C&T

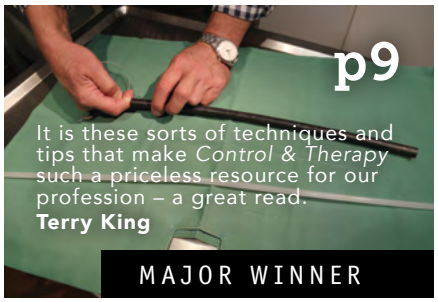
Issue 282 | March 2016

Centre for Veterinary Education

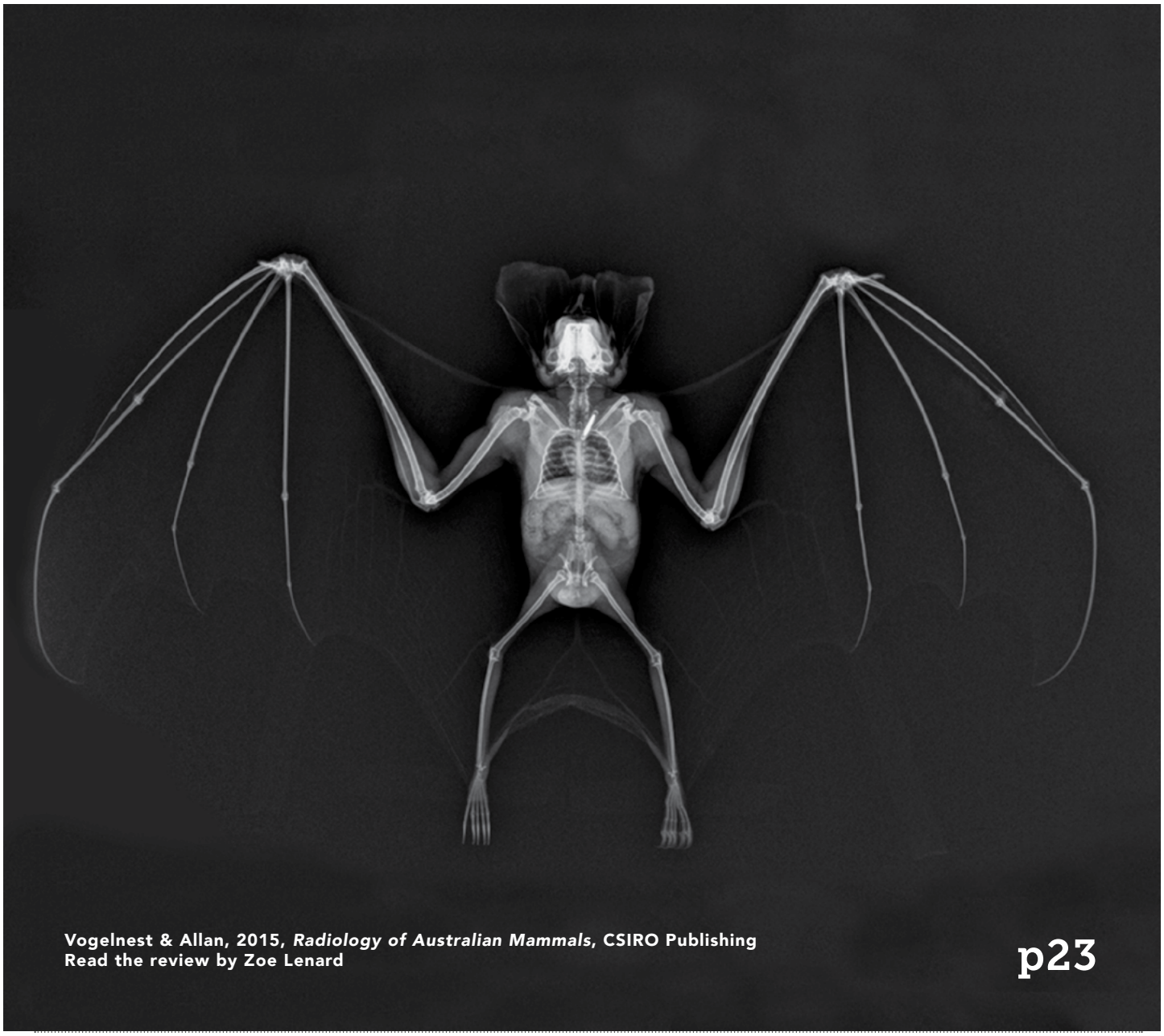
Control & Therapy Series

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



C&T

Issue 282 | March 2016

Control & Therapy Series

Centre for Veterinary Education

Australia's Leading Veterinarian Forum

PUBLISHER
Centre for Veterinary Education (CVE)
Tel: + 61 2 9351 7979
Fax: + 61 2 9351 7968
cve.publications@sydney.edu.au

www.cve.edu.au

Veterinary Science Conference Centre
Level 2, B22 Regimental Drive
The University of Sydney, NSW 2006
Print Post Approval No. 224792/0012

