Animal Welfare & Ethics
Spirited debate and comments from veterinarians around Australia

Focus on Feline – An extra 16-pages of feline articles, sponsored by the Valentine Charlton Bequest

Aussie Ingenuity in action...
Fish Hooks – The Non-Surgical Approach by Jamie Andrews

Vogelnest & Allan, 2015, Radiology of Australian Mammals, CSIRO Publishing
Read the review by Zoe Lenard
Fel-O-Vax FIV kittens become cats that keep coming back

Based on a survey of 213 clinics around Australia\(^3\), 76% of vets believe that FIV vaccination of kittens is likely to increase the number of repeat annual vaccinations throughout a cat’s life.
As I write this column, the Medical Imaging conference is on its final day. Over the week, 30 hours of CPD will have been presented by some of the best imaging and medical specialists in Australia. We often ponder whether or not long conferences still have a place in the current continuing professional development environment, but judging by the many interactive question and answer sessions and the feedback from the participants, many still feel that conferences provide some of the best CPD. Four to five days can lead to a deeper understanding of the subject matter, not just a superficial overview. Apart from the opportunity to listen to and question leaders in their fields, the interaction with colleagues during meal breaks often helps to recharge the batteries.

In 2016 the CVE will be holding another two ‘major’ conferences, each with 4 days of lectures followed by a final Masterclass day, which provides a special day for people with a deep commitment who like to be challenged by a more intimate and interactive format. There will also be many shorter one and two day seminars as well as a variety of hands-on workshops. We believe that this mix caters for a range of individuals, who may be at different stages of their professional careers and recognises the varying demands on everyone’s time.

Of course, in this digital age not everyone can spare the time to attend face-to-face events, which is why the CVE has been expanding the offering of our short online courses – TimeOnline. This year there are many new programs and others are being prepared for release later this year or early next year. No matter where you live in the world, these courses offer over an average 10 hours of CPD that can be taken in your own time – spread over a couple of days or a couple of weeks. The discussion forum for each course enables all of the participants to communicate with each other and the tutors, with additional interactivity, timely feedback and reinforcement, which provides increased depth of learning.

As with everything offered by the CVE, we strive to ensure that quality is at the forefront of our minds. We do not produce programs merely to satisfy regulatory requirements for structured CPD points. Rather we make sure that what we offer satisfies the thirst for knowledge across the veterinary profession and are proud of our quality control. Our aim is to keep the profession up-to-date with information which can be applied in practice across all levels and is of benefit to both your clients and your patients.

This issue of CAT is another 64-page edition containing a broad offering. The C&T has always been a forum for ideas and debate and this issue is no different, containing some divergent opinions across a couple of topics. If you find articles that we publish do not sit comfortably with you, feel free to write your own rejoinder or contribute in some other meaningful way. Being a Centre within the University of Sydney, we welcome healthy discussion and debate.

Dr Hugh White
Director, Centre for Veterinary Education
The C&T is the brainchild of Dr Tom Hungerford, one of the founders of the PGF* (established in 1965) and the first Director (1968–1987), who wanted a forum for uncensored and unedited material.

‘…not the academic correctitudes, not the theoretical niceties, not the super correct platitudes that have passed the panel of review…not what he/she should have done, BUT WHAT HE/SHE DID, right or wrong, the full detail, revealing the actual ‘blood and dung and guts’ of real practice as it happened, when tired, at night, in the rain in the paddock, poor lighting, no other vet to help.’

The first C&T, contributed by Dr R M Kibble from Kurring-gai Animal Hospital, Turramurra North, NSW was on ‘Infertility – Uterine Conditions’ and was published on 29 April 1969. CVE Members are reminded that this and other C&Ts, Perspectives, Proceedings and veterinary publications are available to CVE members through the CVeLibrary. Contact cve.enquiries@sydney.edu.au or call us at +61 2 9351 7979 if you’ve forgotten your Username and Password for access.

Thank you to all contributors
…and more C&T articles and Perspectives are needed

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The property is a well-managed, self-replacing beef herd that has been ‘closed’ with the exception of bull introductions. All bulls are tested negative for Pestivirus prior to introduction.

On 23rd April, blood and faecal samples were collected from 2 calves and 3 cows and submitted to the lab. The bull was euthanased and a necropsy examination conducted.

Background

Sporadic deaths had been occurring in 3-6 month old calves in one mob since early March. The syndrome had been investigated by Dr Vic Coleman of Keith Veterinary Clinic, and samples taken from 2 affected animals, including 1 at necropsy examination. Affected animals treated with antibiotics appeared not to respond to treatment and died.

Samples were submitted to the lab on 17/3/15 and 9/4/15 with Infectious Bovine Rhinotracheitis (IBR) as the presumptive diagnosis.

By April 2015 a total of 4 calves had died; one 4-year-old bull was very unwell and no definitive diagnosis had been made.

PIRSA was aware of the investigation and became involved when there was a report from Dr Coleman of oral ulceration and salivation occurring in an affected animal. In discussion with Dr Coleman it was arranged that a PIRSA vet would visit the property and collect samples to rule out foot-and-mouth disease (FMD) and vesicular disease, as well as collect further samples to try and achieve a diagnosis of the problem.

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I enjoy reading C&T more than any other veterinary publication.

Terry King

WINNERS

MAJOR PRIZE WINNER

Fish Hooks – The Non-Surgical Approach
Jamie Andrews

CVE PUBLICATION PRIZE WINNERS

Lymphosarcoma & Concurrent Cryptococciosis in a Cat
David Fowler

Treatment of Herpetic Keratitis with Topical Aciclovir in a Persian Cat
Wye Li Chong

Sonographic Characterisation of the Urogenital Tract of the Koala (Phascolarctos Cinereus) for Standardised Investigations of Urogenital Pathology
Caroline Menschner & Kathryn Stalder

Publication Prize Winners are entitled to a CVE proceedings of their choice: www.vetbookshop.com

*The Post Graduate Foundation in Veterinary Science of The University of Sydney (PGF) was renamed the Centre for Veterinary Education (CVE) in 2008.

Figure 1. Angus calves on poor, dry pasture.
Clinical Signs
Affected calves were observed to be ataxic, salivating, and had developed stiffness in the front legs, and then were found dead after 3 or 4 days of disease progression. None of the affected animals appeared febrile, and no lameness was reported. With the exception of the 4-year-old bull, no other animals in the herd of 123 Angus cattle appeared affected in any way. The bull had been progressively declining in body condition, and developed scours since March. It was euthanased on 23rd April.

The bull was in score 1 body condition, with evidence of scours, and appeared very weak. Temp 39.4°C, with otherwise normal clinical signs.

Necropsy Findings
There were no obvious gross pathological signs in the bull except that the abomasum appeared slightly enlarged and of a granular appearance; portions of the ileum appeared to be thickened but draining lymph nodes appeared normal. The anterior portion of the brain had a darkened appearance. A large amount of fine sand was found impacted in the rumen and abomasum.

Lab Results
Samples were tested negative for exotic diseases and IBR.

Liver levels of copper were low in 1 calf sampled on 9/4/15, except that the abomasum appeared slightly enlarged and of normal copper levels. The copper levels were low in 1 sample indicating that there may be an underlying copper deficiency in the herd.

1 calf (No. 4) was Antigen positive for BVDV, meaning that it is a persistently infected (PI) animal and exhibiting symptoms of mucosal disease; this calf later died.

No definitive diagnosis from laboratory samples was suggested for the bull, although the signs displayed and the observation of a large amount of fine sand in the rumen and abomasum suggest that the animal may have been deteriorating due to sand impaction. This is a rare and seldom diagnosed condition in cattle.

Only one easily available journal article was available on line at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3003582 and there are few textbook references available that I could find. However, the symptoms were consistent with chronic abomasal irritation, if not mild left abomasal displacement. Treatment as suggested by some authors is to drench with paraffin oil and provide other supportive treatments. Antibiotics may assist in treating the diarrhoea.

This was an unusually dry period in this region and in many areas there was no pasture feed left, so cattle were being totally hand-fed. In these conditions, outbreaks of infectious disease are possible including parasitic and bacterial diseases such as salmonellosis. If possible, cattle should not be fed in very sandy areas and attention should be given to deficiencies of copper and vitamin A that may occur in the absence of green feed.

Since there is evidence of recent exposure of the herd to pestivirus, and 1 PI animal was identified, it might be wise to have a plan to test the remaining female weaner calf cohort with an antigen test, and the cows in this group as well, to attempt to identify further PI cattle.

There were other reports of deaths and illness in beef cattle in this area at the time, and in some cases sand had been observed in large amounts in rumen, omasum and abomasum.

and 1 calf tested positive for Pestivirus using PCR, with others returning 2+ or 3+ ELISA antibody results, indicating recent exposure to the virus.

Faecal cultures were negative for common enteric pathogens (Salmonella etc).

The bull had raised pepsinogen levels and a FEC of 100 eggs per gram, low blood calcium and raised urea levels. Faecal cultures were negative for common enteric pathogens.

Conclusion
Laboratory results for tested calves did not identify a specific cause of disease or death, although there is a possibility that bovine viral diarrhoea virus (Pestivirus) may have been implicated in some of them. Liver copper levels were low in 1 sample indicating that there may be an underlying copper deficiency in the herd.

Pestivirus, and 1 PI animal was identified, it might be wise to feed in very sandy areas and attention should be given to deficiencies of copper and vitamin A that may occur in the absence of green feed.

Antibiotics may assist in treating the diarrhoea.

Figure 1. This cat had a pruritic skin condition with most irritation centred on the head.

Victor filmed this broodmare with stringhalt in China in response to C&T No. 5482. We apologise that we inadvertently left it out of the December 2015 issue.

What is your Diagnosis: C&T No. 5502
Cat With An Itchy Head

Pete Coleshaw
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Frances Harvey
What is your Diagnosis: Triboiculid Mites

Harvest mites are my obsession! They are on ‘steroids’ in my neck of the woods. Dogs and cats.

Interestingly, they’re much easier to catch on cats, than dogs, but dogs have a characteristic pattern, hard to find mites....Tatty hair inside of front legs, crook of the elbow, band across the front connecting the elbows – best place to find – front leg nail bed and crook of the elbow....Cats,
Richard Malik  
richard.malik@sydney.edu.au

FYI, I have only seen a handful of cases, and always in cats. They are more common in some places, such as where Frances works. Pete had a nice photo – see below – but it’s masses of mites in cytology, not the orange spots on the cat.

Figure 4. Harvest in near fold plus ticks.

Figure 5. High power harvest 1. Note the orange pigment seen macroscopically also evident microscopically.

50cm length of stiff 10mm diameter black poly pipe with it. I gently pushed the plastic tube down the fishing line into the dog until I felt it engage the shaft of the hook in the distal oesophagus. I wound the fishing line tightly around my index finger at the top of the pipe to tension it and disengage the barb. Then a short sharp distal tap on the top of the tube freed the hook, a slow retrieval half twist and gentle pull followed. Liquid diet and good antibiotic cover for 1 week concluded the treatment.

Two kookaburras, 1 tortoise and 4 dogs later and all successful with no surgery. My last case was a dog with a hook embedded in the gastric lining and this too was successfully retrieved with no surgery.

Tip – for very small patients use the inner plastic tube from a Mares uterine catheter.

My practice is in a rural area and fish hook ingestion is quite common here. Mostly dogs are the offenders, with cats and wild-life infrequent patients. My previous approach was general anaesthetics – D/V and lateral x-rays and surgical removal. Thoracic surgery is not for the faint-hearted or ill-equipped and referral costs preclude this as an option in most cases.

Three years ago a patient presented with a fish hook embedded in the proximal oesophagus. I managed to remove it under general anaesthetic by grasping it with forceps and pushing distally to disengage the barb. Follow up with a liquid diet, good antibiotic cover and all was well.

The next case was a distal oesophagus and I had to retrieve the fishing line from the oesophagus with a spey hook. I tied more fishing line onto it until I had approx 60cm clear of the dogs nose, tied a small bead on the end and threaded a new CVE Members are reminded to check the CVELibrary for other articles on this subject.

Download Peter Howe’s article in the eBook: Fish Hook Removal C&T No. 4912 Peter Howe Issue 251, June
The relative advantages of endoscopy:-
- Depth of penetration of the hook (barb) can be assessed prior to trying this technique.
- The extent of the damage to the oesophagus/stomach mucosa can be assessed post-retrieval.
- The one- or two-tube (obturator) technique can still be employed to prevent iatrogenic damage in the fishhook retrieval.

Just an added suggestion to be considered:

Jamie Andrews says:
‘…threaded a 50cm length of stiff black poly 10mm diameter line into the dog until I felt it engage the shaft of the hook in the distal oesophagus. I wound the fishing line tightly around my index finger at the top of the pipe to tension it and disengage the barb. Then a short sharp distal tap on the top of the tube freed the hook, a slow retrieval half-twist and a gentle pull followed.’

This technique can be made marginally safer by employing a second shorter but larger bore tube (two-tube technique) – one tube fits inside the other, the inner tube being longer than the other; the more rigid endotracheal tubes, PVC pipe, flexible clear tubing (Bunnings) are all suitable.

Once the distal end of the smaller (inner) tube is resting on the inner curve of the fishhook within the oesophagus, slide the larger (shorter) tube over the smaller inner tube. Once the barb is disengaged and freed from the oesophageal wall with the gentle tap as described above, hold the inner tube stationary and slide the outer tube over it to effectively shelter the hook within the larger tube. Then pull both tubes out simultaneously.

Thanks for a great tip and very useful technique.

Fish Hook Dislodger C&T No. 312 Granger, Chapman & Christie (Issue 7, January 1975)
The Elusive Fish Hook: Know How C&T No. 3913 Mark Debritz (Issue 31, April 1999)
Fish Hook Remover C&T No. 4912 Peter Howe (Issue 251, June 2008)
5a. Would ACAT (aged care assessment teams) teams be able to act as a resource for the older owner as well, and community care people in general, for any sole pet owner?

The Red Cross has a telephone checking-in-service; does it also have the capacity to run a ‘permission to access the house register’ and be the point of contact as soon as an alert is raised?

5b. Should sole owners be encouraged/allowed to enrol on a database with The AWL or RSPCA, which both have emergency boarding programs, or with the Red Cross?

5c. Could the medical-alert -button also have a sister button that, again, could be left in a standard place which the paramedics can push and activate as they leave? This alerts the service that a home-alone-pet situation has occurred, with permission given for Council or Police or RSPCA to respond to that home-alone-pet as soon as possible.

5d. Could the pet-home-alone poster featured in this C&T article (and available to download in the eBook or from www.cve.edu.au/candt2016) act as a de facto alert and permission to the paramedics that the household needs their animal rescued promptly?

5e. Should the neighbour pet-watch scheme be a legal extension of the AWL/RSPCA boarding programs? That way, any insurance issues and privacy act rules can be automatically covered by these existing charity organisations.

I personally believe these animals are better off in actual homes rather than commercial or charity shelters. Sole, isolated owners tend to live quieter lives - many of the dogs don’t know they are dogs! Extracting these pets to be then placed in noisy high-traffic Veterinary or RSPCA settings is not ideal. Most veterinary cages are not suitable for extended stays for any animal, let alone a healthy one. The RSPCA kennels would be slightly better as they are designed for longer-stay clientele but animals suddenly plucked from their homes need to be able to have quiet and peace and space to move around in an environment that mimics their normal daily home life. We don’t put left-alone children into the local doctor’s surgery.

What can be done NOW:

- If you know someone on your street that lives alone, offer to be a contact for their pet if the owner is taken ill or injured suddenly.
- If you live alone; make sure you fill out the emergency pet contact wallet card with the details of the preferred emergency contact.
- If you don’t wish to name a contact; then fill out the wallet-card that allows the authorities to send someone to your home and rescue your pet ASAP.
- Place a copy of the card in full view on your fridge.

We need YOUR help!

If you have any ideas or suggestions, please send them to me at homealonepet@outlook.com. I can compile a dossier so we can start to make things happen.

Currently, I’m getting graphics to use in some advertisements to feature true stories on animals we ourselves have helped rescue, fostered and then rehomed; but, if you have any stories to share on pets you know that ran into trouble like this, then happy to hear them as well. Spread the message, talk to family, alert friends…

Home Alone Pets: Do YOU have a Plan B?

