory submission form. The fee for samples examined by Karen is $160 excl GST.

What is your diagnosis?



The image to the left, (Figure 1), is from a canine blood film. The animal concerned presented for vomiting and diarrhoea. Routine serum biochemistry revealed a mild ALP elevation only. FBC revealed a mild to moderate regenerative anaemia and adequate platelet numbers.

Can you identify the predominant abnormality evident in the image and the significance?

Please see the answer later in the newsletter.

< ***Figure 1:*** Canine blood film (x100 oil)

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Call for Fanconi Cases

Acquired renal tubulopathy (also called Fanconi syndrome) is continuing to occur in dogs in Australia and overseas (http://news.vin.com/VINNews.aspx?articleId=29411). Treat-associated renal tubulopathies in Australian dogs were initially found to be due to Kramar chicken treats. A large number of cases occurred between 2007 and 2009 and the incidence of acquired tubulopathy in dogs dramatically decreased once the treats were withdrawn from the market (Thompson et al 2013). However, this problem has not gone away entirely and cases are still being intermittently diagnosed. Some cases have been responsive to removal of pork based treats such as pigs ears but worryingly, some cases have been associated with quality commercial diets and have responded to diet change. In Labradors especially there has been a link to copper-associated hepatitis (Langlois et al 2013) so this is not only a small breed issue, as was largely the case with the original dogs.

The most identifiable pathology is glucosuria in dogs with normal blood glucose. Urinalysis is essential in every ill pet and glucosuria is one change that cannot be ignored in a diagnostic investigation. Some dogs do have increased liver enzymes also. The cause of this problem is just not known but the pet food industry is co-operating with investigations into the problem.

Vetnostics has supported Sue Foster to collect and follow up cases of acquired renal tubulopathy and record them for diet and case analysis from 2008 until present. Tests such as urinary fractional excretion of electrolytes have been offered at no charge if a full dietary history has been made available to Sue. This assistance has made it possible for Sue, Linda Fleeman and Mary Thompson to continue following the problems during and beyond the time of the initial epidemic.

Sue, Linda and Mary are still actively collecting cases of acquired renal tubulopathy. They request that all suspected cases are lodged via PetFAST, which is the Australian reporting system for suspected adverse events to pet food, pet treats, and pet meat. For Vetnostics clients, if you can contact Sue and provide additional details via an Acquired Renal Tubulopathy Report Form, that would be very helpful to the ongoing research into this problem. Any suspect food or treats should be kept for possible analysis.

Further information can be obtained by contacting Sue Foster on 0423 783 689.

References

1. Thompson MF, Fleeman LM, AE Kessell AE, LA Steenhard LA, SF Foster SF. Acquired proximal renal tubulopathy in dogs exposed to a common dried chicken treat: retrospective study of 108 cases (2007–2009). Aust Vet J 2013;91:378-373 (http://onlinelibrary.wiley.com/doi/10.1111/avj.12100/abstract;jsessionid=ED9E2B11F45F00141828DDCE4678FA51.f01t03)
2. Langlois DK, Smedley RC, Schall WD, Kruger JM. Acquired Proximal Renal Tubular Dysfunction in 9 Labrador Retrievers with Copper-Associated Hepatitis (2006–2012) J Vet Intern Med 2013;27:491-499 (http://onlinelibrary.wiley.com/doi/10.1111/jvim.12065/abstract)

Synacthen® (tetracosactrin)
Supply shortage

Current available information indicates that the supplier of Synacthen® will only have further stock available in Australia by late December 2013. This is likely to be of relevance to veterinarians as stocks dwindle with an ongoing requirement for ACTH stimulation testing.

Our recommendation therefore after reviewing the literature is the use of Synacthen® Depot and the following protocol:

1. **Collect a basal (0 hour) blood sample into a serum tube**
2. **Inject 250ug of Synacthen® Depot IM**
3. **Collect a further blood sample into a serum tube 60 minutes later**
4. **Label sample times clearly on the tube**
5. **Clearly indicate on the submission form that Depot Synacthen formulation was utilised as well as indicating dosage and blood sampling times**
6. **Tick ACTH stim (code VAS) as normal on submission form**

It is imperative that a thorough clinical history is provided with these submissions to allow accurate and useful interpretation and advice. Many thanks.

Reference

Ginel PJ, et al: Evaluation of serum concentrations of cortisol and sex hormones of adrenal gland origin after stimulation with two synthetic ACTH preparations in clinically normal dogs. AJVR 2012; 73: 237-241.