DIRECTOR
Hugh White BVSc MVSc MACVSc

EDITOR
Elisabeth Churchward
elisabeth.churchward@sydney.edu.au

VETERINARY EDITORS
Richard Malik
Hugh White

ADVERTISING
Ines Borovic: *ines.borovic@sydney.edu.au*

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From the Director

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 on 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 tutor, 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 C&T 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

Calendar

Conferences

MELBOURNE
Valentine Charlton Feline Conference + Masterclass
Monday 20 – Friday 24 June, 2016

BRISBANE
Small Animal Emergency Conference + Masterclass
Monday 24 – Friday 28 October, 2016

Seminars

SYDNEY
Sports Medicine: Theory & Practice
Friday 11 – Sunday 13 March, 2016

HOBART
Clinical Pathology Seminar
Sunday 13 March, 2016

CAMDEN
Avian Medicine: Theory & Practice
Saturday 19 – Sunday 20 March, 2016

ADELAIDE
Critical Care Seminar
Sunday 1 May, 2016

CANBERRA
Clinical Pathology Seminar
Sunday 29 May, 2016

PERTH
Feline Medicine Seminar
Saturday 23 – Sunday 24 July, 2016

SYDNEY
Ophthalmology: Theory & Practice
Friday 26 – Sunday 28 August, 2016

PORT MACQUARIE
Critical Care Seminar
Sunday 9 October, 2016

SYDNEY
Dentistry: Theory & Practice
Saturday 22 – Sunday 23 October, 2016

TOWNSVILLE
Feline Medicine Seminar
Saturday 12 – Sunday 13 November, 2016

2016

March

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Hands-on Workshops

SYDNEY
Approaches to Bones & Joints Workshop
Friday 13 May, 2016

SYDNEY
Ultrasound Workshop
Friday 20 & Saturday 21 May, 2016

SYDNEY
Hip & Stifle Workshop
Saturday 20 August, 2016

SYDNEY
Bone Plating Workshop
Sunday 21 August, 2016

SYDNEY
Anaesthesia Workshop
Friday 26 August, 2016

SYDNEY
Stress Free Surgery Workshop
Saturday 17 September, 2016

TimeOnline – Online CPD

PodcastPLUS

Head to our website for upcoming topics: **www.cve.edu.au/timeonline** **www.cve.edu.au/podcastplus**

Calendar Key

- DE Super Early Bird ends
- DE Early Bird ends
- Conferences
- Seminars
- Hands-on Workshops
- TimeOnline start dates
- PodcastPLUS
- School holidays (NSW)

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THANK YOU TO ALL CONTRIBUTORS

Follow Tom Hungerford's 'goanna track to success'...

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

Thanks to every author who contributed articles or comments to the *Control & Therapy Series* (C&T) and to those who supplied images and visuals. Without your generosity the Series would cease to exist.

WINNERS

MAJOR PRIZE WINNER

Fish Hooks – The Non-Surgical Approach
Jamie Andrews

CVE PUBLICATION PRIZE WINNERS

Lymphosarcoma & Concurrent Cryptococcosis 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 Marschner & 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.

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

Terry King

LARGE ANIMAL

Disease Investigation Report

Jeremy Rogers

Senior Veterinary Officer, Biosecurity
e jeremy.rogers@sa.gov.au
t (08) 8391 7100
f (08) 8388 8455
w www.pir.sa.gov.au

Primary Industries and Regions
South Australia – PIRSA
Murray Bridge SA 5255

C&T No. 5523

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.

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.

Figure 1. Angus calves on poor, dry pasture.

Clinical Signs

Affected calves were observed to be ataxic, salivating, 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 scouring, 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, and 1 calf tested positive for Pestivirus using PCR, with others returning 2+ or 3+ ELISA antibody results, indicating recent exposure to the virus.

Figure 2. Recumbent bull in BCS 1.

Figure 3. Fine sand in the abomasum.

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.

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 wasno 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. ■

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RE C&T No. 5482 – **What's Your Diagnosis**

Victor Epstein

Veterinary Surgeon
Flinders Island TAS 7255
e vicepstein500@gmail.com

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.