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Toll Free: 1800 785 642 | Email: vi@provet.com.au
Medical Management of Gallbladder Mucocoele

Heather Shortridge
Locum Veterinarian
heathershortridge@gmail.com
C&T No. 5527

‘Sindy’, a 15-year-old female Shetland Sheep Dog, presented just before Christmas 2013, for inappetence of several days’ duration. Bloods were run in house, which showed elevated white blood cells (36.04, reference range 6-17) and azotemia with both urea and creatinine elevated (urea 19.9 (2-5.9), creatinine 145 (27-124)).

The results were discussed with the owners and amoxycillin clavulanic acid injections, and intravenous fluid therapy (lactated Ringers solution) were commenced. Sindy was also started on omeprazole.

Sindy’s condition improved in hospital and she began eating. As it was Christmas, it was decided to see how she did at home, and she would re-visit for further work-up as required. Sindy was discharged on Christmas Eve, onto a renal diet and with Ipakitine®. She was also treated at home with amoxycillin and clavulanic acid injections, and intravenous fluid therapy. (lactated Ringers solution) were commenced. Sindy was discharged to her owners to continue medication at home.

Three months later, Sindy re-presented after a few days of just wanting to be left alone and not eating much food again. Sindy was admitted and her urea and creatinine rechecked just wanting to be left alone and not eating much food again.

Blood results three months post initial presentation

I had Sindy over to my colleague Philippa for an ultrasound. Philippa found Sindy’s abdomen to be hyperechoic cranially, and particularly in the region of the gallbladder, which had a very bright hyperechoic wall, with hyperechoic contents and spherical patchy hypoechoic areas. Sindy was painful on palpation of the gallbladder. A diagnosis was made of gallbladder mucocoele.

We had not seen one of these cases before, but discovered that Shetland Sheepdogs are one of the overrepresented breeds for this condition. Further research suggested that, generally speaking, this condition is best managed surgically due to the risk of gallbladder rupture. However, given her advanced age, and being aware that medication is palliative treatment only, Sindy’s owners elected to try medical management.

Sindy remained in hospital on fluids, and amoxycillin, and was started on 75mg ursodeoxycholic acid (‘Destolit’) once daily, and 225mg S-Adenosylmethionine with 24mg of Silybin (‘Denamarin’) once daily. She was also given daily maropitant (‘Cerenia’) injections for nausea. Two days after this, Sindy’s bilirubin and ALP were repeated and both had worsened:

- TBL 38 (2-10)
- ALP > 2000 (20-150)

Sindy’s condition was clinically improving and she pulled her drip out the following day. She no longer seemed to be painful in her cranial abdomen. Sindy was discharged to her owners to continue medication at home.

Sindy continued on Denamarin and Destolit with periodic rechecks. As at 6 months post diagnosis, my colleagues report she was going well, and re-presented for a dental, which was performed uneventfully.

While gallbladder mucocoele is generally best managed surgically, this is a case where medical management has so far resulted in a good quality of life, and at the time of writing Sindy continues to be well.

Editor’s Note: It is possible that this dog had both mucocoele and a bout of pancreatitis.

Figure 1. Sindy.

- Platelets 621 (200-500)
- with other analytes within normal reference intervals.

Tinkerbell Microbiology

‘Tinkerbell’, a 10-year-old DLH cat, first presented to us in March 2012 with a history of vomiting almost daily for several weeks. She seemed otherwise well and screening bloodwork (haematology and biochemistry) were unremarkable. A dietary trial using Hill’s i/d food was commenced. She was seen a month later because no improvement had been observed, she was basically vomiting after every meal. It was revealed the owner was feeding some things other than the Hill’s i/d. More in-depth diagnostics were declined at that stage so we transitioned her to an exclusive diet of Hill’s z/d and commenced a prednisolone trial. Later the same month, we caught up with the owners. They had found it impossible to give prednisolone tablets, however vomiting had effectively resolved since the switch to Hill’s z/d. The diet was maintained.

Six months later in October 2012, ‘Tinkerbell’ was seen for a routine health check. She appeared well to the owners, with only very occasional vomiting. 0.65kg of weight loss over the prior 6 months was noted and discussed. In December 2012 she was seen with further weight loss, a ravenous appetite, and possible abdominal mass noted on palpation. Total T4 was checked and was normal. We undertook abdominal ultrasound in January 2013. A 4cm diameter round, mildly irregular mass was detected in the cranioventral abdomen (Figure 1). This mass was closely associated with the liver and ventral to stomach. It was suspected that this mass was separate to the liver and the stomach however attachment to stomach could not be discounted. Small intestinal wall thickening (3.1 mm in places) was noted.

A fine needle aspirate of the abdominal mass was performed and sent to a pathologist for cytological examination. The cytology report (population of atypical intermediate-sized to large round cells possessing round occasionally indented mildly irregular nuclei with clumped chromatin) gave us a semi-definitive diagnosis of ‘Lymphoma, probable’. At that stage the owners declined more detailed or invasive investigation such as tissue biopsy as they would have been reluctant to pursue multi-agent chemotherapy. In lieu of this, a typical low-grade lymphoma protocol was commenced because we felt it would offer some palliative benefits with minimal cost. The regimen consisted of prednisione 10 mg/day and chlorambucil pulse therapy (4mg daily on four consecutive days out of every 21 day period).

Upon starting this regimen, ‘Tinkerbell’ improved rapidly with weight gain, improvement in demeanour and, amazingly, within 3 weeks of starting, the abdominal mass was not detectable on palpation. She continued to do well aside from some weight fluctuations, and by 4 months after starting on the regimen, she was back to the weight she had been 12 months earlier when she was doing well on an exclusive diet of Hill’s z/d.

In July 2013, after 6 months on the immunosuppressive drug regimen, she was presented because of acute onset of neurological signs – ataxia, proprioceptive deficits in the forelimbs, absent menace response, absent visual and tactile placing reflexes. Her palpebral reflex and PLR’s were normal. Diagnostically we considered CNS neoplasia (lymphoma), hypertension (from any cause), or (assisted by immunosuppression), toxoplasmosis or cryptococcosis. Clindamycin was commenced in case of toxoplasmosis and on Sue Foster’s advice, we put in an order for pyrimethamine from a compounding pharmacist. Serum was sent for a latex cryptococcal antigen agglutination test. Hypertension was ruled out using a Cardell BP monitor. Immunosuppressive drugs were halted immediately.

Figure 1. Mass (irregular 4 cm diameter) present in the dorsocranial abdomen.
were submitted to Assoc. Professor Mark Krockenberger. A post mortem examination was performed and tissues were subsequently euthanized. By that stage there was a (new) mass palpable along the urinary bladder. By October 2013, she had developed intermittent ataxia and was taken to the RSPCA Animal Referral Hospital for further investigation. Her weight had dropped precipitously, and she had abdominal pain. The high grade lymphosarcoma is the most significant finding, but it is not rule out the possibility and the most likely scenario is multifocal infiltration of the stomach wall. Large firm white mass dorsal to urinary bladder, suspect enlarged medial iliac lymph node. Large intestinal wall contained obvious intra-mural thickenings (lymphoid tissue?). Large firm white mass dorsal to urinary bladder, suspect enlarged medial iliac lymph nodes. Unfortunately, I failed to collect blood for a repeat LCAT at the time of post-mortem, so we will not know to what extent Cryptococcus may have played a role in her eventual deterioration and death.

Comment courtesy of: Mark Krockenberger

On examination of tissues from this case, there is a spectacular multifocal large cell high grade lymphosarcoma with marked multifocal infiltration of the stomach wall, liver, submucar lymph node, large intestinal MALT, kidney and meninges. On examination of the post mortem tissues, I am unable to confirm a focus of cryptococcosis in this case. This does not rule out the possibility and the most likely scenario is that this case represents a small focus of localised disease secondary to lymphosarcoma. The high grade lymphosarcoma is the most significant disease process in the case.

Figure 2a. Gross Photograph of the cat’s eye.

Figure 2b. Gross Photograph of the cat’s kidney.

Figure 2c. Gross Photograph of the cat’s liver.

Note: Magdoline posted this question whilst employed at the RSPCA.

Question

Just wanted your opinion on oral terbinafine for feline ringworm. Due to cost of itraconazole, is the compounded or human generic terbinafine just as effective? I have seen conflicting reports on it but wanted your advice given how cheap it is and the dermatologist at Animal Referral Hospital (Dani Hoolahan) says it would be effective in a shelter environment and only $15 for a 6 week course. Would this also be the case for dogs? We just had over 25 dogs with ringworm from a hoarder. It is our policy to use Griseofulvin if large dogs due to cost; however, if terbinafine is effective and has minimal side effects, we would switch over.

Comment courtesy of: Jeffrey So

I am not the expert. But I have a friend who has done a lot of the research – Kimberly Coyner in the States. You will not get a better human on the planet – she loves helping shelters.

In cats, I think Sponoran is still best – but terbinafine isn’t far behind. There is a new kid on the block – a new Australian Itraconazole formulation made by Mayne Pharmaceutical is better than the original – and you use at half the dose. Robin can tell you more about it – and they might make you a good offer if you buy in bulk. Kim will be interested too.

In the dog, griseofulvin is probably still quite effective – I haven’t treated ringworm in the dog for a long time, whereas Sally’s cat adoption kittens often have ringworm.

Kim – can you tell Mags about your terbinafine research?

PS Robyn – Mags is head vet at RSPCA and a sensational person. Kim is one of the top shelter ringworm researchers in the USA. A trial of your new Itraconazole in the RSPCA setting could be a great thing to do to assess the vet market (and to measure blood levels).

Comment courtesy of: Magdoline Awad

**Discussion on Generic Human Terbinafine**

**Magdoline Awad**

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**Note:**

Magdoline posted this question whilst employed at the RSPCA.

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**Comment courtesy of:**

**Richard Malik**

CVE Valentine Charlton Consultant

richard.malik@sydney.edu.au

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Lucien was a 14-week-old MN Russian Blue kitten presented on Anzac Weekend 2014 for symptoms of ‘cat flu’ and 3 days of anorexia.

Physical examination showed a stunted but quiet, alert and responsive kitten weighing 0.52kg with a temp of 39.5°C. Both eyes were discharging serous fluid and the nose, which was crusted with mucus, was cleared. The nostrils then continued to produce a steady drip of mucopurulent discharge.

On questioning, the owner reported that the kitten had stopped climbing the kitty kingdom tree climbing thing some 5 days ago, but was otherwise normal in behaviour until anorexia and respiratory signs developed 3 days prior to presentation.

Lucien was given 0.1mL convenia subcutaneously (SC) and admitted. He was offered warm roast chicken which he sniffed but did not eat. If small bits were placed in his mouth he happily chewed and swallowed. I was unable to secure an IV line in the kitten as every time I touched a vein it blew to the size of a marble, so 20mL of 5% glucose* was given an IV line in the kitten as every time I touched a vein it blew to the size of a marble, so 20mL of 5% glucose* was given. He was given 20mL of 5% glucose* and the morning (I noticed a yellow tinge to Lucien’s ears which was not noted in consult. He was continuing to eat balls of chicken or Hill’s a/d™ so bloods were taken for an urgent biochemical profile. I did an in-house PCV which showed a Hct of 22, icteric serum and almost no buffy coat, and a very rare platelet here and there on smear. Differential diagnoses at this stage were: hepatitis and feline infectious anaemia due to haemotropic mycoplasmas, amongst others.

BLOODS:

- Na 143 (134-161)  
- K 3.5 (3.7-4.9)  
- Bicarb 14 (15-24)  
- Crea 35 (40-190)  
- Glu 11.6 (9.9-8.3)  
- Bilir 89 (<17)  
- AST 12,230 (1-60)  
- ALT 5626 (1-80)

So, being a Saturday night on a long weekend with what was by now a very yellow kitten (in 6hrs!) which was also very unwell, I called a pathologist after hours. My eternal gratitude to Bruce Duff from Vetnostics who pulled over on the side of the road somewhere to talk me through the results, and really confirmed my suspicions by now. We both decided the results showed a massive cholangiohepatopathy with some muscle necrosis and possible disseminated intravascular coagulation. Whilst pancreatitis was a potential diagnosis with associated triaditis, Bruce and I were both pegging systemic toxoplasmosis for this poor kitty.

By now it was 8pm and Lucien was becoming more depressed, more yellow, was now anorexic and reluctant to move. I called the owners into the clinic to cuddle him, and to discuss the extremely guarded prognosis for Lucien. They remarked at how yellow he was, but were happy for me to try to save him. So I started Lucien on 15mg/kg of Clindamycin and took him home with me for the night. However, as the night progressed poor Lucien became quite dull, and was beginning to have difficulty breathing. I was continuing fluids at home, but by midnight it was apparent he was suffering and unlikely to turn around, so I made the decision to euthanase him.

I informed the owners who requested cremation. I also asked if I could do a necropsy and take some samples for histology and they were kind enough to let me do this.

Necropsy (some 20hrs after death by the time I got to it, being on call as well) showed scant abdominal fat, hepatoMEGA-megally (past the navel!) with small pocks in it, and friable (presumably necrotic centres). All mesenteric LNs were enlarged and gastrointestinal tract normal. Lungs were atelectatic with purple discoloration (may have been post-mortem change), small amount of pleural effusion and no ascites. I took liver and lung samples for histopathology.

By now the pathologist’s comment was in on the bloods: Anaemia is non-regenerative. Primary BM disease a possibility but a viral study is worth considering pending histopathology results. Marked thrombocytopenia confirmed, scant small clumps evident. Neutrophs show toxic change and had a prominent left shift. Occasional blast cells identified.

My liver sample showed marked autolysis. Hepatic sinusoids showed fibron thrombi with multifocal to coalescing necrosis of surrounding tissue. Some portal veins had endothelial cells containing 6-12 banana shaped 1-2um zoids.

Definitive Diagnosis: Marked acute multifocal to coalescing necrotising hepatic with intrahepatic portal collateral trachyzoites consistent with acute systemic toxoplasmosis.

It’s bad, but I got pretty excited then. I spoke to Sue Foster (I love that woman; always there when you need to discuss something) who has done a lot of work on toxo, and we decided to do a viral panel as there were possibilities that Lucien was FIV or FeLV (or both) positive which allowed me to do a viral panel as there were possibilities that Lucien was FIV or FeLV (or both) positive which allowed the toxo to go rampant (which also means Lucien was likely infected in uterus, in which case the breeder should be notified), or the toxo itself has caused the stunting, and Lucien was infected either late in uterus, or just after being born. So… results for FIV and FeLV were both negative. Bugger. Sue also got excited for me and sent a sample off for PCR, which also came back negative. Bugger.

So, no causal agent found for Lucien’s rampant toxo infection. He was tiny for his age, though reportedly quite a normal kitten until days before presentation. The cessation in climbing was probably, in hindsight, the start of it all as the toxo began to replicate in his muscles, then moving to his other organs? Who knows? However, it was an interesting case even if I never got a definitive cause. Poor Lucien though, he never really had a chance, and it was heartbreaking for the family and especially the kids whom Lucien was bought for. My everlasting gratitude, as always, to all the people at Vetnostics who are always so helpful on turn around, so I made the decision to euthanase him.
Treatment of Herpetic Keratitis with Topical Aciclovir in a Persian Cat

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Note: Wye Li saw this case whilst employed at The Gables Veterinary Group in Canberra.

A 9-year-old male neutered Persian cat was presented for routine grooming. Anisocoria was noted incidentally with moderate medial canthal discharge and crustation. Upon clinical examination, it was noted that the right eye was incoitic with mild corneal cloudiness as well as superficial corneal neovascularisation. Conjunctival hyperaemia was not present. A large geographic ulcer was evident after application of fluorescein dye (Figure 1). Further questioning of the owner was unrevealing as they had not noticed any signs of upper respiratory disease and had perceived the cat to be healthy. The patient vaccination status was up-to-date.