It is imperative that a thorough clinical history is provided with these submissions to allow accurate and useful interpretation and advice.

It has also come to our attention however the Synacthen® Depot is additionally in short supply. In this context, the following advice/guidelines may prove useful:

As Synacthen® is in short supply, use a low dose ACTH protocol e.g. 5 µg/kg IV for small dogs and 1 µg/kg IV for large dogs and store the remaining sample for future monitoring. Both doses have been proven to produce maximal cortisol secretion in healthy dogs (Martin et al 2007). If using really low doses, close attention with regards to timing of the post-stimulation sample is required. Timing needs to be PRECISELY one hour post injection for doses of 1 µg/kg or less (*Martin et al 2007*).

Intramuscular dosing with 5 µg/kg has also been shown to cause maximal cortisol secretion (Behrend et al 2006). In the author's opinion, IV dosing is preferable unless patient difficulties preclude it's use, as it ensures that the required dose does reach circulation.

Only a small amount of Synacthen® is administered when using a 1 - 5 µg/kg dose so freeze any remaining sample. Draw up the left-over Synacthen® in a 1 ml syringe (or draw up accurate doses into multiple 1 ml syringes), leaving a small air space at the end of the syringe. Cap each syringe, label it with dog name and date (frozen Synacthen® is stable for 6 months; Frank and Oliver 1998) and place it in the freezer. When that dog needs another ACTH stimulation test, thaw it, draw up the required dose and re-freeze the remainder.

By doing this, multiple doses can be obtained out of one vial. This significantly decreases the cost of monitoring treatment: the owner can be billed for the whole vial initially but thereafter, until another vial is required, there is no more cost for Synacthen®, just fees for cortisol measurement and procedure.

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Serum Amyloid A (SAA)

SAA is an acute phase protein, ie. it increases as part of the acute phase inflammatory response. Many processes may result in an increase in SAA, including infections, neoplasia and surgery amongst others. SAA is a major acute phase protein in the horse, superior in this respect when compared to fibrinogen. SAA shows a rapid increase associated with acute inflammation, followed by an equally rapid decrease once inflammation is resolved. SAA is thus of use in horses for confirming the presence of inflammatory disease as well as for monitoring response to treatment.

Vetnostics is pleased to be able to offer SAA testing daily. The sample required is a serum sample and the cost of the assay is $32 incl. GST.

It is available both as a stand-alone test and as an add-on to an existing profile.

Please request Serum amyloid A (code SAA) under Other Tests on the request form.

What is your diagnosis? Answer:

The predominant finding on this blood film is the presence of multiple eccentrocytes (numbers 1-4 in the image being examples) which are also known as blister cells. The significance in this case is that they support oxidative damage to the erythrocytes, further corroborated by the identified Heinz Bodies (numbers 5 and 6). Of particular importance with regard to the eccentrocytes is that they appear smaller and more dense than regular erythrocytes and may therefore be mistaken for spherocytes.

Oxidative damage to erythrocytes may occur with onion/garlic toxicity and acetaminophen toxicity amongst other causes.

Apart from further erythrocytes, the further complete cells visible consist of three thrombocytes and a heterophil.

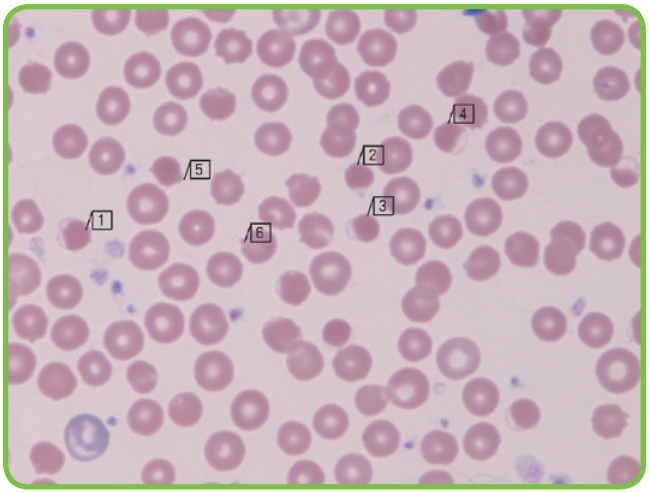

Figure 1: Canine blood film (x100 oil)