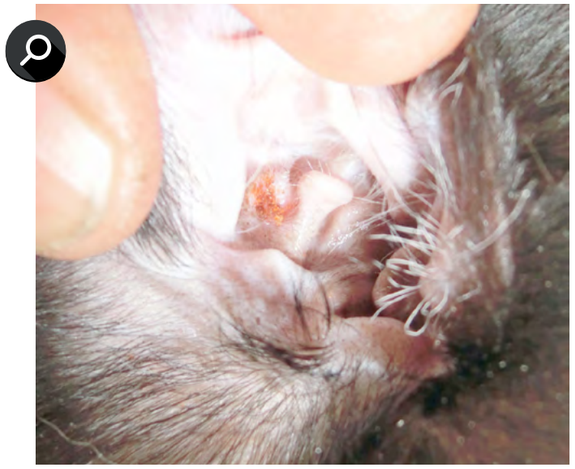

Figure 1. This cat had a pruritic skin condition with most irritation centred on the head.

ISFM FORUM

SMALL ANIMAL

Answer to What's Your Diagnosis: C&T No. 5502 Cat With An Itchy Head

Pete Coleshaw

Jaffa's Health Centre for Cats
52 St Francis Road Salisbury UK
p 01722 414298
e jaffa@jaffavets.com
w www.jaffavets.com

C&T No. 5524

Frances Harvey

What is your Diagnosis: Trobiculid 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,

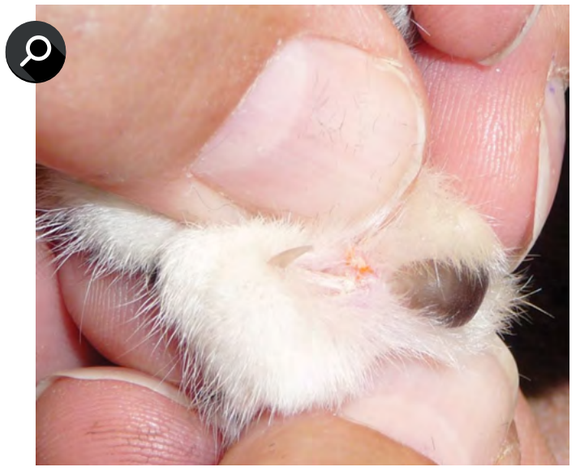

Figure 2. Harvest mites near the nail bed.

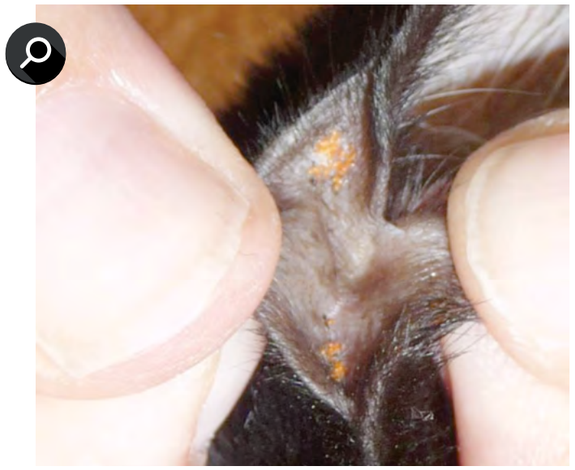

Figure 3. Harvest mites in the ear pouch.

ear pouch, claw pouches, orange splodges grossly, heaving under magnification .

I, sadly, use prednisone EOD and Comfortis®. I know it's only got a flea license, but it seems to have an effect. The literature suggests fipronil topically, but it really doesn't seem to work, maybe because it's too difficult to apply. I think Seresto® collars may have a really big place here, but I hate collars on free range cats; they're expensive and I haven't got a client to bite yet. ■

Reply No. 1

Richard Malik

e 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.

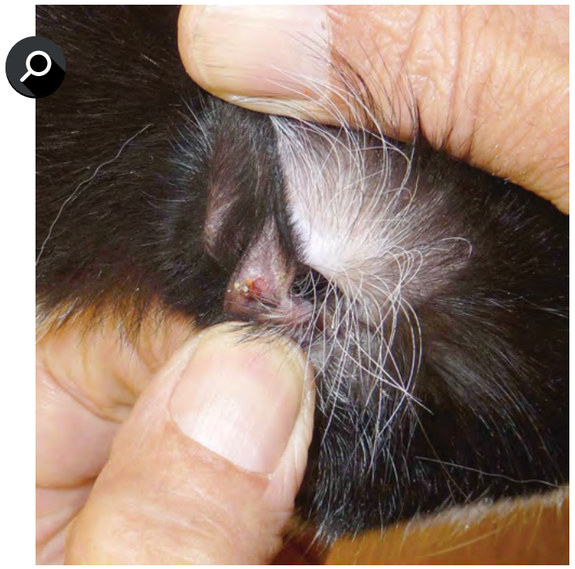

Figure 4. Harvest in near fold plus ticks.