Discussion
Feline Herpesvirus-1 (FHV-1) is the most common viral pathogen of domestic cats worldwide with up to 97% of cats having serologic evidence of exposure (Field et al, 2008). It is the only documented viral cause of feline keratitis and is primarily a feature of recrudescent disease of adult cats. Recrudescence occurs as a result of life-long latency within the trigeminal ganglia, and can range from brief episodes of conjunctivitis to chronic ulcerative keratitis with progression to sequestrum formation or stromal keratitis (Andrew, 2001; Hartley C, 2010). FHV-1 induced corneal ulceration results from direct viral cytolysis in the basal cell layer of the corneal epithelium, often beginning in a dendritic fashion that coalesce with chronicity to a map-like appearance results from direct viral cytolysis in the basal cell layer of the corneal epithelium, often beginning in a dendritic fashion that coalesce with chronicity to a map-like appearance. Recrudescence occurs as a result of life-long latency within the trigeminal ganglia, and can range from brief episodes of conjunctivitis to chronic ulcerative keratitis with progression to sequestrum formation or stromal keratitis (Andrew, 2001; Hartley C, 2010). FHV-1 induced corneal ulceration results from direct viral cytolysis in the basal cell layer of the corneal epithelium, often beginning in a dendritic fashion that coalesce with chronicity to a map-like appearance results from direct viral cytolysis in the basal cell layer of the corneal epithelium, often beginning in a dendritic fashion that coalesce with chronicity to a map-like appearance. Recrudescence occurs as a result of life-long latency within the trigeminal ganglia, and can range from brief episodes of conjunctivitis to chronic ulcerative keratitis with progression to sequestrum formation or stromal keratitis (Andrew, 2001; Hartley C, 2010). FHV-1 induced corneal ulceration results from direct viral cytolysis in the basal cell layer of the corneal epithelium, often beginning in a dendritic fashion that coalesce with chronicity to a map-like appearance results from direct viral cytolysis in the basal cell layer of the corneal epithelium, often beginning in a dendritic fashion that coalesce with chronicity to a map-like appearance. Recrudescence occurs as a result of life-long latency within the trigeminal ganglia, and can range from brief episodes of conjunctivitis to chronic ulcerative keratitis with progression to sequestrum formation or stromal keratitis (Andrew, 2001; Hartley C, 2010).

Figure 1. Geographic ulcer with small superficial neovascularisation coursing from 12 o’clock of cornea to most dorsal aspect of ulcer.

Based on clinical signs and signalment, a presumptive diagnosis of herpetic keratitis was made. No further diagnostic procedures were performed. The ulcer was debrided and the patient promptly treated with atropine 1% eye drops (one-off), triple antibiotic (neomycin, bacitracin, and polymyxin B) ointment BID, doxycycline 10mg/kg SID per os, as well as topical 3% acyclovir ointment 5 times daily. Upon revisit 1 week later, the ulcer had markedly decreased in size (Figure 2), with complete resolution of clinical signs the week after. Topical aciclovir was continued for 1 week post restoration. Oral isosorb 500mg BID was recommenced but declined.

Figure 2. 1 week later showing marked reduction in size of ulcer. Clinical resolution was noted a week after.
and the presence of neovascularisation confirmed chronicity of the ulcer which further increased the index of suspicion. However, a definitive diagnosis using viral isolation, immunofluorescence, immunohistochemistry, or PCR study was not obtained due to financial restraints.

The treatment of FHV-1 keratitis and keratoconjunctivitis is complicated by the lack of efficacious economic and readily available antiviral medication in the author’s geographic surroundings (Canberra). Topical aciclovir was chosen to treat the present case due to its convenient availability from the local chemist, as well as its relative low cost compared to other anti-viral medication. In vitro efficacy of aciclovir have been investigated by Maggs & Clarke (2004) and Williams et al (2004), both of which showed aciclovir to have a low efficacy compared to other antivirals (Figure 3); systemic doses associated with systemic toxicity were needed to reach clinically effective concentration at ocular surface. Hence, in a clinical trial performed by Williams et al (2005) in vivo, 4 cats with confirmed FHV-1 with geographic icicles were treated with frequent (5 times daily) topical application of 0.5% aciclovir ointment; all 4 cases resolved within 12-16 days with no toxicity. The authors concluded that despite the low in vitro efficacy of aciclovir, high drug concentrations when applied topically frequently provides viricidal effects on ocular surface without toxic effects. No in vivo comparison of aciclovir with other antivirals has been investigated to date. The present case was in line with clinical trial with resolution of clinical signs within 2 weeks of commencement of therapy. The use of oral aciclovir was considered in this case and would have afforded a more efficacious and convenient dosing regimen; however, financial considerations excluded its use. The success of aciclovir in this case was largely due to the owner being a retiree and hence able to commit to frequent medication.

The use of topical triple antibiotic ointment and oral doxycycline were largely prophylactic and empiric as coinfections with Chlamydia felis is common (Aroch et al, 2013). In hindsight, due to lack of notable chemosis and conjunctivitis in the present case, secondary infection with Chlamydia felis was likely absent. However, doxycycline has been found to have anti-inflammatory effects through inhibition of cytokine IL-1B, anti-collagenase properties through reduction of matrix metalloproteinases in tear film, and may have a positive effect on corneal epithelial cell migration (Liddle, 2008). These properties would support the use of doxycycline in any cases of keratitis; however, much of the information in Liddle’s (2008) review was extrapolated from human literature, as such these effects may not be inferred on feline patients. Furthermore, in one study only low concentration of doxycycline was found in tear fluid. This is hypothesised to be due to doxycycline being highly protein bound in plasma of cats, thus fluids with low protein content such as tear fluid can only carry a low concentration (Hartmann et al, 2008), thus an appreciable level within the tear film may not be reached in cats to achieve the anti-inflammatory, anti-collagenase, and epithelial migratory properties discussed.

Conclusion

The use of topical aciclovir provides a cheaper alternative to oral famciclovir for the treatment of persistent herpetic keratitis in cats; however, much consideration has to be given to client compliance in order to assure success due to its frequent dosing regimen. ■

References

Feld HJ, Blauwe S, Mohammadi MT (2006), Hepatitis: latency and therapy – From a veterinary perspective, Avitar/Research Vol 71 pg 127-133
Liddle VL (2004), The Novel Action of Doxycycline in Ocular Disease, Aust Vet Pract Vol 34 No 1
Williams DL, Robinson JC, Lally E, Field HF (2005), Efficacy of topical acyclovir for the treatment of feline herpetic keratitis, results of a prospective clinical trial and data from in vitro investigation, Veterinary Record Vol 157 pg 254-257

Have you ever been faced with an injured possum, lame wombat or wattle with a wobbly tail and wanted to take a radiograph to help you decide what was going on? Only then to be faced with a grim situation: on the radiograph you can see bones and they are leg-bones, but they don’t really bear any resemblance to a dog or cat. What are they? Should they look like that? Tarsal bones designed for hopping are not likely to look like those designed for walking. Is that normal?

It takes a back: perhaps you would like to radiograph an Australian mammal, but are not sure how to approach this task. Can you (or should you) even sedate native mammals? What’s the correct sedative dose for an echidna, platypus or bat? What is the dental formula for a wallaby, and what does kumyaw look like?

Radiographic anatomy of individual species has been published piecemeal over the last hundred years or so, but it is difficult to find it all in one place. The species of wallaby or possum in your practice might be different to pictures on the internet, and North American mammals are just not the same even if we squint…

Radiology of Australian Mammals presents the first detailed reference for normal anatomy of Australian mammals and...
Diarrhoea is an extremely common problem in both cats and dogs. Mild, transient diarrhoea is rarely a cause for concern but severe or chronic diarrhoea can be damaging to a pet’s health. Faecal tests for gastrointestinal pathogens (bacteria, viruses and parasites) should be considered whenever an animal has chronic diarrhoea. Routine flotation remains an important first step and can be easily done in practice. Wet preparations of fresh faecal mucus can also be useful in animals with signs of large bowel diarrhoea, and can be easily done in-house. On the whole, nematode and cestode infections have become less common as the result of widespread use of monthly products to prevent heartworm, fleas and endoparasites, although whipworm can still be problematic in certain jurisdictions. Some intestinal parasites such as Giardia duodenalis, Cryptosporidium parvum, Dientamoeba fragilis or Blastocystis hominis can be zoonotic (to a variable degree) and pose a significant health threat to people. Interpretation of results is highly dependent on the clinical scenario, so Veterinary Pathology Diagnostic Services (VPDS) of The University of Sydney makes a point of assisting practitioners with interpretation of results. It is well placed to do this as its staff are leaders in their fields, with a deep understanding of the test, the pathogen it is detecting (which may be viral, bacterial, protozoan or a helminth), and its impact on animal management.

The Faecal Pathogen Panel (‘Poo panel’ for short) offered by VPDS uses the principle of Multiplexed Tandem PCR employing 2 sequential PCR steps, adding to both sensitivity and specificity. Controls for PCR inhibition and DNA quality are included for each sample. Samples that fail quality control (QC) are repeated free-of-charge and unusual results are confirmed by follow-up testing in consultation with the veterinarian. This panel is designed to detect all known sequences of Campylobacter, Salmonella, Giardia duodenalis, Cryptosporidium (C. parvum, C. hominis), Trichomonas foetus, Toxoplasma gondii, Dientamoeba fragilis, Blastocystis hominis, Parvovirus, Distemper virus, Feline Coronavirus, and Canine Coronavirus. Let’s go through a typical clinical scenario where Veterinary Pathology Diagnostic Services (VPDS) and its multiplex PCR testing might be useful.

A Birman kitten recently acquired from a breeder appears to have chronic diarrhoea, which seems to be halfway between small bowel and large bowel in nature. Any in house test for Giardia coccidian antigen is positive, so the kitten is treated with high dose metronidazole. There is a transient improvement in clinical signs, but the kitten develops neurological signs, and so the treatment is stopped and the owner is cranky. A fresh poo specimen is submitted to VPDS. Faecal flotation is done first, and identifies a large number of eggs typical of a coccidian species in the enterocyte of the small bowel. The multiplex PCR panel is positive for Giardia, Trichomonas and Feline Enteric Coronavirus, although the C value for Giardia is very high. The interpretation of this result is that the kitten has a polymicrobial infection, suggestive of faeco-oral spread, with coccidiosis and trichomoniasis being the most likely enteropathogens responsible for the clinical signs being observed. The high C value for Giardia is most consistent with it having been a problem recently, but with the metronidazole having reduced the numbers of Giardia trophozoites in the intestinal mucus. Our recommendations are to first treat the cat using toltrazuril (Baycox; pig formulation) to clear the coccidians and then with a 2-week course of ronidazole (from BOCNA Compounding) to address the Trichomonas. During this time, feeding small meals of a highly digestible, high protein, low residue canned diet would be ideal, and a course of probiotics might be useful after finishing both anti-parasitic agents. Remember that there is physiological inflammatory bowel disease for some weeks after such enteric infections with an associated dysbiosis in the enteric microbiota, which...
takes a few weeks to resolve. The presence of Feline Enteric Coronavirus is common in cats that have recently been purchased from a cattery or shelter, and good litter tray hygiene, the use of clay litter and time is usually all that is required to deal with this likely self-limiting infection. It might be worth testing any in-contact cats who might have acquired either infection from the kitten, and the breeder should probably be contacted to alert them to an on-going problem in their cattery.

VPDS also performs a wide range of other sensitive molecular diagnostic methods for pathogen detection, including: highly sensitive and specific conventional and real-time PCRs; immunohistochemistry and in-situ hybridization for fresh and formalin-fixed tissues; immunofluorescence for detection of pathogens in body fluids, cytological preparations and faecal parasites; and molecular identification of bacteria and fungal fungi from cultures. For example, in the recent outbreak of feline calicivirus virulent systemic disease we have been able to apply specialised immunohistochemistry techniques to identify virulent systemic feline calicivirus FCV-VSD to be the cause of recent deaths in cats.

Detection of pathogens associated with companion animal and wildlife species is an area of strength for the group, and they welcome the opportunity to discuss development of new assays for support of animal management or research in these specialist areas. Plans are underway for release of an Avian Pathogen Panel (psittacine beak and feather disease virus, Avian Borreliosis, Avian Polyomavirus, Chlamydia psittaci, Psitticine Herpesvirus) and a Feline Anaemia Panel (FIV, FeLV, haemotropic mycoplasmas) early in 2016.

For more details, and for sample submission information, visit: sydney.edu.au/vetscience/vpds/molecular.shtml or gary.muscatello@sydney.edu.au

### Additional pathogen detection assays available from VPDS:

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### CVE Clinical Competency Awards for 2015

Each year we take great pleasure in inviting each Australian and New Zealand faculty of Veterinary Science to choose a recipient for our CVE Clinical Competency Award.

This prize of CVE$1,000 towards CVE CPD is offered to the graduating student who has been recognised by their faculty as being the most competent in clinical skills over the clinical portion of his or her undergraduate years.

We congratulate the recipients, wish them well as they embark on their veterinary careers, and look forward to welcoming them as Recent Graduates to upcoming CVE continuing professional development courses.

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Rollover names below:

- Charles Sturt University: Clare A Ferris (pictured)
- James Cook University: Troy Burg
- Murdoch University: Stephanie Nicholas
- University of Melbourne: Susan Ciaravolo
- University of Queensland: Katherine Law
- University of Sydney: Sarah Matthews

Massey University & University of Adelaide prize winners to be announced in a later issue.

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Any discussion of the factors that have caused the declination of Australia's fauna and ways to redress them should be welcomed. Dr Harvey's article 'The Debate About Feral Cats' (C&T 5517) unfortunately invokes emotion and ethics to support a case to 'forget the past and embracing the entirety of Australia's modern ecosystem'. Even accepting the situation as it is today is not the situation which will exist in 5 or 50 years' time. All the environmental elements are in dynamic balance 'accepting the situation as it is today' is to accept a trajectory to further extinctions and further environment degradation. As appealing as such a simple option might be, it is not a solution at all.

It is also pretty meaningless to draw comparisons between the intensive veterinary care of a terminal pet patient 'loved, owned domestic cat' and feral animals. The domestic cat has probably, throughout its life, been supplied with meat provided via the slaughter of healthy meat-producing species at the hands of man. The same ethical and welfare considerations should apply to this as the killing of Cecil the lion or any other animal. Killing on an industrial scale is the situation as it is today’ is to accept a trajectory to further extinctions and further environment degradation. As appealing as such a simple option might be, it is not a solution at all.

Let us look at the whole picture. The domestic cat, Felis domestica when released into a habitat becomes a predator and can decimate many of the other species native to that habitat. Do native species of birds, reptiles and small mammals not need protective action on the part of us humans that introduced the cat in the first place? Are these species any less important?

Controlling feral cats by trapping and euthanasia by itself had indeed been shown to not be successful. To be successful in the extensive habitat we have here in Australia there needs to be a multiple, intensive approach. Our human population needs to be educated to our responsibility as stewards of this environment. There needs to be Government participation. As is the case with dogs, cats need to be registered and microchipped. The registration fee needs to differentiate between intact and surgically sterilised individuals and a further reduction of the yearly fee for the construction of a cat enclosure. A public education program must be instituted for both adult cat owners and volunteers need to take a program to the classrooms of young children. Remember, confined cats have a life expectancy of between 4.5 and 5 years longer than cats allowed to be outside and free. If trap and neuter programs are used, the cats should not be released but kept in confined cat colonies. A team of biologists from New Zealand are very successful in eliminating cats from island environments using panteucopina virus. In remote areas where there are no natural predators, (Dingos and Tasmanian Devils) using the virus can be considered.

I am sure that there are other strategies that can be included in a program. We as a profession are responsible for the care of all the species; except one must look at the whole picture and consider all the species we share this planet with.

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I would like to voice my concerns regarding this article, which in my view is misleading and patronising. In the first paragraph 'The ABC recently broadcast greyhound live baiting that is now prohibited by the legislation' – this is misleading as it was prohibited many years ago.

Further down, ‘Despite the appointment of Animal Welfare officers by the abattoir management, the cruelty seems to be increasing according to reports from Animal Liberation activists’ – this is hearsay.

In 2011 secret footage of an Indonesian abattoir broadcast on ABC TV’s Four Corners led to the suspension of live cattle exports to Indonesia – this was a knee-jerk reaction by the ALP, which did not consider the fact that shutting down the market so suddenly led to thousands of cattle starving in northern Australia (not to mention loss of income by Australian farmers).

Finally the quote from Sir Paul McCartney: ‘if slaughterhouses had glass walls, everyone would be a vegetarian’. I visited many abattoirs as part of my Veterinary degree in Italy, and to this day I still enjoy my meat! Sir Paul, being a vegetarian, represents a minority view.

When Meat & Livestock Australia commissioned an independent team of consultants to assess animal welfare in Indonesia from arrival to slaughter in 2010, the live export trade to Indonesia was over 20 years old. It was the slaughter descriptions provided in this report (Caple et al 2010), which led to the investigations by both Animals Australia and the ABC. It was particularly significant that the report noted that ‘Slaughtering was observed to deliver the single biggest animal welfare and the greatest adoption of stunning in the slaughter of Australian cattle in Indonesia should be an aspirational goal’ (my emphasis). The 2011 trade suspension, regrettable as the fallout
Thus, even without trade suspension, starvation, and appalling body condition in cattle in the northern herds (referred to by Dr Viscardi) are not at all uncommon and do not require a trade suspension to occur. I travelled in the Kimberley in 2014 after an excellent wet season in 2013/2014 and the robust recovery of the live export trade. The photos I took in early September (still months away from the wet season and any feed) show significant degradation of the rangelands with its resultant effect on animal welfare.