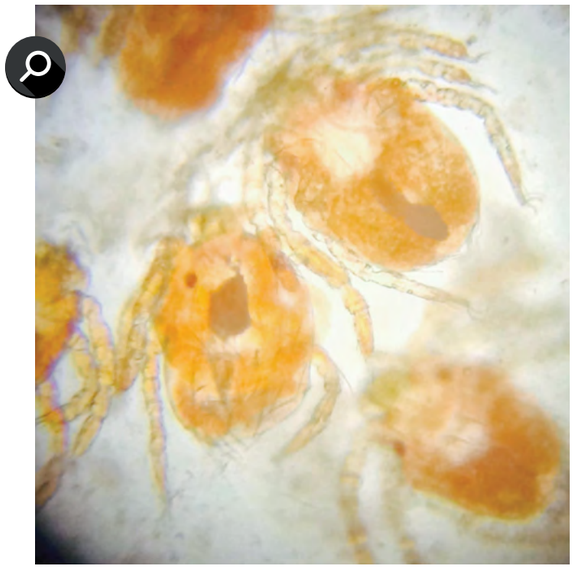

Figure 5. High power harvest 1. Note the orange pigment seen macroscopically also evident microscopically.

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MAJOR WINNER

SMALL ANIMAL

Fish Hooks – The Non-Surgical Approach

Jamie Andrews

Highett Street Veterinary Hospital
34 Highett Street
Mansfield VIC 3722

w www.highettstreetvet.com.au

C&T No. 5525

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 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. ■

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 Remover C&T No. 4912 Peter Howe (Issue 251, June)

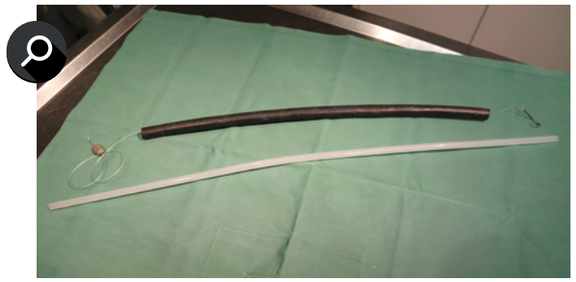


Figure 1.

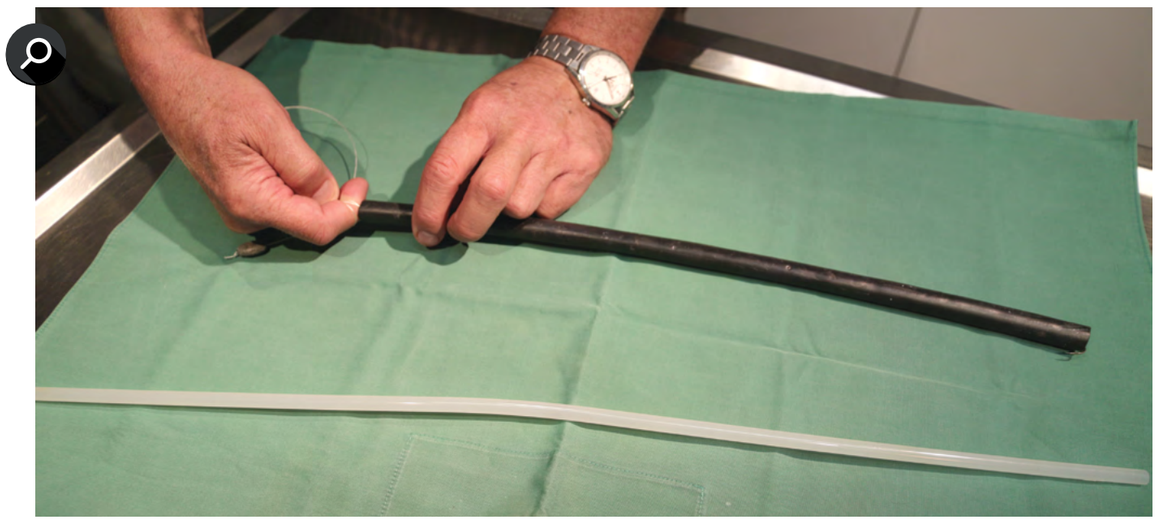


Figure 2.

Figures 1 & 2. Many thanks to Jamie and Head Nurse Jo Richmond who, in the absence of a patient, re-enacted the fish hook removal for this article (twice – the second time against a green drape) for the benefit of our readers.

Comment courtesy of:

Terry King

Vet Specialist Services
Shop 14, Hometown Corner Lexington &
Logan Roads QLD 4119

e TKing@vss.net.au

I read with much interest Jamie's technique for retrieval of ingested fish hooks. This Victorian Alp method matches that of the saltwater fisherman technique of using the Fish Hook Dislodger (C&T 312, 1975 – Granger, Chapman & Christie) and the elegantly described modified version of this device and method (C&T 4912, Peter Howe) and of course the Queensland-Easy method using large ET tubes (C&T 3913, Mark Debritz, 1996).