These welfare issues exist with or without any trade suspension.

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The issue of feral animals in Australia is highly complex, ecologically, ideologically, it is a minefield. Ethically and philosophically, it’s a nightmare of complex ideas. As Peter Doherty said very recently – ‘it’s very healthy to have a good argument’, so Andrea Harvey started the ball rolling, and we have had views from Mike Barnyward (who was a researcher and raccoon) and James Harris (who has done just about everything you can in a veterinary career and, just to make things interesting, is now a Tasmanian). I could not resist weighing into the arguments. Stop reading now if you think I have a cogent answer to this issue. Because it’s hard to have a narrative thread when addressing a hyper-complex issue. I just want to say some things in point form:-

1. The horse has not just bolted. It has left the district. Shutting the stable door is not likely to help. By this I mean that Australia was an island continent full of unique marsupials and, apart from bats, hardly any placental mammals. As soon as man arrived, he/she proceeded to cause havoc. And I don’t just mean the first fleet from nany England. I mean the Asian seafarers that released the dingo-precursor for 14 thousand years ago. Just introducing this single highly intelligent placental mammal apex-predator changed so much, as existing marsupial predators simply were not in the race in terms of on-going natural selection.

2. Australia is the feral capital of the planet. We have the obvious things – rats (black and Norwegian), mice and rabbits. We have more camels in central Australia than they do in Arabia. We have more brumby than there are mustangs in the USA. Pigs and goats do exceptionally well here, as do feral cats, the subject of the initial debate, and foxes, intermediate in size between cats and dogs. The most common vertebrate in eastern Australia is... the cane toad, ironically introduced to control cane beetles (even though the best evidence-based research at the time predicted it would be a disaster). Some people argue the dingo has been here so long it should not be classed as being feral, but 8,000 years doesn’t seem that long ecologically. And then there are a range of feral plants – prickly pear, serrated tussock, bracken, gorse, blackberry and so forth. And of course the worst feral introduction is us, by which I mean non-Aboriginal humans. I am certainly no expert, but although there is good evidence indigenous people shaped the land by the use of fire, their population was small and well distributed, and perhaps their overall impact was negligible, or limited.

3. Perhaps the most informative story of feral pests in Australia is the rabbit. The impact this feral pest had was enormous. When the rabbit plague was at its worst, there was no sympathy for the rabbit. They were not considered cute. Probably they were thought of the way people currently think about rats, mice, cockroaches, cane toads and ex-prime ministers. There were no advocates for the welfare of this creature (there probably would be now). It was a vilified pest. Interestingly, the government was prepared to do something about this, and put their money where it counted. There is a terrific book called Pasteur’s Gamble (www.stephendandocollins.com/australian-history/pasteurs-gamble) about early attempts to develop biological control of this pest, and genuses based around Pasteur and Koch were in the race for the pot of gold. Eventually, the pox virus causing myxomatosis had a huge impact, and the role of Ian Clunies Ross (a veterinarian and then head of CSIRO), Sir Macfarlane Burnett (Walter and Eliza Hall Institute) and Frank Fenner (JCSMR, ANU) cannot be easily forgotten or over-looked. There was no collateral damage and, for a substantial period, things were greatly improved. Later release of haemorrhagic rabbit calicivirus was likewise effective; perhaps more controversial. For plants, biological control of Opuntia is the poster boy for control of feral plant pests, and where I live we desperately need something like this for serrated tussock (introduced in the saddle of an Afghan soldier when they build the Ghan railway line).

4. Right now there are government investment in the control of feral pests has been pitiful. This reflects overall investment in scientific research in Australia, including all aspects of veterinary science. The Invasive Animals Co-Operative Research Centre tries very hard, but is underfunded. There is not the political will to invest in the science which will make a difference. The goal is 20 years does not allow for investment in a problem that might take 10-20 years to pay off. Immunocastration using biological vectors should be possible in this world of advanced molecular biology, but it’s expensive and needs farsighted politicians to support it. I just don’t think Barnaby Joyce has the clout or the vision to pull off something like this. Maybe Alan Finkel, our new Chief Scientist, will make a difference; he has the intellect and the track record and he thinks about the big picture.

5. Mike Barnyard is completely correct in terms of the value of eradicating feral pests from islands, or to create fence-off sanctuaries. But this is laborious and expensive. A terrific idea for islands with treasured birdlife, and worth every cent. There are many islands left and we should do more to support such initiatives. Doing it on the mainland is no easy task, but it could be done if we are prepared to put in the funding. The great advantage of the island’s strategy is that it is much cheaper. However, the cost of eradicating feral pests in the mainland is so high that it might be cheaper to give up on mainland Australia altogether. It was a vilified pest. Interestingly, the government was prepared to do something about this, and put their money where it counted. There is a terrific book called Pasteur’s Gamble (www.stephendandocollins.com/australian-history/pasteurs-gamble) about early attempts to develop biological control of this pest, and genuses based around Pasteur and Koch were in the race for the pot of gold. Eventually, the pox virus causing myxomatosis had a huge impact, and the role of Ian Clunies Ross (a veterinarian and then head of CSIRO), Sir Macfarlane Burnett (Walter and Eliza Hall Institute) and Frank Fenner (JCSMR, ANU) cannot be easily forgotten or over-looked. There was no collateral damage and, for a substantial period, things were greatly improved. Later release of haemorrhagic rabbit calicivirus was likewise effective; perhaps more controversial. For plants, biological control of Opuntia is the poster boy for control of feral plant pests, and where I live we desperately need something like this for serrated tussock (introduced in the saddle of an Afghan soldier when they build the Ghan railway line).

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that, in my lifetime, there has been more attention on the potential extinction of Tassie Devils than any other terrestrial wildlife. The loss of unique frog populations, interesting small marsupials in WA and various rare birds have never really generated much ‘traction’ by the mainstream population or media. Yet the disease that threatens Devils is, by all accounts, a spontaneous event not related to any introduced feral species, global warming or anything else that we have done recently. Although I congratulate people who have intervened to preserve this iconic marsupial, the resources devoted to this endeavour might have been equally spent in many alternative ways. Maybe natural selection would have favoured spontaneous eradication of the disease? Maybe unaffected Devils should have been released back onto the mainland as some people have proposed, and not just in well-protected islands? Others will have a more informed view. It is fascinating to read about the unintended effects of reintroducing wolves into parts of the USA where they had formerly been hunted out, and how this has impacted on beavers, elk and the whole tapestry of the land.

6. What about feral pigs, goats, horses, camels, cats, foxes and dogs? This is where it becomes philosophical and highly emotional, and Dr Harvey’s opinion piece taps into this complexity. Peter Singer would wade in, I am sure, given half a chance. Let me explain what I feel, as a nominal ‘animal lover’ who owns a 600 acre hobby farm.

7. I hate rats and mice. They breed prolifically, eat grain and other foods, harbour pathogens and attract snakes to the house and bird sheds. I use anti-coagulant rodenticides and dispatch any rodents found to be dying slowly of their effects. I try as best I can to keep the place clean, and not leave feed around, but I cannot convince the chooks and other birds to do the same in their cages. I do not lose sleep over killing these rodents. But many people in cities keep them as pets; they are cute, can be loving and share 99% of our DNA.

8. I cannot stand watching the ‘damage’ feral pigs make to the pastures when rooting, especially in winter when the ground is soft after rain. A commercial elite shooter and his dogs are enlisted to take care of the problem. It’s a temporary fix, because you never get all the young ones, and others always move in. I take no pleasure from facilitating this; the methods used are not always acceptable ethically. The alternative is feeding them grain poisoned with 10-80, and this is complex and labour-intensive to make sure non-target species are not poisoned. I do not know enough about pigs’ effects on the whole ecosystem, but I look forward to the day someone develops a contagious disease which will provide effective biological control. I am sure with effort that this will be possible.

9. We have feral goat herds in the area, some quite large, plus lots of sporadic groups of goats. They occasionally come through the property, I don’t mind them at all, and I get upset when they are killed by helicopter shooting on nearby land controlled by the National Parks & Wildlife Service. I especially dislike that nothing useful is done with the carcasses; it seems just a complete waste. The locals say the goat herd is changing colour, as darker animals are better camouflaged and not so easy to shoot from the choppers.

10. Finally, I abhor the shooting of brumbies, and have yet to read convincing evidence that they have any important environmental impact in most jurisdictions where they are shot. If you read through the recent papers, they keep referencing government reports, which reference other government reports, without any really good peer-reviewed papers showing exactly what adverse impact they have on the environment. They make wonderful saddle horses, and some of them are my best friends.

11. What about feral cats, dogs and foxes? When driving to and from the farm – dingos and foxes look attractive, intelligent animals to me – I swerve and brake to stop hitting them with my car. When a fox kills one of my geese, I am the first person to lay 10-80 baits – and I am very happy if feral cats and dogs take them as well. I would prefer to use TAP, and cannot understand why it’s taking so long to be released into the market as it promises to be much safer to use an agent with an antidote (methylenene blue) which can be given orally should a farm dog eat the baits.

12. There is almost no spill-over from unwanted domestic cats from suburbs into the Australian bush. Most of the young cats that are not taken in by well-meaning people die of infectious diseases, poor nutrition and predation. They have negligible impact on cats in the bush, which are a prolific, self-perpetuating population. No amount of early neutering will impact on the feral cat problem, except immediately around human civilisation.

13. Are my views justifiable? Using logic or philosophy – not a chance! I love my small ruminants and don’t want a dingo-hybrid-dog to kill them. To me, they are family. And I don’t want a fox to ‘take’ my chickens, ducks or geese. The birds are trained to come in at night, but the feral carnivores do not always play by the rules. But I am not under the illusion that controlling these animals around our property will make any impact on the wider problem. I am certain most programs that control feral cats are likewise a waste of time and effort. There has to be a more robust solution – and likely it will be immunocarcinogen or the use of a contagious infectious disease for which a vaccine can be made to protect owned cats (as was the case for Caliciviruses and rabbits).

14. Now, the really hard question: Can a vet who loves all animals condone carnivores like dogs and cats? Cats and dogs are carnivores, and I feed my cats raw meaty bones for their optimal health, combined with commercial canned food. But it’s only through mental compartmentalisation that I can attend to my large collection of birds (chickens, bantams, geese, peafowl, turkey, ducks, parrots, etc.), while feeding chicken drumsticks to my cats at home. Logically, this is nuts! Likewise, it seems to be that its nuts to be a vegan and own a cat or dog, as you are locked into feeding it fresh meat or rendered animal protein to maintain its optimal health (I guess you could base their rations on rendered fish, which I honestly do not think of as being a sentient species).

15. Mental compartmentalisation is a very human thing to do – we do it with our kids and families all the time. It’s a sad reality that many cats and dogs in Australia are much better fed, housed and medicated than people in impoverished nations, possibly including some of our indigenous Aboriginal population. If you own a large dog and feed it a diet of premium dog food and raw meaty bones, and total the cost or nutrient value, you could probably save the lives of at least 2 human beings somewhere on the planet. Having said this, you could save many more if you stop sending your kids to private school(s), or stopped subscribing to cable television. But how do you value the companionship a cat or small dog brings to a lonely pensioner? Or a young kid in a dysfunctional home?

16. Although I am striving for hyperbole, there are important points here – and they have been raised or addressed by the different correspondents. How can vets who love sentient species eat meat, or feed it to our pets? I have loaded many cattle onto trucks over the years and, Mario, I can tell you they are not happy on the way to the saleyard. The animals on live export ships are even less happy. They know something bad is going to happen. They know they are leaving their friends and relatives.

Although I don’t have answers for the ‘big questions’, I do have some suggestions:

1. For people who like dogs, recommend people get small dogs, not big dogs. Maybe not a popular move, but a very sensible one. They eat less food and live longer, and are less likely to damage others - dogs, cats or people. It may not be a conventional or popular view, but I don’t think we need any dogs weighing more than 25 kg. In fact, my view is that 8-20 kg is about optimal.

2. For many people, an intelligent parrot might be a far better pet than a cat or dog. They are intelligent, engaging, make fantastic companions and live long healthy lives. The trouble with many species is they are loud and raucous, but species like Eclectus & the bill (I am no expert here).

3. And as caring people, if you cannot come at being a vegetarian (and I lack the discipline), then eat more fish, and smaller servings of meat. And try to find someone who sells REAL free range eggs.

4. To feed small obligate carnivores (like cats and small dogs, I think the way forward is to use fresh meat that is not well loved by humans, like ruminant hearts and chicken wings for cats and chicken necks for dogs. Unfortunately, my present crew of cats prefer drumsticks. Without doubt, this is more efficient and healthy than rendering the protein so it’s suitable for use in extruded dry cat and dog rations.
Veterinarians Taking the Lead on Animal Welfare & Ethics: A Summary & Reflection of the Day

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On the 2nd December 2015, over 80 veterinarians and a handful of lawyers, animal welfare scientists and other interested professionals gathered at the Centre for Veterinary Education (CVE) at The University of Sydney to attend a symposium entitled ‘Veterinarians taking the lead on animal welfare and ethics’. This excellent attendance was a good start to the symposium in demonstrating that veterinarians are taking a serious interest in animal ethics and welfare issues. The day had an enticing programme with an impressive line-up of speakers, with: keynote speaker Professor Emeritus John Webster from the UK, the veterinarian credited with the Five Freedoms; animal welfare lecturers from all Australian and New Zealand veterinary schools including Anne Fawcett, Teresa Collins, Susan Hazel, Clive Phillips, Raf Freire, Andrew Fisher, Janice Lloyd and Kevin Stafford; and spokespersons from veterinary animal protection groups: Rosemary Elliott from Sentient, The Veterinary Institute for Animal Ethics, Sue Foster from Vets Against Live Export (VALE), and Alex Burleigh President of the Australian Veterinary Association (AVA) group Australian Veterinarians for Animal Welfare and Ethics.

I was eager to see how the day compared to another animal welfare conference that I attended recently held by the NSW Young Lawyers Society and focusing on animal law. I was left at the end of that day with the distinct impression that our professional skills, our professional training and the animals being hunted. ’They call for ‘statutory codes of practice to be developed to ensure that those engaged in hunting use methods to seek and kill prey animals that will minimise stress and suffering to both the prey animal and any other animals used in the hunting process.’ Since this statement was written, there has been a huge public shift against support for recreational hunting. The AVA’s position statement reads as a moral cop-out, ignoring the unethical nature of the trade.

I had been privileged to have been taught by Prof John Webster as an undergraduate during my veterinary science degree at The University of Bristol. He is undoubtedly a world-class leader in animal welfare and has been for several decades, since being inspired to make a difference after witnessing the horrific conditions that veal calves were reared in. His key messages for the delegates were simple and very wise:

- We need science to improve our understanding of factors that determine an animal’s welfare state, but science never will and never should be the sole foundation for our attitudes and actions towards animals;
- The welfare of a sentient animal is defined by the animal’s perspective and think about how the animal would favour, and not simply act towards solutions that the animals themselves would favour;
- The responsibility of the animal welfare scientist is not just to seek the truth but also to guide public opinion towards solutions that the animals themselves would favour;
- Veterinarians need to always consider things from the animal’s perspective and think about how the animal would be feeling in any given situation (which he termed reverse anthropomorphism);
- Veterinarians need humanity and courage in fighting for the interests of animals. Our professional skills, scientific knowledge, and ethical arguments are of no concern if we don’t act to create solutions that the animals themselves would favour, and not simply act according to regulations.
members, some of whom may be employed by industries that perpetuate inhumane practices. When seeking advice on animal welfare matters, the media, government and industry bodies will call first upon professional associations. This may raise questions about whether the advice provided is in any way influenced by the desire to maintain relationships with powerful industry stakeholders. This isn’t just a problem in Australia; in a commentary by Professor Andrew Knight on how the welfare standards of veterinary associations in the UK lag behind the general public, he stated ‘Notwithstanding individual, courageous exceptions, it appears that a certain critical mass of public opposition to animal exploitation must be present before the veterinary profession finds the courage to add its weight to the debate en masse. Such “leadership from the rear” is neither courageous nor honourable.’

Dr Elliott asked the audience: ‘Is this what we are striving for? Why are we still allowing industry to dictate what our profession accepts about how animals are treated? We know that the welfare needs of animals do not change, regardless of the situation they find themselves in.’

Dr Anne Fawcett considered some of the ethical dilemmas in companion animal practice and, in particular, raised the critical deficiencies in the conclusions of this paper. She asked the audience: ‘Why are we still allowing industry to dictate what our profession accepts about how animals are treated? We know that the welfare needs of animals do not change, regardless of the situation they find themselves in’. Dr Fawcett also discussed how often people can get away with ‘doing the wrong thing’, but it is actually when we are trying really hard to ‘do the right thing’ that conflicts more frequently come to the fore. In other words, sometimes the area in which we are working or people with whom we are working make ‘doing the right thing’ in the interests of animals harder than ‘doing the wrong thing’. In contrast, it is easy to do the wrong thing by animals; they can’t speak back, they can’t bad-mouth us, they can’t make complaints, send us threats, sack us from our jobs, exclude us from a community, or take legal action against us. Perhaps that is why the veterinary profession is lagging behind in the animal protection movement; because doing the right thing is often not the easy pathway. Perhaps as a profession we are not always strong and courageous enough to ‘do the right thing’, particularly when conflicts of interest arise, making this pathway particularly uncomfortable. A final and important conflict of interest that wasn’t discussed on the day but is highly significant, is simply the desire to ‘fit in’ and ‘be liked’. This often means conforming to entrenched practices rather than challenging them. Is this why many in the profession cop out when it comes to standing up for animal welfare when there are complex conflicts of interest, because we follow the path of least resistance and ‘take the easy way out’? It is only a courageous person that questions common belief, and this not infrequently may result in significant personal and professional compromise.

A recent paper published in the Australian Veterinary Journal (AVJ) by Moore et al, ‘Risk factors for mortality in cattle during live export from Australia by sea’, analysing voyages from 1995 to 2012, was discussed by three of the speakers, Dr Sue Foster, Dr Rosemary Elliott and Prof Andrew Fisher. It was interesting and insightful to hear their different conclusions on this paper. Prof Andrew Fisher used it as evidence of the live export industry making positive steps to investigate and improve animal welfare and praised them for these efforts and this success, with the conclusion of the paper being that the industry had achieved a marked reduction in mortality rates since 2000. Dr Rosemary Elliott took interpretation of the paper a step further by noting that it was industry-funded research and, acknowledging that this may introduce conflicts of interest, she looked further for any evidence that such conflicts of interest may have influenced the researchers’ conclusions. Dr Elliott noted that outliers such as disasters due to ventilation failures or bad weather had been excluded from the data set. Dr Sue Foster also noted this and had taken this another step further by seeking further information about the voyages that had been excluded from the data set. These outliers were found to include several voyages where mortality was up to an astonishing 75%, due to issues with rough seas and inadequate ventilation, both factors that were not identified as significant risk factors in the conclusion of the paper. Dr Sue Foster then showed that all the high mortality voyages after the period of study had been due to rough seas, inadequate ventilation or both, i.e. the inherent risks of the live export trade that had been overlooked or excluded in this industry-funded AVJ paper. This begs the question as to why a Professor of Cattle and Sheep Medicine, and ANZCVS Fellow in Animal Welfare, would fail to notice these critical deficiencies in the conclusions of this paper. Is the veterinary profession truly standing together on important animal welfare issues or are conflicts of interest pulling the wool over our eyes?

Prof Kevin Stafford highlighted that you can never be liked by all parties when it comes to wild horse management, and advocated the shooting of wild horses for managing their populations. He did, however, acknowledge that none of the currently utilised methods of wild horse control in Australia could be called humane in the true sense of the word, which means tender, sympathetic, and kind. Mass killing of any animals is neither tender, sympathetic nor kind. This led me to wonder: is ethical blindness enforcing us to accept an alternative definition of ‘humane’, influenced by our own views, rather than admit that the entrenched...
practices we condone are inhumane? As an area close to my heart, and one in which I am currently undertaking a PhD, I would argue that we can do better than this and MUST do better than this. The path ahead is not an easy one – there are never simple solutions to complex issues – but as veterinarians we MUST strive for better solutions that are in the best interests of the animals and not kid ourselves that mass killing of healthy animals is anything near acceptable as a long-term solution.

Prof Stafford thoughtfully discussed the many complexities surrounding decision-making in wild horse management, outside of animal welfare concern, including environmental issues, politics, tradition, public views, resources and, importantly, finances. He raised the difficult role of politicians in their position of deciding whether to spend a particular pot of money on improving management of wild horses or improving child health care and education. I am not belittling the difficult dilemmas for the politicians, but luckily we are veterinarians, and not politicians. The decisions on complex issues like wild animal management, racing regulations, live export etc, will ultimately be made by others outside the profession and it is up to them to consider other factors. It is our job to be advocates for the animals and advise on the most ethical and humane decisions for them. If the veterinary profession does not do this, then animal welfare will never be high on the political agenda.

Perhaps this is one of the problems in our profession: We try to take on too much responsibility in too many areas. Juggling lots of conflicting interests is extremely challenging and emotionally stressful, and undoubtedly one of the contributing factors to the high suicide rate in our profession. As veterinarians, we may be: business owners, industry employees, or veterinarians for sport animals and livestock; we may have human clients, strive to be responsible citizens, or wear other hats outside of being a veterinarian. But if this symposium taught me anything, it was that we MUST as veterinarians ALWAYS have as our primary goal the best interests of the animals. If we deviate from this we are letting down the animals and our profession. If we can remember this simple priority first and foremost perhaps it will make decision-making simpler for us, and the conflicts easier to manage. In this quest to always advocate for the best interests of animals, we can use science, but we must also use empathy and common sense, and we must open our eyes to what is happening around us, and be courageous enough to challenge entrenched practices that compromise animal welfare.

Prof John Webster insightfully commented at the end of the day that there had been much discussion throughout the symposium about how WE felt about various animal welfare issues, and conflicts of interest, but with the exception of a couple of speakers, there had been little discussion about how the ANIMALS might feel within the various contexts and industries discussed.

Prof Webster challenged Dr Alex Burleigh by asking if he had tried ‘Stockstill’ (electro-immobilisation) on himself, which he had not. The purpose of the question was not about arguing whether electro-immobilisation was ethical or humane, but it was to make the point that we need to put ourselves in the situations of animals before we can make a judgement about whether a particular practice is ethical or humane, and this is what Ruth Harris, the first real pioneer of animal welfare, and author of ‘Animal Machine’ did. In this modern day, with so much knowledge and resources at our fingertips, we need to reflect on the fact that Ruth Harris in the 1960’s may have had a better understanding of animal welfare than many of us have now, by simply putting herself in the shoes of the animals, and really truly considering how they might ‘feel’.

How many of us have tried living cramped so tightly together with thousands of others that we can barely move? How many of us have tried living in such cramped conditions that it gives rise to fighting and cannibalism? How many of us have tried giving birth and feeding babies whilst confined in a crate preventing us from even turning around? How many of us have tried running frantically for our lives whilst being shot at from the ground or helicopter as our friends and families fall dead around us? How many of us have had our newborn baby taken away from us and slaughtered so that we can provide milk for another species? How many of us spend most of our life pregnant having another baby year on year? How many of us have tried running as fast as we can to the point of incurring severe limb injuries and often in extreme heat whilst being whipped to run faster? How many of us have laid in our own faeces on a long sea voyage for days with inadequate ventilation and extreme heat, whilst friends around us were dying of pneumonia and heat stroke? How many of us really understand how the animals are FEELING in these practices and industries that our profession condones?

Dr Jeni Hood also spoke wisely, simply and powerfully, saying: ‘In the quest to be scientific we can miss the obvious. We can be informed by science but we need to be advocates for animals and this requires empathy as well as science.’ It doesn’t matter what credentials or publications in animal welfare we have if we cannot empathise with how these animals are likely to be feeling. Dr Elliott went on to say: ‘Society judges vets by very high standards – our actions and our INACTION may be interpreted as condoning animal cruelty, and this risks leaving us behind in what many have conceptualised as the next social justice movement. And yet, we must remember we are in a powerful position to effect change, as members of a respected profession with an enviable knowledge of animals and their care.’ She showed the audience a number of images similar to those in figures 5 to 8, and asked ‘do we find them acceptable, knowing animals have the capacity to experience pain and distress? And joy? Are we doing enough to advocate for the higher order needs of animals (such as the ability to maintain social relationships and engage in positive experiences) rather than setting the bar at trying to reduce pain and suffering? And are we even achieving that?’

Considering Dr Elliott’s very pertinent and worrisome points, it would appear very clear that the veterinary profession is not taking the lead on animal welfare and ethics and is, in fact, being left behind by other stronger and more courageous professions and organisations in the animal protection movement. Dr Elliott advised ‘we are best placed to find our niche in the animal welfare movement by being vets – without apology – which means approaching all issues of animal welfare and ethics with a fresh view. Knowing that animals have the capacity for both suffering and pleasure, we have no excuses: we must advocate for them using sentience as the foundation of ethical decisions. The moral implications of animal sentiment will take us to some uncomfortable and confronting positions, and how we deal with this will determine the views others hold of our profession.’

Prof John Webster summed up that a few movers and shakers will make the biggest difference to advances in animal welfare; let’s be those movers and shakers and not remain fence-sitters. I confess that I have not always been courageous; I am as guilty as others of ‘sitting on the fence’, and wanting to take ‘the easy option’. I am proud to be in the veterinary profession, and I want to fit in and be liked. I don’t want to be disliked by the AVA, by the speakers I have disagreed with here, by other members of our profession, or by industries that condone poor animal welfare outcomes. I have many friends in the farming industry and in equine sporting industries. I have friends who are hunters, and many of my friends eat meat, and I don’t want to be disliked by any of them. But I have been inspired by the speakers at this symposium and intend to follow Dr Elliott’s advice to strive to be a strong and kind vet, an animal advocate, without apology, and to do the right thing by speaking out for the interests of animals above any personal conflicts of interests, highlighting the deficiencies of our profession in this regard, and encouraging other veterinarians to do the same.

Others may not have made an oath, or may not abide by it, but in July 2000 at Bristol Veterinary School, UK, in the presence of the board of the Royal College of Veterinary Surgeons (RCVS), I made the following oath: ‘I promise and solemnly declare that I will pursue the work of my profession with integrity and accept the responsibilities

**Interesting podcast**

From Radio National, Life Matters
to the public, my clients, the profession and the RCVS, and that ABOVE ALL my constant endeavour will be to ensure the health and welfare of animals’.

The oath itself contains factors that will cause conflicts of interest; however, it is the RCVS and not me that capitalised the ‘ABOVE ALL’, making it very clear where a veterinarian’s priorities should lie within any conflicts of interest. I will therefore stand by my oath, without apology. We can make animal welfare as complex as we like, we can hide behind the science, and hide behind the clouds of ‘lack of evidence’, but the take-home message is simple. If the whole veterinary profession followed this without exception, we surely would be taking the lead on animal welfare and ethics, and would be surely making a positive difference to the lives of billions of animals in doing so.

Dr Elliott challenged the audience by asking: ‘When we consider the sheer numbers of animals who suffer in intensive industries, with estimates of approximately 76 million sheep, 2 million pigs, 99 million chickens and 29 million cattle at any one time in Australia, how can we justify remaining silent as a profession while organisations such as Voiceless, Animals Australia and RSPCA Australia, and the legal profession through their involvement in these organisations, and others such as the Barristers Animal Welfare Panel, take the lead in educating the public and advocating for animal welfare reform? The veterinary profession has a clear role to collaborate with these other organisations and professions in the animal protection movement’.

But until we can unite as a profession with the simple goal of being animal advocates as our primary purpose, then I think it is difficult to know exactly how asymptomatic a cat with a borderline anaemia is since they are so good at adapting to their illness. I would certainly consider treatment if there are other clinical signs which could be impacted e.g. poor appetite, generally below par etc which could reflect poor energy levels. Otherwise, watch and wait and change the plan if there is a deterioration in complete blood count/clinical status.

I have a renal case with an asymptomatic packed cell volume (PCV) of 21. The September 2013 JFSM article lists criteria for deropoeitin use and commented re limited data regarding efficacy and safety and suggests not treating till symptomatic. I wonder if advice has altered with more-widespread use? I guess my thrust is whether renal-induced anaemia becomes more refractory to erythropoietin if left until more severe?

Reply

Sarah Caney
CVE/ISFM Feline Medicine DE Course Tutor

I don’t think that there is currently much published evidence to support decision making for you or answer the questions you pose.

For me, a PCV of 21 is at the borderline of ‘to treat or not to treat’. I would definitely check iron levels as many of my patients are borderline or actually iron deficient and that is something that can be addressed easily and inexpensively.

I think it is difficult to know exactly how asymptomatic a cat with a borderline anaemia is since they are so good at adapting to their illness. I would certainly consider treatment if there are other clinical signs which could be impacted e.g. poor appetite, generally below par etc which could reflect poor energy levels. Otherwise, watch and wait and change the plan if there is a deterioration in complete blood count/clinical status.

Interesting Link

Regional Nerve Blocks Key to Delivering Quality Dental Care
Brett Backman

Sonographic Characterisation of the Urogenital Tract of the Koala (Phascolarctos cinereus) for Standardised Investigations of Urogenital Pathology

Winners: Kathryn Stalder,1 Caroline Marschner1 & Richard Malik,1 Damien Higgins,1 Larry Vogelnest,2 Graeme Allan1,3 & Mark Krockenberger1

Background: Urogenital tract disease is the most important clinical manifestation of chlamydiosis in free-living koala populations in Australia. Chlamydiosis is a contagious, venereally transmitted endemic disease of considerable importance in most koala populations. Structural urogenital pathology is an important feature of this disease; that is why a systematic diagnostic ultrasonographic protocol was developed to standardize the sonographic examination of koalas. As the urogenital anatomy of marsupials differs significantly from that of domestic species such as the dog and cat, the protocols used in companion animals were adapted for the purpose.

Results: Using a variety of acoustic windows, described, it is possible to consistently obtain diagnostic images of the kidneys, bladder, prostate, bulbourethral glands, uterus, ovaries and testes. A detailed description of the sonographic examination technique, equipment requirements, reference data for the dimensions and echo-texture of normal urogenital organs are provided. Pathological sonographic findings observed commonly in association with urogenital chlamydiosis are described and verified by gross necropsy observations and histopathological findings.

Conclusion: A standardised ultrasonographic protocol for the examination of the koala urogenital tract is described, enabling effective use of this diagnostic tool in the management of individual koalas.

Abstract

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Conclusion: A standardised ultrasonographic protocol for the examination of the koala urogenital tract is described, enabling effective use of this diagnostic tool in the management of individual koalas.

Key words: Koala; Phascolarctos cinereus; protocol; ultrasonography; ultrasound; urinary; genital; Chlamydia
is also reflected by the rate at which koalas with chlamydial disease are admitted to care.\cite{16}

Many of the severe structural changes associated with chlamydial disease in koalas can occur without clinical signs,\cite{16} yet detecting these is crucial to therapeutic and prognostic decision making. Abdominal radiography is a poor diagnostic tool in the koala as there is minimal intra-abdominal fat to provide visualisation of most of the organs. Ultrasonography has become a common diagnostic tool for characterising urinary and reproductive tract diseases in small animal medicine\cite{17} and principles of the ultrasonography are fundamentally the same regardless of species. Diagnostic ultrasonography offers the ability to identify and examine individual viscera in koalas\cite{17} and was recently demonstrated to be an accurate tool to evaluate the urogenital tract in this species.\cite{17} Anatomic differences between the reproductive tracts of marsupials and small eutherian carnivores (cat, dog, ferret) include the arrangement of the accessory sex glands in the male koala, the paired uteri in the female and prominent epipubic bones, which interfere with certain acoustic windows used for abdominal and especially pelvis imaging. For this method to be effectively utilised to assess pathological changes, knowledge of normal sonographic anatomy and dimensions are necessary to accurately differentiate abnormal from normal.

The aims of this study were to first develop an ultrasonographic protocol suitable for the evaluation of the urogenital tract in male and female koalas; to establish normal ultrasonographic references in terms of size, shape, position and echogenicity of organs in the urogenital tract of healthy adult male and female koalas and finally to apply this information in the context of identifying pathological structural changes and validating these changes with gross and histopathological findings.