It is these sorts of techniques and tips that make *Control & Therapy* such a priceless resource for our profession – a great read.

Being in a referral practice situation for half my veterinary career, I'm used to having all the toys available as well as the personnel, technical assistance and experts (surgeons – some would call them experts!) on hand if needed to assist or bail me out in these situations. It's so humbling to be brought back to reality when you learn of colleagues achieving the same outcome so effectively, safely and economically.

The clear advantages of this type of technique described by Jamie Andrews:-

- Obvious saving of time and manpower for the clinician, expense for the client, and likely morbidity for the patient (including the dreaded postoperative complications of oesophageal dehiscence +/- stricture formation).
- The oesophagus is a much more forgiving organ than was once believed, and these manoeuvres usually only cause small, almost insignificant, tears in the mucosal and submucosal wall that heal without consequence.
- If the hook cannot be freed from the oesophageal wall in this way, it usually means the hook and barb have penetrated the outer wall of the oesophagus and then surgery is indicated with thoracotomy, if in the distal oesophagus. Not for the faint-hearted.

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 (overtube) 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 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 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) all are suitable.

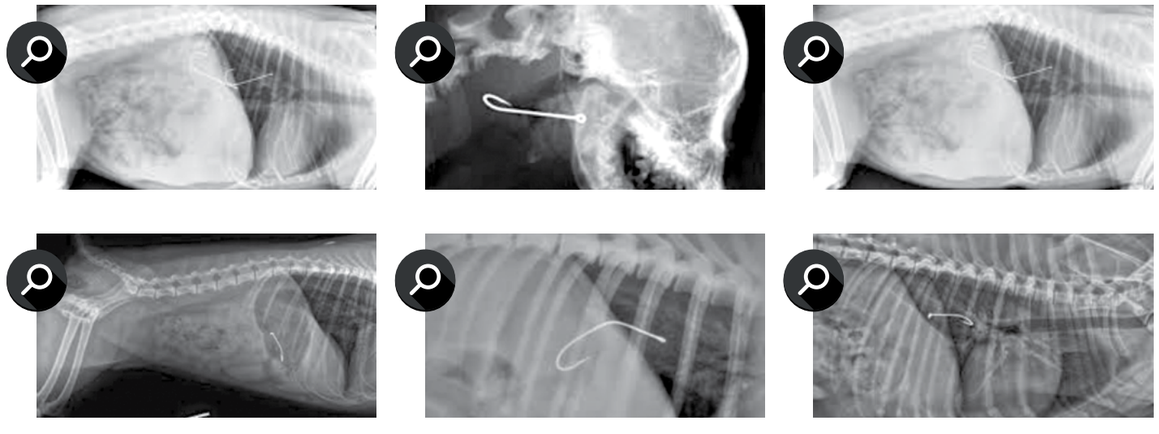
Once the distal end of the smaller (inner) tube is resting on the inner curve of the fishhook within the oesophagus, slide the larger (shorter) tube over the smaller inner tube. Once the barb is disengaged and freed from the oesophageal wall with the gentle tap as described above, hold the inner tube 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)



SMALL ANIMAL

Behind the Locked Door
The Crisis Confronting Pets & Paramedics

Aine Seavers

Oak Flats Veterinary Clinic
58A Central Avenue, Oak Flats NSW 2529

e reception@oakflatsvet.com.au OR
e homealonepet@outlook.com

C&T No. 5526

Triple 0 Call-Out; But what about the Companion Pet?

As a society we are becoming increasingly insular and disconnected from those around us. Many family members live thousands of miles away and often, as people age, there is simply no family left with which to connect.

Animals, especially dogs and cats, fill the void and provide much love, comfort and companionship to all of us, but especially to those living alone. These companions are of great benefit to our health and wellbeing. Pet ownership is to be supported and encouraged.

However, these pets have needs that must be met on a daily basis. When those needs can't be met, for whatever reason intentional or otherwise, the pet suffers.

We need to have an emergency back-up support or care plan for our pets. Like the adverts on TV for Bush Fire Survival – the time to make emergency plans is long before you ever might need them.

The problem of the 'home-alone-pet' arises most often when the sole human carer is suddenly taken ill. 000 is called and our wonderful paramedic heroes arrive to, in most cases, transport the human patient to hospital. Sometimes the patient will return home but sometimes they never do return.

What happens to their pet in those early days?

On the day of the 000 call, the paramedics have very little time to concentrate on the well-being of the family pet. But that doesn't mean that knowing they have left a pet behind does not dwell on their minds and negatively impact on their own lives. Often the paramedic only has enough time to tip their own water bottle out to leave for the animal, secure the property and leave. The paramedics have enough to be dealing with; they shouldn't be left to carry the weight of this pet issue as well.