### Animals & Methods

To establish technique and reference intervals, ten Chlamydia PCR-negative and Cryptococcus serum antigen-negative adult koalas (7 female, 3 male), were chosen from a captive population at Taronga Zoological Park. The koalas comprised one juvenile (1 to 2 years), two young adults (2 to 5 years), six middle-aged (5 to 10 years) and one aged adult (over 10 years). Animals were examined during seasonal reproductive quiescence (between April and June). The investigation was approved by the Taronga Conservation Society Australia and University of Sydney Animal Ethics Committees. For the ultrasonographic investigations of diseased urogenital tracts, deceased koalas were used. These koala cadavers were presented frozen and thawed, or fresh (approximately 24 hours after death or euthanasia) for routine necropsy at the University of Sydney following euthanasia by koala care groups or veterinarians for welfare reasons at remote centres.

#### Part 1: Establishing an ultrasonographic protocol and reference intervals

**Ultrasonographic protocol:** Koalas were anaesthetised using a semi-closed circle system (Midjet Mark 3**, CIG using an Abbot Isoflo® out-of-circuit precision vaporiser). Anaesthesia was induced by mask using isoflurane (initially 5%) in oxygen (5L/min) and maintained using isoflurane (1-2%) after endotracheal intubation. The anaesthetised koala was placed in dorsal recumbency on a heating pad. Fur was not clipped from the ultrasound sites, but 70% ethanol and then ultrasound coupling gel were applied in liberal quantities to improve acoustic coupling. The ultrasound machine was B mode, grey scale ultrasound machine (Toshiba Just Vision 200 SSA 320 A) with a multi-frequency micro-convex transducer (5-7 MHz). Due to unforeseen circumstances this ultrasound machine was not available on one occasion, so a second B mode grey scale ultrasound machine (Aloka 500) with 5 MHz linear transducer (model UST-974-S) was used. The images obtained from each animal were printed by a Sony Video Graphic Printer (Model Number UP-895 CE) onto Sony Type 1 URP-1106 High Quality Printing Paper. Additional images were acquired for publication after the end of the study using two additional machines. One was a GE BT12 e Logiq e with two transducers, being a microconvex transducer producing 4.0-10.0 MHz frequency and a small footprint linear array transducer producing 8-18MHz. The other machine was an Essa equipped with a microconvex 5.0-8.0 MHz transducer and a linear 6.0-12.0MHz transducer. Images from these machines were stored digitally on a hard drive and output directly to a computer for archival storage.

**Examination of the female urogenital tract:** The transducer head was initially placed in the pouch, along the ventral midline between the epipubic bones, parallel to the long axis of the koala, to facilitate visualisation of the urinary bladder. Sagittal and transverse views were obtained. Five measurements of wall thickness were made in each view. The urinary bladder was described in terms of its size, shape, position, internal echogenicity and wall thickness. While evaluating the bladder in the transverse plane, the left and right uterus were visualised consistently. The transducer head was then gradually rotated to trace each uterus cranially along its sagittal plane. Measurements of ureter luminal diameter and wall thickness were obtained and described in terms of size, shape, position and echogenicity.

**Figure 2. Dorsal scan of the right kidney from a healthy koala.**

**Figure 3. Sagittal scan of the urinary bladder, using colour flow Doppler to highlight the ureteric jet (colour coded red/yellow).**

**Figure 4. Sonographic images of the uteri and urinary bladder in healthy koalas.** In A (transverse view of the pelvic contents), the urinary bladder (UB) is in the near field, while the right uterus is evident as a circular structure delineated by arrows. In B (sagittal view of the pelvic contents), the urinary bladder (UB) is visible in the near field, with the right uterine horn adjacent (highlighted by arrows).

**Figure 5 (Right). Multilobulated paraovarian cysts from a koala Chlamydia positive.** The right ovary from a healthy koala is illustrated in A (defined by 2 marking points). In B, image of a Chlamydia diseased animal was obtained in situ after euthanasia, while C was taken prior to necropsy, but ex situ (in organ bath). D demonstrates the gross appearance of the lesions at necropsy.
To obtain views of the kidneys in a dorsal plane the transducer was placed on the lateral flank, immediately ventral to the transverse processes of the lumbar vertebrae. For the sagittal views, the transducer contacted the ventral body wall, caudal to the last rib, parallel to the long axis of the koala, a few centimetres lateral to the midline. Five measurements of the dimensions of each kidney were taken at its maximum, where the characteristic mid-sagittal pattern (diverging renal diverticulae and blood vessels evident as two hyperechoic parallel lines) was observed.11-13 Transverse views were obtained by rotating the transducer 90° in a clockwise direction.

Examination of the male urogenital tract: The scrotum was reflected and the urinary bladder identified and described as in female koalas. The prostate was imaged by tracing the neck of the bladder caudally and directing the transducer head under the pubis, into the pelvic canal.

If the testes were of equal size, only one was examined. Five measurements of testicular dimensions were obtained from both sagittal and transverse planes. To evaluate the bulbourethral glands the transducer was placed between the epipubic bones, scanning laterally and caudolaterally.

Table 1: Ultrasonographic appearance of the urogenital tract of healthy koalas

<table>
<thead>
<tr>
<th>Structure</th>
<th>Ultrasonographic Appearance</th>
<th>Organ Size (mm)</th>
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</thead>
<tbody>
<tr>
<td>Bladder (n=10)</td>
<td>The urinary bladder appeared as a distinctive oval to round shaped organ, with a thin wall and an anechoic lumen. An outer hypoechoic serosa and inner hypoechoic submucosa separated by a hypoechoic muscularis layer. The innermost hypoechoic mucosal layer was not consistently differentiated. Jets of urine were easily seen entering the urinary bladder at the uretero-vesicular junction in both transverse and sagittal sections. The presence of echogenic material within the bladder lumen was noted in 3 koalas, urinalysis findings from these koalas were unremarkable.</td>
<td>Bladder wall thickness: 1.5-5.5mm, depending on the degree of distension.</td>
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<tr>
<td>Kidney (3 males; 6 females)</td>
<td>The fibrous renal capsule appeared as a thin hypoechoic structure adjacent to the peri-renal fat surrounding the kidney in both the transverse and sagittal planes.</td>
<td></td>
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<tr>
<td>Renal capsule</td>
<td>The renal cortex was uniform in texture with a finely mottled appearance. It was less echogenic than the renal capsule, but hypoechoic relative to the renal medulla.</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>Arcuate arteries were visible as circular and linear hypoechoic areas at the cortico-medulary junction. Interlobular arteries and their associated adipose tissue appeared as circular areas of increased echogenicity between adjacent renal pyramids.</td>
<td></td>
</tr>
<tr>
<td>Vessels</td>
<td>The fibro-fatty renal capsule appeared as a thin hypoechoic structure adjacent to the peri-renal fat surrounding the kidney in both the transverse and sagittal planes.</td>
<td></td>
</tr>
<tr>
<td>Transverse section of uteri</td>
<td>The right and left uteri appeared as round, homogenously hypoechoic organs located dorsal or dorsolateral to the urinary bladder and ventral to the colon.</td>
<td></td>
</tr>
<tr>
<td>Longitudinal section of uteri</td>
<td>The urinary bladder appeared as homogeneous hypoechoic structure.</td>
<td></td>
</tr>
<tr>
<td>Sagittal section of uteri</td>
<td>The uterine lumen was seen in longitudinal plane and was narrow, anechoic to hypoechoic and bounded dorsally and ventrally by echogenic uterine walls.</td>
<td></td>
</tr>
<tr>
<td>Uterine Lumen (n=2)</td>
<td>The urinary bladder (n=10)</td>
<td></td>
</tr>
<tr>
<td>Testis/Epididymis (n=2)</td>
<td>The head of the epididymis, located at the cranial pole of the testis, was almost isoechoic to the testicular parenchyma, whereas the tail of the epididymis was readily distinguishable by its anechoic to hypoechoic appearance.</td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td>The tunica albuginea is visible as a clear hypoechoic line surrounding the testis and epididymis.</td>
<td></td>
</tr>
<tr>
<td>Epididymis</td>
<td>Round to ovoid homogenous structures of medium echogenicity. The Tunica albuginea is visible as a clear hypoechoic line surrounding the testis and epididymis.</td>
<td></td>
</tr>
<tr>
<td>Sagittal section of prostate</td>
<td>The prostate was round to ovoid, relatively homogeneous and of intermediate echogenicity. Difficulties were encountered in this plane and measurements are unlikely mid-sagittal and in maximal dimension.</td>
<td></td>
</tr>
<tr>
<td>Transverse section of prostate</td>
<td>The prostate was heart shaped, echogenicity and texture varied from hypoechoic peripherally (within glandular tissue) to hypoechoic centrally (fibromuscular tissue).</td>
<td></td>
</tr>
<tr>
<td>Prostatic urethra</td>
<td>The prostatic urethra was seen as a hypoechoic circular area present in the dorsal third of the prostate. Its connection to the urinary bladder could not be visualised.</td>
<td></td>
</tr>
<tr>
<td>Lateral Bulbuourethral Glands (n=2)</td>
<td>The bulbuourethral glands were oval in shape and typically glandular in appearance, with hypoechoic parenchyma of medium to fine echo-texture.</td>
<td></td>
</tr>
<tr>
<td>Urinary Bladder (n=10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Part 2: Comparison of ultrasonographic observations and measurements of the urogenital tract with gross and histological necropsy findings in koalas with urogenital chlamydiosis

Two different ultrasound machines were utilised to examine koala cadavers. The first machine was an ATL-Ultrasound machine with linear multi-frequency 5-10 MHz and curved 7 MHz or 4-7 MHz transducers. The use of the 10 MHz transducer was preferred when examining the kidneys as it provided superior resolution. The use of this large transducer head was, however, impractical in the inguinal region because of its large footprint. The second machine was a Toshiba Sonolayer V-SSA 90 A with a curved 3.75 MHz transducer.

Ultrasonographic and necropsy techniques: The ultrasound protocol developed in Part 1 was utilised for the examination of the cadavers but, due to the marked structural changes induced by chlamydial disease in some individuals, additional methods were used during necropsy to confirm the identity of the structures observed. To assess females for the presence of ovarian bursal cysts, the transducer was placed in the pouch, on the ventral midline, and directed 45° to the ventral body wall, or with the transducer placed lateral to and directed under the epipubic bones. To confirm identity of structures observed by ultrasound, the urogenital tract was removed en bloc and placed in a water bath and the organs imaged sonographically a second time. Sagittal and transverse images were obtained of each organ and measurements were taken using both electronic callipers and a tape measure placed on the glass surface of a light box.

A thorough necropsy was then performed on each cadaver using standard veterinary protocols. At the same time swabs were collected from the penile urethra of a male koala (n=1) and from the urogenital sinus of females (n=7) for polymerase chain reaction (PCR) testing for Chlamydia spp. nucleic acid. 18 In all cases, infection with Chlamydia spp. was confirmed by PCR testing. The urogenital tract was then fixed in 10% neutral buffered formalin for 24 hours prior to paraffin embedding, sectioning (5μm) and staining with haematoxylin and eosin, followed by microscopic evaluation.
Results

The sonographic appearance of the urogenital tract of healthy koalas is presented in Table 1. Sonographic data from the urogenital tract of koalas with urogenital chlamydioidis and gross and histological necropsy findings are presented in Table 2. Representative sonographic images are shown in Figure 1-6.

Discussion

This study obtained valuable information regarding the sonographic appearance of the urogenital tract of normal koalas and koalas with urogenital chlamydioidis. The concept of utilizing ultrasonography as a diagnostic tool in koalas is not novel, but, to date, practitioners have not had the benefit of either a description of the normal sonographic anatomy of the koala urogenital tract nor a validated systematic examination protocol.

The techniques described have been used for 10 years in a hospital with a high caseload, with findings confirmed at necropsy. Following development of technical proficiency, this ultrasonographic protocol has proven to be accurate in identifying urinary tract changes and the presence of parovarian cysts. This demonstrates the wide applicability of this technique; even where high-resolution units might be unaffordable. However, recent advances in the sensitivity of ultrasonography technology and its availability will continue to enhance this technique as an important diagnostic procedure for the prognostic assessment of chlamydioid disease in koalas.

Features of koala sonographic examinations that differed from those of small companion animals are highlighted. General anaesthesia was required to minimise stress to both the animal and technicians and to improve the quality of images obtained; however, this is not mandatory in the field. Fur was not removed from the areas examined sonographically.

Anecdotally, koala fur is slow to regrow, and its thermoregulatory function is likely of particular importance in this species, which exists on a limited energy budget. Thorough wetting with ethanol and generous quantities of acoustic coupling gel allowed sufficient contact of the probe with the skin to permit adequate imaging of the urogenital tract in most positions; however, the denser fur present on the lateral aspect of the animal made the examination more challenging. To prevent further evaporative cooling, any residual ethanol and coupling gel was wiped away using a towel at the completion of the procedure. The pouch of the female koala proved to be a useful acoustic window to the urinary bladder and uterus. As it is an area of glabrous skin, ultrasound beam transmission was greatly enhanced compared to other sites. Lactating koalas and/or those with pouch young should not be examined in this way to minimise the risk of impacting the pouch young.

The epipubic bones present in the caudo-ventral abdominal wall of both sexes restricted access to the lower urogenital tract to some degree. Transducer selection was therefore influenced not only by image resolution and depth of penetration required, but also by size and shape of the transducer’s footprint. Ideally, a paediatric phased array transducer of high frequency and small footprint should be utilised for scanning in this location to circumvent shadowing of the epipubic bones. The volume of urine in the bladder was a limiting factor in some ultrasonographic studies, especially when attempting to image the prostate. When relatively empty, the urinary bladder was situated within the pelvic canal, too far caudal for trans-abdominal assessment of the prostate. Despite the fact that the females were expected to be reproductively inactive during the time of these studies, it is probable that breeding history and physiological status will affect the sonographic appearance and size of the uterus. Further studies at different stages of the reproductive cycle are therefore required.

Table 2. Comparison of ultrasonographic data from urogenital tract with gross and histological necropsy findings in koalas with urogenital chlamydioidis

<table>
<thead>
<tr>
<th>No.</th>
<th>Ultrasonographic Appearance</th>
<th>Necropsy Findings</th>
<th>Histology Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urinary Bladder (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Bladder lumen contained foculent echogenic material and the bladder wall was thickened (&gt;5.5mm using electronic calipers).</td>
<td>The bladder wall confirmed to be &gt;5.5mm using manual calipers.</td>
<td>Severe chronic active, predominantly plasmacytic cystitis in association with smooth muscle hyperplasia.</td>
</tr>
<tr>
<td>2</td>
<td>Wall appeared thickened, with an irregular serosal surface obvious in the longitudinal plane. In the transverse plane almost no lumen was discernable, due to both its small size and the reduction in contrast between echogenic material within the lumen and the thickened bladder wall. Pyroechoic foci were evident within the bladder wall in both planes.</td>
<td>The bladder was grossly distorted in shape and size and its wall appeared thickened. Nodules were present on the dorsal serosal surface. The right ureteric papilla was almost completely effaced by proliferative changes in the mucosa. This resulted in a distalisation of the right ureter.</td>
<td>Moderate smooth muscle hyperplasia and fibrosis were evident. Additionally, mild to moderate neutrophilic foci were present in the subepithelium. One small focus of lymphocytes and plasma cells was present in the submucosa of the right ureter.</td>
</tr>
<tr>
<td>3</td>
<td>A thickened urinary bladder wall (4-9mm) with a very little lumen visible with a heterogeneous mass lesion of moderately increased echogenicity was visible between the hypoechoic submucosa and the hypoechoic muscularis layers. The echo-texture in this region was more granular than that of the surrounding bladder wall.</td>
<td>A severely distorted bladder with a thickened wall (7-10mm). Polypoid growths were present on the luminal mucosal surface, some corresponding to the position of the ureteric papillae and others scattered elsewhere.</td>
<td>The mass lesion was situated between the submucosa and the mucosalis, consisting of spindle shaped cells of morphology and arrangement consistent with leiomyoma. A similar tumour was observed in the walls of the urogenital sinus.</td>
</tr>
<tr>
<td><strong>Kidney (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Right kidney appeared misshapen and of increased echogenicity compared to the apparently normal left kidney.</td>
<td>A misshapen right kidney, with small indentations evident on the capsular surface.</td>
<td>Evidence of infarction and segmental cortical necrosis.</td>
</tr>
<tr>
<td>2</td>
<td>No abnormalities</td>
<td>One kidney misshapen</td>
<td>Multifocal pyogranulomatous interstitial pyelonephritis. The presence of inflammation in the renal medulla and the wedge-shaped pattern of inflammation suggested an ascending infection.</td>
</tr>
<tr>
<td>3</td>
<td>No abnormalities</td>
<td>No abnormalities</td>
<td>Mild chronic pyelonephritis</td>
</tr>
</tbody>
</table>

Table 2. Continued.

<table>
<thead>
<tr>
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<th>Necropsy Findings</th>
<th>Histology Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uteri (n=2)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Uteri exhibited mural thickening (&gt;4mm) and an irregular mucosal surface. The walls were anechoic to hyperchoic and varied in thickness from 4-8mm. Uterine lumen appeared anechoic due to the presence of intraluminal fluid.</td>
<td>Uteri were enlarged, with thickened walls surrounding fluid-filled lumens.</td>
<td>Moderate to severe chronic lymphocytic and histiocytic inflammation was observed histologically, with scattered plasma cells present in the muscularis.</td>
</tr>
<tr>
<td>2</td>
<td>Hyperechoic and homogenous mass could be appreciated at the caudal dorsal aspect next to the urinary bladder.</td>
<td>No abnormalities</td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td><strong>Para-Ovarian Cysts (n=6)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-6</td>
<td>The cysts appeared as thin walled structures with anechoic lumens and distal acoustic enhancement. In some cases, echogenic material was present within the cysts. Ex situ, it was possible to visualize ovarian tissue in association with the parovarian cysts. The ovaries were small, flattened ovoid structures (5 x 8mm) with an irregular outer surface as a consequence of protruding follicles. Ovaries were hypoechoic with variably sized anechoic regions throughout, presumably corresponding to follicular activity.</td>
<td>Five out of the six animals showed mild chronic prostatitis and bronchial thickening. Cysts ranged up to 20mm in width and 83mm in length. They varied in shape from ovoid to spherical. Some were simple cysts, whilst others were multi-lobulated or compartmentalised. The fluid within the cysts varied in appearance from colourless to yellow or brown and from clear to turbid.</td>
<td>Ovarian bursitis was present in two cases. This was characterised by a mild to moderate plasmacytic inflammation with varying degrees of fibrosis.</td>
</tr>
<tr>
<td><strong>Prostate (n=1)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>The prostate ranged from an anechoic to hypoechoic echogenicity. The glandular portion was mottled in appearance. The outer fibromuscular capsule, more evident ex situ than in vivo, was discernable as a hypoechoic band surrounding both prostatic lobes. A focus of increased echogenicity was also present centrally, surrounding the prostatic urethra.</td>
<td>No abnormalities</td>
<td>Mild chronic prostatitis and fibrosis</td>
</tr>
</tbody>
</table>
Conclusion
The data obtained in this study are particularly valuable in the determination of prognosis for koalas with urogenital disease, as chronic fibrotic lesions resulting in structural alterations are unlikely to resolve with treatment. The major pathological sequelae of chlamydiosis in a koala, the presence of para-ovarian cysts, are readily recognised during sonographic examination. In less severe cases, where bladder wall thickening might be observed due to oedema of the mucosa or submucosa, ultrasonography and the availability of reference intervals for structural dimensions will be useful in monitoring the response to therapy. The ultrasonographic appearance of other organs such as the liver, spleen, gastrointestinal tract, adrenal glands, lymph nodes and heart could also be investigated, and techniques for imaging these organs standardised. This would widen the applicability of diagnostic ultrasound in koalas, facilitating detection of diseases affecting other organ systems. Correlations between sonographic, gross necropsy and histopathological findings should be investigated further using larger numbers of fresh koala cadavers. A comprehensive atlas of high quality images would be a useful resource for practitioners.

Competing interests
The authors declare that they have no competing interests.

Acknowledgements
This study was conducted in 2003 as part of the thesis submitted to the University of Sydney for the degree of Bachelor of Science (Veterinary). The authors wish to acknowledge the support of veterinary and nurse staff at Taronga Zoo, Coffs Harbour Zoological Park, Port Macquarie Koala Hospital as well as Veterinary Imaging Associates. Richard Malik’s position is supported by the Valentine Charlton Bequest. Caroline Marschner’s position is partially supported by the Koala Health Hub, University of Sydney.

References
Structurally, nothing abnormal was detected; no foreign body, but NO gastrointestinal motility at all surgery. We passed a urinary catheter – no mechanical obstruction – and his bladder was easily expressible. We biopsied the gastrointestinal in 8 places, then woke him up again.


 We started him on prokinetic – initially metoclopramide continuous rate infusion and cisapride (0.5 mg tid). That went nowhere for the next 2 days – frequent regurgitation, constant drooling, the dog was very distressed and required manual bladder expression.

 Two days after the surgery I added IV metoclopramide boluses on top of the continuous rate infusion, doubled the cisapride dose to 10g tid, and added ranitidine at the textbook dose. Thirty minutes after the double dose of cisapride the dog looked normal! We offered food and it ate ravenously, without regurgitating. His bladder function had returned to normal; he had been eating normally for 24 hours, and now looked like a completely normal dog.

 Gastrointestinal biopsies are now back, and the following are selected comments accompanying the diagnosis:

**Diagnosis:**
1. Small Intestine - Enteritis, lymphoplasmacytic, diffuse, marked, chronic and marked lamina propria fibrosis
2. Stomach, tunica muscularis - Hemorrhage, multifocal, mild

**Comment:** The histomorphology of enteric mucosa is compatible with marked chronic inflammation. Moderate increase in number of lymphocytes, plasma cells and moderate lamina propria fibrosis is compatible with inflammatory bowel disease (IBD). Canine IBD is an idiopathic condition. In this case, the World Small Animal Veterinary Association (WSAVA) gastrointestinal standardization group guidelines are used for histopathologic evaluation. There is no evidence of neoplasm or infectious agents in any of the examined tissue sections. However, this inflammatory lesion may not have caused clinically significant microscopic changes in the submucosal and intermuscular ganglia within the examined tissue sections.

**Provisionally then we had:**

1. Oral ulceration
2. A history of foreign body ingestion
3. Severe generalised gastrointestinal dysmotility, AND
4. Apparent functional bladder atony.

**HK Diagnosis Report**

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Elise Robertson
I’ve just cut them down to level of pad to help with locomotion and not snagging on the carpet... the cat was fine and not uncommon for them to be FeLV/FIV negative. I’ve seen in both negative and positive cats... prognosis – good as they seem to manage well. It’s usually something else that causes them problems – in my experience, it’s been considered an incidental finding.

Andy Sparkes
ISFM Veterinary Director
andy@icatcare.org
Agree – these look like classic cutaneous horns.

Can be associated with FeLV, papillomavirus or squamous cell carcinoma (or pre-malignant change) although the site and number would make the latter very unlikely.

Conservative management is probably best unless they are causing a lot of discomfort, in which case surgical excision at the base may be an option to try to remove them and prevent regrowth.

Richard Malik
CVE Valentine Charlton Consultant
richard.malik@ucm.edu.au
Love to see some photos. From your smart phone. I can arrange a PCR for papillomavirus from John Munday. If it’s a young cat and it might be papilloma related, what about using Aldara after trimming back?

Elise Robertson
Richard, do you think azithromycin could also work, like in those cases of cyclosporin-induced gingival hyperplasia? Somewhere, I think I remember reading something about its use in papilloma virus but could be oh so wrong! I’m not sure re mechanism of action. I’m happy to admit not knowing and keen to learn from others’ experiences!

Response from Emir
Great feedback. Thanks! I’ve never used Aldara. Is it safe in cats? Don’t they lick it? What sample do you need for PCR?

Reply 3
Richard Malik
If you cut any of the lesions off – keep them in ethanol – that will preserve them and be gentle to the DNA – and I can arrange for someone to do some PCRs – most likely John Munday in New Zealand – he will probably do it for interest, if the client can cover the postage.

Aldara is safe in cats – just apply sparingly to all affected lesions every 2nd day – but it’s expensive. STOP the cat licking it off for 15 minutes, then it doesn’t matter what happens.

I have never seen a case that is as extensive as your patient. It might be prudent to test for FeLV, as others have suggested.

John Munday
BVS, PhD, Diplomate ACVIM
j.munday@massey.ac.nz
Of course! I remember these were always thought to be caused by FeLV so the fact that this cat is not positive for this virus makes this interesting by itself. I guess it is too late now, but if he can get some into formalin (maybe bring the cat back in and remove some more) histology would be really good. I can do histology from ethanol, but it tends to look rubbish. If he can’t get more samples, maybe chop in half and put half in formalin and half in ethanol.

John to Emir
Sorry for not contacting you sooner. The easiest thing to get into the country is formalin-fixed paraffin-embedded blocks. However, failing this, best idea is to fix things in formalin and send them in a tissue wrapped in formalin. Check with your courier company first though, they may be less happy, although presumably this will be a really small piece of tissue and so you can put it in a small screw-top tube. For me, just the horn isn’t likely to contain much PV DNA, it will be in the cells right at the base of the horn if PV is present. Having a piece at the interface of the horn and the epidermis would be really useful to look for PV inclusions also. I guess see what you have.

RICHARD – can you find any more samples of these? It would be good to do a case series if we can.

Response from Richard's request to look for papilloma virus in these lesions.

Response to Richard's request to look for papilloma virus in these lesions.

Response to Richard's request to look for papilloma virus in these lesions.

Figure 3a.

Figure 3b. Another photograph of the patient’s forelimb and radiographs to show there is no bony involvement.
There are three different interpretations for these results:

- Firstly, it is possible that the cutaneous horns were not caused by papillomavirus infection. Histological evidence of papillomavirus infection is present in many papillomavirus-induced lesions in animals. Therefore, the absence of any detectable papillomavirus cytopathology in the cutaneous horns also provides some evidence that these lesions are not caused by papillomavirus infection.
- Secondly, it is possible that formalin-fixation could have fragmented the DNA so much that amplification was not possible. This fragmentation is difficult to prevent, but it is currently recommended that the time of fixation is minimized to try to prevent fragmentation in the sample. The presence of fragmentation can be tested in most samples by amplifying DNA from a ‘house-keeping’ gene. This was not done in the present case as this was likely to be negative due to the absence of any cells with intact nuclear material.
- Thirdly, it is possible that the horns were caused by a papillomavirus type that was not amplified by the three sets of consensus primers that were used.

Overall, while it was definitely worth investigating these lesions for the presence of papillomavirus DNA, these results do not support a papillomavirus cause for cutaneous horns in cats. Currently, the greater role of papillomaviruses in skin diseases in the veterinary species is starting to be recognized and the spectrum of disease caused by these viruses is expanding. The techniques to investigate the presence of papillomaviral DNA in samples are routinely used at Massey University and we are happy to investigate the presence of papillomaviruses in any lesions for which there is evidence supporting a viral etiology. I am always happy to be contacted regarding a possible papillomavirus involvement in a lesion at jmunday@massey.ac.nz.

Comment from Emir

Meanwhile the ‘horns’ have grown again, so we could get new samples.

Note from CVE Director, Hugh White

In 2013 the CVE agreed to fund a half-time Postdoctoral Researcher in keeping with the terms of the Valentine Chantlon Bequest (www.cve.edu.au/bequests), namely the investigation and treatment of feline infectious diseases. The research scientist would focus on the study of diagnostic and treatment options for various infectious diseases of cats, both in the laboratory, and in relation to clinical trials. The successful candidate was Dr Ángeles Sánchez-Pérez, a career scientist of some 20 years standing.

To new CVE Members and Readers who may have missed the previous Perspective in our March 2015 Issue 278, please read it here:

Perspective 114: Flow Cytometry as a Tool to Assess Mycoplasma Infection of Feline Red Blood Cells

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PART I: Understanding PCR

What is PCR?

The Polymerase Chain Reaction (PCR) is a molecular biology technique designed to make multiple copies of a particular DNA sequence, making it a useful technique for clinical diagnostics and research.

This article explains the basic PCR principles as well as some applications of the technique, such as RT-PCR and qPCR. RT-PCR extends the power of PCR, a procedure that can only amplify DNA, to the amplification of RNA; while qPCR allows quantification of the target DNA, making this procedure essential to assess gene expression, and to determine bacterial or viral infection load.

The PCR Mixture

The essential components for the amplification reaction are shown in Fig. 1A. The reaction must include: the original DNA (template DNA), DNA primers, deoxynucleotides (dNTPs), DNA polymerase enzyme (Taq), a buffer, and Mg²⁺.

Template DNA: The size of the template DNA can vary from a whole genome to just a small DNA fragment, but must contain the sequence to be amplified by PCR (depicted in blue in Fig. 1).

The DNA molecule is composed of two polynucleotide chains (strands), made from dATP, dCTP, dGTP and dTTP. The nucleotides in each chain are joined to one another, by covalent bonds between the sugar of one nucleotide and the phosphate of the next. These strands run in opposite directions (antiparallel), 5' to 3' for the Positive strand and 3' to 5' for the Negative strand (Fig. 1B). The Positive strand (or Coding strand) has a nucleotide sequence that directly corresponds to the mRNA (the codons that are translated into protein). The Negative strand, also known as the Template strand, is copied (used as template) during the mRNA synthesis. The two DNA strands can only join together, to make double-stranded (ds) DNA, if they are complementary to each other, and this complementarity is

Ángeles graduated with BSc Honours in Zoology from the University of Salamanca (Spain, 1979), where she completed her PhD on Microbiology (1981). During her postdoctoral research at the Roche Institute of Molecular Biology (IL, USA; 1982-85), working on equine (Vesicular Stomatitis Virus) and human viruses, she met her Australian husband, Michael. After a couple of years in ‘neutral territory’ (Nippon Roche Research Center, Japan), she migrated to Australia in 1987 to work at the CSIRO. After taking leave to attend to her family, Ángeles started work at the University of Sydney (1997), School of Medical Sciences, where she has worked in a variety of roles, as a teacher, Molecular Biologist and Flow Cytometrist. Ángeles currently combines her position at the Faculty of Veterinary Sciences with her role as the Bosch Live Cell Analysis Facility Officer.
Figure 1. PCR Reaction Components.

(A) The essential reaction components are the Template DNA (containing the DNA sequence to be amplified, in blue), DNA Primers (starting point for the polymerase), dNTPs (the DNA building blocks), and DNA polymerase (the enzyme that catalyses the reaction), as well as a buffer (to provide the right pH and ionic strength) and cofactors (Mg²⁺).

(B) DNA complementarity: The two DNA strands are complementary to each other and the DNA primers are complementary to the ends of the DNA sequence to be amplified. The nucleotide bases provide the complementarity and share hydrogen bonds (A=T, C=G).

(C) The DNA polymerase adds nucleotides at the 3’-end of the primer and moves in the 5’- to 3’- direction using the complementary DNA strand as template.

Figure 2. The three stages in the PCR cycle: 1- DNA Denaturation, 2- DNA Annealing and 3- DNA Elongation.

Denaturation is required to separate the two DNA strands, the temperature is then reduced to allow the primers to find the complementary DNA template region and anneal to it. The third step (DNA Elongation) involves the DNA polymerase, which uses the primer as starting point and polymerises a new DNA chain by using the opposite DNA strand as a template. The PCR cycle produces an additional copy of the template DNA. The template DNA is represented in blue and the newly synthesised DNA, containing the DNA primers, is depicted in green.

Figure 3.

1- DNA Denaturation
2- DNA Annealing
3- DNA Elongation

2- Annealing: This step requires a lower temperature (40 to 60°C), depending on the length and composition of the DNA primers, to allow stable association (annealing) of the primers with the complementary regions in the newly separated single DNA strands (Fig. 2-2). The Forward primer anneals to the negative DNA strand, whereas the Reverse primer anneals to the positive DNA strand (Fig. 1B).

3- Elongation: During elongation (Fig. 2-3), the Taq DNA polymerase uses the single-stranded DNA as template and adds complementary dNTPs to the 3’-ends of each primer, generating a section of double-stranded DNA in the region selected for amplification (Fig. 1C). This reaction is carried out at around 72°C (70 to 74°C) and is very fast. At this temperature, Taq can replicate a DNA fragment 1000 nucleotides long in less than 10 seconds. The high elongation temperature also helps maintain the specificity of the PCR primers, as lower temperatures would allow the primers to anneal to sequences that are only partially complementary.

After the elongation step, the original target DNA has been copied. Thus, after the first PCR cycle there are two copies of the target DNA: the DNA present in the original sample (in blue in Fig. 2) and the new duplicate DNA (primers and DNA strains in green in Fig. 2). After this, the new PCR cycle starts, this time also using the new duplicated DNA as template (Fig. 3).