Reporting the home-alone-pet can be done by the paramedic alerting the RSPCA but again, days may pass before the RSPCA can attend. A young healthy dog might recover OK from such a gap in care. Not so an elderly dog, or medicated pet, or any cat. After 48 hours left alone, these pets are in big trouble...!

So what can we as a society do?

I don't have the answers and I am interested in feedback and suggestions.

I will be running with this topic and taking it to veterinary and animal welfare and council bodies but the more thoughts we can collect on the issue before-hand, the better.

My first thoughts are:

1. We need to put together some good TV ads highlighting the situation and the means to address it.
2. The current wallet-card system (of which we have samples at the clinic) for those who live alone need to be expanded into 2 forms.
   - The current wallet-card alerts the finder that the ill/injured person has an unattended pet at home and provides an emergency contact number for a person who can look after that pet.
   - We need a second card that indicates the person has an unattended pet at home but no emergency contact is available. Therefore, the card-holder gives permission for an RSPCA officer, Council Ranger or Police Officer to attend the home and rescue the dog immediately.

Figure 1. The wallet-card currently in use is supplied by ATX.

These cards need to be prominently displayed in a standard location in houses so the paramedics know where to quickly look to retrieve it.

Currently, a notice has to be posted on the door of the house and if no one contacts authorities in 72 hours, then the house can be entered and the pet retrieved. We need to remove that waiting period and get to the pets faster.

3. Is there a need for, or a way to have, a register of approved persons, happy to provide emergency home shelter or foster care for such an animal?
4. Is there a way to have a Neighbourhood Pet-Watch sticker, where approved homes close to where pets live can be identified and pets housed there until their own owner returns home?

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.**

hospital or institution when rescued. The human child goes into emergency foster-care homes. We need to offer similar to our pet dependants. Otherwise, their quality of life suddenly crashes around them just as their stress levels soar. ■

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…

SMALL ANIMAL

Medical Management of Gallbladder Mucocoele

Heather Shortridge
Locum Veterinarian
e 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 (2.5-8.9), creatinine 145 (27-124)).

The results were discussed with the owners and amoxicillin 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 amoxicillin and clavulanic acid. However, she would not eat the renal diet and was transitioned back to her normal food as she continued to be well. By the 5th of January she was completely back to normal.

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 on our dry chemistry analyses. While her urea was elevated at 17.6, her creatinine at 76 was well within the reference interval. Full bloods were then run on our other machine, with the following results:

- Albumin < 10 (25-44)
- Globulins 0 (problem with machine?)
- ALP 438 (20-150)
- ALT 664 (10-118)
- TBIL 23 (2-10)
- BUN 16.2 (2.5-8.9)
- TP 53 (54-82)
- WBC 27.6 (6-17)
- Neutrophils 25.36 (3-12)

Figure 1. Sindy.

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

Blood results three months post initial presentation

I handed 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 amoxyclav, 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:

- TBIL 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.*

WINNER

SMALL ANIMAL

Lymphosarcoma & Concurrent Cryptococcosis in a Cat

David Fowler
t +612 4423 1688
e davidfowler1@outlook.com

North Nowra Veterinary Hospital
205 Illaroo Road
North Nowra NSW 2541

C&T No. 5528

Tinkerbell Microbology
Download here

'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 prednisolone 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.

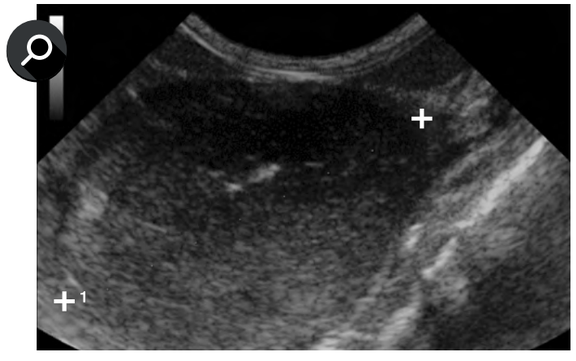


Figure 1. Mass (irregular 4 cm diameter) present in the dorsocranial abdomen.

'Tinkerbell' seemed to stabilize and then improve within a few days. This was put down to cessation of immunosuppressive drugs at the time. The LCAT came back positive, with a titre of 128. At that stage, on Richard Malik's advice, we asked the pathologists to review the original cytological samples from 6 months earlier to see if there was any sign of *Cryptococcus* on these specimens. None was found.

We soon commenced 'Tinkerbell' on fluconazole, taking the view she would need to be on it for life. We intended to re-start the immunosuppressive drugs to palliate her lymphoma, and this would put her at ongoing risk of recurrence of the fungal infection. We re-started her on prednisolone and chlorambucil a week after starting fluconazole. The neurologic signs had abated by that stage, and she continued to do well for many months, notwithstanding gradual weight loss. By October 2013, the abdominal mass was palpable again, but she still seemed well in herself.