These PCR cycles are repeated multiple times (PCR usually involves 30 to 40 cycles) and each PCR cycle exponentially increases the amount of target DNA in the reaction (Fig. 3). If we assume that the PCR process is 100% efficient, one single DNA fragment present in the reaction would produce 2 in cycle 1, 4 in cycle 2, 8 in cycle 3, 16 in cycle 4 and so on. This means that there would be 1 624 in cycle 10, 1 048 576 in cycle 20, 1 073 741 824 in cycle 30, and 1 099 511 627 776 in cycle 40. The number of target DNA fragments present in the PCR sample is given by the formula: y = 2^n, where n is the number of target DNA fragments and x is the number of PCR cycles.

The PCR amplified DNA fragments, known as amplicons, in the first cycles can have different lengths (as the original amplification template can contain sequences outside the DNA primers, Fig. 1 open DNA chains), but eventually, through repeated cycles of PCR, the majority of templates will be restricted to the size of the DNA segment of interest, as they will have been generated from DNA copies that include both PCR primers. The figures have been simplified by showing only the template DNA area but, as seen in Fig. 3, with every PCR cycle, the percentage of the original DNA template in the reaction diminishes, until most of the reaction templates only contain the DNA area amplified in the reaction (primers and DNA strains in green).

A temperature profile for a typical PCR protocol with Taq polymerase is shown in Fig. 4. The first cycle includes a
In 1971, Kleppe described a novel in vitro method to replicate a short DNA template. The technique was developed in H.C. Khorana’s laboratory (1968 Nobel Prize winner for his work on the Genetic Code) and used DNA polymerases and primers, and it is generally considered as a precursor to PCR.

Kary Mullis developed the PCR technique in 1983, while working for the Cetus Corporation (awarded the Nobel Prize in Chemistry for this discovery in 1990). In his 1990 publication in Scientific American, Mullis describes that the idea of PCR came to him ‘…during a moonlit drive through the mountains of California’. He was trying to develop a technique for sequencing DNA mutations. PCR was patented in 1985 by Mullis and assigned to the Cetus Corporation, and the first PCR paper was published also in 1985. This publication earned Saiki (the first author) a stern letter from the United States Government, admonishing him for publishing a report on ‘chain reactions’ without the required prior review and approval by the U.S. Department of Energy.

In 1989, the Science Magazine selected the Polymerase Chain Reaction as the major scientific development of the year and chose the DNA polymerase molecule as its first ‘Molecule of the Year’. The reason given was: ‘Few technologies in the life sciences can claim to have been as pivotal as the polymerase chain reaction (PCR). Some might point to DNA cycle sequencing or the cloning of genetic material as groundbreaking techniques. And they are. However, neither would be possible—or at least practical—without an initial amplification step provided by PCR’. In its recognition of PCR, Science did not only acknowledged Mullis, but also ‘the novel application of the heat stable Taq polymerase by scientists at Cetus that really allowed PCR to flourish’. …

In the early stages, PCR was performed manually and it was a tedious procedure that tested the patience of researchers. In addition, PCR was originally carried out with a non-thermostable DNA polymerase (the Klenow fragment of DNA polymerase I), and fresh enzyme had to be added to each PCR cycle. Moreover, the extension cycle had to be performed at a relatively low temperature (57°C), thus allowing partially matched primers to anneal to the template DNA. As pointed out in the PCR Collection Science Magazine, the PCR technique did not flourish until the heat stable Taq polymerase was used in the procedure.

Chien, Edgar and Trela discovered Taq Polymerase in 1976. Taq is a thermostable DNA polymerase, named after the thermophilic bacterium Thermus aquaticus from which it was obtained. The great advantage of this enzyme is that Taq polymerase remains active after repeated incubations at 95°C thus surviving the multiple PCR cycles. Addition of a thermostable DNA polymerase made PCR easier to perform and also considerably simplified PCR automation.

Finally, on November 1987, both the Taq enzyme (Ampli Taq DNA Polymerase) and PCR-1000 Thermal Cycler (an automated PCR instrument) became commercially available. Since then, the PCR technique has improved and evolved, becoming an essential application in a wide variety of fields. This is reflected by the number of publications obtained using the key word ‘PCR’ on the National Library of Medicine (NCBI PubMed). As seen in Fig. 5, the exponential increase starts in 1988, the year after the Taq enzyme and Thermal Cycler became commercially available.

Evolution of PCR:

From RT-PCR to qPCR and Multiplex PCR

Since the discovery of PCR this technique has been evolving. Some modification include new DNA polymerases with higher ‘proof-reading’ ability or more stable at higher temperatures, thus improving the specificity and fidelity (accuracy or ‘faithfulness’) of the reaction; while other variations have been designed for specific applications and are now regularly used in molecular genetic laboratories. Only three of these applications will be included here: RT-PCR, Real-Time qPCR and Multiplex PCR as they currently have the greatest significance in veterinary science.

In 1987, Powell et al. described a technique (RT-PCR) that extended the power of PCR to the amplification of RNA. RT-PCR (Fig. 6) uses a reverse transcriptase to copy RNA (that cannot be amplified by PCR) into cDNA, hence making it possible to amplify the sequences using PCR. This technique made it possible to use PCR to detect and analyse rare mRNA transcripts and other RNAs present in low abundance.

Higuchi et al. monitored the accumulation of DNA products during PCR by addition of a fluorescent compound that binds to DNA. They found that the fluorescence detected was directly proportional to the concentration of PCR products. This discovery represented the start of quantitative real-time PCR, also known as quantitative PCR (qPCR).

Current qPCR technology uses two approaches to quantify the PCR products: fluorescent DNA-binding dyes and oligonucleotide probes (summarised in Fig. 7), and this approach has become the standard method for many laboratories.

In 1996, Muyzer et al. described a new technique called Multiplex PCR. This technique allows the simultaneous and independent amplification of several DNA samples in one PCR reaction. The advantage of using a PCR machine with multiple reaction chambers allows a remarkable increase in the speed of PCR by running several samples at the same time. The determination of as many as 96 samples in a single assay is now possible with this technique.
**A. SYBR Green**

![SYBR Green Diagram](Image)

**B. TaqMan® Probe**

![TaqMan® Probe Diagram](Image)

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**TaqMan® probes are dual-labelled oligonucleotides with a fluorescent label on one end and a quencher on the other (Fig. 7B). As far as the oligonucleotide remains intact, the quencher absorbs the fluorescence produced by the label. During the PCR elongation phase, the exonuclease activity of Taq polymerase cleaves the probe and releases the label. The free label is no longer quenched by the quencher and emits fluorescence, which is directly proportional to the amount of PCR product accumulated in the reaction.**

**Multiplex PCR** allows the amplification of several different DNA sequences simultaneously. Fig. 8 depicts the amplification of three different DNA sequences in the same reaction. It requires the use of three separate PCR primers (each primer set is designed to amplify one of the DNA targets) and three different TaqMan® probes. Each TaqMan® probe contains a sequence complementary to one of the amplicons and uses a different fluorescent reporter. FAM and HEX can be excited with the same wavelength (488 nm), but emit light at different wavelengths, the peak is at 518 nm (yellow-green) for FAM and 566 nm (orange) for HEX. Cy5 is excited with a different laser (633 nm) and the peak emission is at 662 nm (red). This means that the three dyes can be excited in the PCR reaction, and hence the three reactions can be carried out in the same PCR tube.

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**Figure 7A.** Current qPCR approaches: (A) Fluorescent DNA-binding dyes

SYBR Green is the most popular DNA-binding dye currently used. This dye emits low fluorescence when it is free in the PCR solution. On the other hand, the DNA-bound compound is highly fluorescent, and the fluorescence intensity is proportional to the concentration of the PCR product.

**(B) Oligonucleotide Probes**

TaqMan® is a dual-labelled probe that contains a fluorescent dye and a quencher. The probe is cleaved during the PCR elongation phase, releasing the reporter. The free reporter emits fluorescence and the intensity of the fluorescence increases as the PCR product accumulates.

**PART II: PCR in the Veterinary lab**

**Uses of PCR**

The PCR technique has revolutionised DNA and RNA (RT-PCR) detection, hence this technique is used in a wide variety of veterinary tests. PCR is most often used in the veterinary lab for diagnosis of infectious diseases or in genetic testing and Fig. 8 lists some examples of PCR based tests currently available for cats and dogs. Some of these tests use multiplex PCR (Fig. 8) and can detect and quantitate, in the same assay, a variety of different microorganisms that could be responsible for the clinical signs. This is achieved by designing a panel of PCR primers that can amplify and identify the different pathogens responsible for common clinical signs.

In this way, there are panels for feline and canine diarrhoea, respiratory disease, etc.

**Avoiding false positives:** As seen in Fig. 10, sensitivity, one of the great advantages of PCR, can constitute its main limitation when the technique is used to determine the causative agent of an infection. On the one hand, the sample can be contaminated at the point of collection causing a false positive PCR result, while on the other hand PCR can identify organisms that are present at a subclinical level or are not viable. It is then essential that sample collection for PCR analysis is carried out as aseptically as possible, making sure that there is no cross-contamination. Diagnostic laboratories are subjected to strict rules, such as the use of different equipment and separate areas for the different sample preparation stages (nucleic acid extraction, reaction preparation and PCR amplification), and this, together with PCR test validation (including the use of positive and negative controls), ensures the reliability of the PCR tests.

An understanding of the pathogenesis of disease is also needed for PCR result interpretation. For example, the presence of Feline Coronavirus (FCoV) in feline effusions supports a diagnosis of Feline Infectious Peritonitis (FIP). However, the virus can be present in the blood of the animal in early infection with enteric FCoV and does not necessarily signal the deadly disease FIP. A cat with a lifelong infection with feline herpesvirus may reactivate shedding of the virus due to other diseases and, while the reactivation will not necessarily cause clinical disease, it can be detected in PCR tests, making it difficult to determine with certainty the role of the aetiological agent in causing the current clinical signs. Equally, a dog recently vaccinated with a modified live vaccine against canine parvovirus will potentially contain attenuated virus within a faeces sample for a few weeks and so submission of a sample of faeces for analysis requires careful interpretation by the lab and the veterinarian.

Accordingly, PCR test results should be interpreted within the context of the animal's clinical signs, other diagnostic tests and the limit of detection determined by the lab's specific PCR test.

While PCR cannot distinguish between a live and a dead microorganism, PCR tests can be designed to distinguish between vaccine and wild-type strains. Based on nucleotide sequence differences, Wilkes et al.11 describe the highly sensitive (can detect as few as 5 virus genomic copies) real-time RT-PCR assay they developed to detect the modified canine distemper viruses (CDV) used for vaccination. This assay uses PCR primers capable of amplifying all the viral strains, but distinguishes vaccine strains from the wild type infectious agents by the use of a probe specific to the vaccine strains. In addition, PCR can identify fragile pathogens that cannot survive outside their animal host.
Aiding false negatives: The right type of sample must be provided for testing; for example, EDTA-treated blood is good for analysing haematrophic mycoplasma. PCR samples should be collected prior to antibiotic, antifungal or antiviral treatments or, when determining freedom from infection, at least two weeks (better 4 weeks) after withdrawal of medications. Proper maintenance and handling of the specimens is essential to maintain the nucleic acid integrity. Specimens should be kept at 4 to 8°C, they should not be frozen or touch the ice pack in the transport container. Fresh tissue must reach the testing laboratory within 24 hours of collection, and general samples should be delivered within 48 hours. It is important to note that cell integrity is essential to maintain nucleic acid integrity, as cell disruption causes the release of enzymes that degrade RNA (RNases), DNA (DNases) or proteins (proteases). This is particularly important when dealing with samples that contain faecal material, as stools typically contain many compounds that can degrade nucleic acids and proteins. In particular RNA is a very fragile molecule, very sensitive to degradation, and RNases are ubiquitous (secreted by human skin, present in spores, etc.). In fact, laboratories need to treat all materials used to analyse RNA to remove contaminating RNases.

In summary, if used properly, PCR constitutes the most powerful technique to identify pathogenic microorganisms. The speed of PCR allows prompt and accurate detection of infection in animals allowing early treatment of infected individuals and assisting in the control of infectious diseases. In some diseases, PCR can provide positive identification of the infective agent even before detection by serology (antibody) due to delays in seroconversion in some infections. Conversely in other diseases the identification of the organism by PCR is only possible early in the infection or periodically as the infection load rises and falls, making serology an important option. Again, knowledge of the pathophysiology of infection and disease is essential to determine the best approach for the patient and transcends generalisation made about a methodology for diagnosis.

Interpreting Real-Time qPCR Results
To understand real-time qPCR results, we need to understand a few basic concepts. Fig. 11 is a graphical representation of real-time PCR data using an Amplification Plot. This plot compares fluorescence signal with PCR cycle. In the early stages of PCR, or when the target DNA is not present in the sample, there is little change in the fluorescence signal. This defines the baseline for the amplification plot, which represents the background value for the reaction. The plot in Fig. 11 had the background subtracted from the fluorescence value obtained, thus bringing the baseline to 0.

Figure 10. Advantages and limitations of PCR.
The main characteristic of PCR, its sensitivity, is also responsible for the main caveats of this technique.

Figure 9. Examples of commercially available PCR-based tests in Australia for cats and dogs.
PCR is extremely useful to detect infecting microorganisms and also constitutes the base for veterinary genetic testing.

Figure 10. Advantages and limitations of PCR.

ADVANTAGES OF PCR
Sensitivity: A few DNA copies are usually enough for detection.
Klein & Higuchi calculated that if 0.1mL from a typical PCR amplification (10^12 molecules) was added to the water in an Olympic-sized swimming pool, a 0.1mL aliquot of the liquid would contain 400 amplifiable molecules.
Specificity: Only detects the genetic material recognised by the primers.
Speed: The procedure is very quick, 40 PCR cycles usually take less than 2 hours.
Can detect all forms of organisms: Non-viable or non-replicating forms and organisms that cannot be cultivated.

LIMITATIONS OF PCR
Sample Cross-Contamination: The procedure is so sensitive that minute amounts of cross-contamination can lead to a false positive result.
Detection of Subclinical Infection: PCR sensitivity allows detection of microorganisms at lower levels than those required to cause disease. Healthy animals that have been exposed to the organism can be PCR positive.
Requires Knowledge of DNA Sequence: PCR primer design requires knowledge of the DNA sequence flanking the fragment to be PCR amplified. The primers must also be specific to the target DNA and fulfil other PCR requirements.
Interpretation of Positive Results: Requires extensive knowledge of disease pathogenesis and microorganism biology, as presence of a pathogen may not represent a disease state.

The next phase is called the exponential phase, when the reaction is very precise and specific. Assuming 100% reaction efficiency, each cycle would produce a doubling of the product accumulated in the previous cycle. The threshold should be set at this phase, above the baseline. The next phase is the linear phase, at this stage the reaction components are being consumed, the reaction is slowing and some products are starting to degrade. Finally comes the plateau phase, this marks the stop of the reaction, when no more products are being made and, if left long enough, the products start to degrade.

Fig. 11B shows an amplification plot with three samples (red lines) containing different concentrations of target DNA and a sample (in blue) with no target DNA. This graph also defines the C\textsubscript{t} (threshold cycle), which is the PCR cycle at the intersection between the amplification curve and the threshold line. It is a relative measure of the amount of target DNA in the PCR sample, as the C\textsubscript{t} value decreases with an increasing amount of template. In this way, the sample with the smallest C\textsubscript{t} value (Fig. 11B) contains the highest concentration of target DNA. It must be noted that C\textsubscript{t} values are relative values, and depend on the instrument and reaction conditions used. Hence, the C\textsubscript{t} values obtained from PCR reactions run under different conditions cannot be directly compared. Every PCR cycle can double the amount of target DNA present in the previous cycle. This means that a sample with a C\textsubscript{t} value of 20 contains twice as much original DNA material as a sample with a C\textsubscript{t} value of 21. This same sample would have 4 times as much DNA as a sample with a C\textsubscript{t} value of 22. This same sample would have 4 times as much DNA as a sample with a C\textsubscript{t} value of 22.

However, the C\textsubscript{t} values vary depending on the assay, as the Ct values are not directly comparable. Every PCR cycle can double the amount of target DNA present in the previous cycle. This means that a sample with a C\textsubscript{t} value of 20 contains twice as much original DNA material as a sample with a C\textsubscript{t} value of 21. This same sample would have 4 times as much DNA as a sample with a C\textsubscript{t} value of 22.

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References:

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Synovar Case Study Competition

Congratulations to Dr Anna Katia Loved, Veterinary Surgeon from Redlands Veterinary Clinic who entered the competition as advertised in the January 2016 issue of CVENews.

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