It was not until March 2014 (15 months after the date that the abdominal mass was first detected) that she started to decline. Her weight had dropped precipitously, and she had an obvious ocular lesion (lymphoma vs *Cryptococcus*?). A week later, she had developed intermittent ataxia and was euthanized. By that stage there was a (new) mass palpable in the abdomen in the region of the bladder.

A post mortem examination was performed and tissues were submitted to Assoc. Professor Mark Krockenberger at the University of Sydney (Figure 2 A-C). In terms of gross pathology, the notable findings included:

- Ocular lesion: iris deformed and swollen, proliferating forward into the anterior chamber.
- Generalized mottled/nutmeg appearance to liver with multiple foci of raised white plaques which were firmer than surrounding parenchyma.
- White focal lesions seen in the renal cortices on cross-section.
- The abdominal mass that had been present for 15 months ante-mortem represented a thickening of the stomach wall.
- 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

Associate Professor, Veterinary Pathology | Faculty of Veterinary Science, The University of Sydney

e mark.krockenberger@sydney.edu.au

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, sublumbar 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.

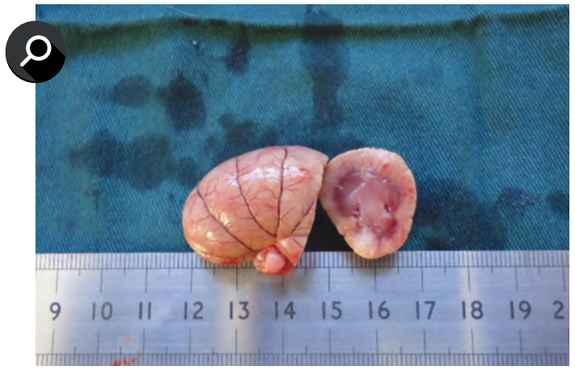


Figure 2b. Gross Photograph of the cat's kidney.

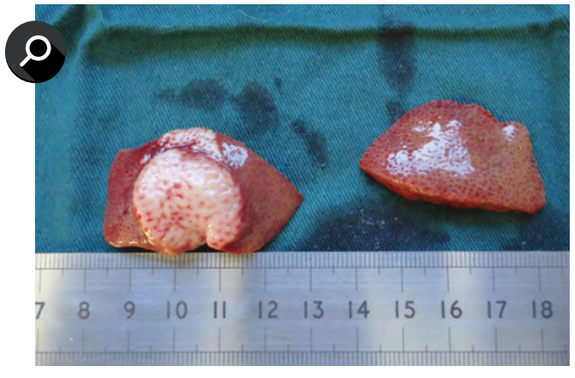


Figure 2c. Gross Photograph of the cat's liver.

SMALL ANIMAL

Discussion on Generic Human Terbinafine

Magdoline Awad

BVSc, MANZCVSc (Animal Welfare), CMAVA

PetSure (Australia) Pty Ltd
Level 9, 465 Victoria Avenue, Chatswood NSW 2067

t +61 (02) 9842 4800
e magdolinea@petsure.com.au

C&T No. 5529

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

Question

Just wanted your opinion on oral terbinafine for 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.

Reply

Richard Malik

CVE Valentine Charlton Consultant

e richard.malik@sydney.edu.au

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 Sporanox 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. Robyn 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).

Reply

Kimberly Coyner

DVM, DACVD

Dermatology Clinic for Animals of Tacoma
5608 South Durango St, Tacoma WA 98409 USA

e dermvetwa@gmail.com

Thank you Richard; I would not classify being drawn into a terrible Vegas shelter and trying to help them with their endemic ringworm as making me one of the top ringworm researchers, but I appreciate it!

Magdoline, since Itraconazole is so expensive in the US, and terbinafine only pennies, we decided to try it (in combination with twice-weekly lime dips and environmental decontamination / improvement) in this huge free-roaming shelter. Karen Moriello was instrumental in formulating the overall plan and monitoring weekly dermatophyte cultures.

We found terbinafine 30mg/kg PO SID x 21 days (2 weeks was not long enough) was curative in the majority of the cats and was well tolerated. I have used compounded terbinafine in 2 cats with good response as well. I have also used it in several dogs with good response, though it takes longer than 3 weeks and does not have the long depot effect in the skin in dogs as it does in cats. I will attach some information for you. ■

Comment courtesy of:

Jeffrey So

BVSc, BSc(Vet) (Hons), GradDipVetClinStud

RSPCA Veterinarian

e jso@rspcansw.org.au

I have used the human generic terbinafine at the dose rate of 30-50mg/kg and have found it to be very effective when animals have been treated for 3 weeks. Besides treating animals with terbinafine we also use the terbinafine cream once daily for 21 days and malaseb or lime sulphur baths or wipes. We have found that after 3 weeks of treatments with terbinafine systemic and topical treatments, as well as baths, that they are getting negative culture results and we have not seen (fingers crossed) any relapse of ringworm infection. So far we have not noted any side effects of terbinafine tablets when given with food and we found it very easy to give a tablet for kittens/cats over 1.5kg.

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In dogs I still tend to use Griseofulvin for cost effectiveness in large dogs. ■

Editor's Note

Lozanoc is an orally-administered antifungal agent with the active ingredient itraconazole.

Lozanoc has approximately twice the bioavailability of the conventional formulation Sporanox. This greater bioavailability compared with conventional formulations is achieved using the SUBA manufacturing process developed by Mayne Pharma International Pty Ltd, located in Salisbury, South Australia.

One capsule of Lozanoc 50mg is therapeutically equivalent to one 100mg capsule of conventional itraconazole. The recommended dose for Lozanoc is therefore half the recommended dose for conventional itraconazole.

Karen Moriello, Kimberly Coyner, Ann Trimmer, Sandra Newbury & Darcie Kunder, 2013, Treatment of shelter cats with oral terbinafine and concurrent lime sulphur rinses, *Vet Dermatol* 2013; 24: 618–e150.

Sandra Newbury, Karen Moriello, Kimberly Coyner, Ann Trimmer & Darcie Kunder, 2015, Management of endemic Microsporum canis dermatophytosis in an open admission shelter: a field study, *J of Feline Medicine & Surgery*, Vol. 17(4) 342-347.

SMALL ANIMAL

Yellow Cats are Never Good

Emma Billing

e emthevet@gmail.com

C&T No. 5530

'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 0.92kg with a temp of 39.3°C. Both eyes were discharging serous fluid and both had third eyelid protrusion. Body condition score was adequate and the kitten was quite affectionate during examination. Chest auscultation showed no wheezes or crackles, and respiration rate was normal, but heart rate slightly elevated. Abdominal palpation revealed an empty gut, liver margins extending beyond the ribs and he was uncomfortable. Skin tent ++. Both eyes were negative on staining with fluorescein, and the nose, which was crusted with mucous, 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 SC, which was repeated every 2hrs or so.

**Editor's Note: Hartmann's Solution or 0.45% NaCl with 2.5% glucose might have been a better choice.*

Figure 1. Lucien at 15wks with his six year old 'brother'.

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Approximately 3hrs later (busy, busy long weekend Saturday 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 sent to Laverty Vetnostics came back as follows (preliminary):-

- Hb 61 (80-140)
- RCC 4.6 (5.5-10)
- **Hct 23** (28-45) ↓
- MCHC 265 (310-350)
- **Plat 25** (200-700) ↓
- **WBC 2.3** (6-16) ↓
- **Neut 0.2** (3.8-10.1) ↓
- **Lymph 0.4** (1.6-7) ↓
- **Bands 1.7** (<0.1) ↑
- ALP 22 (<81)
- CK 2203 (<261)
- RBCs normal, leucopenia ++, neutropenia ++, lymphopenia ++, band neuts +++, thrombocytopenia +++, giant platelets ++
- Na 134 (147-161)
- K 3.5 (3.7-4.9)
- Bicarb 14 (15-24)
- Crea 35 (40-190)
- Glu 11.6 (3.9-8.3)
- **Bili 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-megaly (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 discolouration (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 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. Neuts show toxic change and had a prominent left shift. Occasional blast cells identified.*

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

Definitive Diagnosis: Marked acute multifocal to coalescing necrotising hepatitis with intraendothelial protozoal tachyzoites 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 the toxo to go rampant (which also means Lucien was likely infected *in utero,* in which case the breeder should be notified), or the toxo itself has caused the stunting, and Lucien was infected either late *in utero,* 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

difficult cases. And to Sue Foster who lets me pester her repeatedly and also went in to bat for me, and for Lucien. ■

Postscript: I don't know what the diet was in the cattery; suffice to say the breeders weren't happy at us looking at them as a possible source of infection. The diet at home was supermarket brand kitten food (Whiskas® I think from memory), but adequate nutritionally. The kitten was fed *ad lib*.

Note: We sent blood off for PCR and it was very kindly checked for free by Mark Westman from University of Sydney, who was also interested.

Comment courtesy of:

Vic Menrath

e v.menrath@uq.net.au

The Cat Clinic – 189 Creek Road, Mt Gravatt QLD 4122

I thought the article was a really interesting example of, (in my experience anyway), a very uncommon case of hyper acute Toxoplasmosis in a young cat complicated by (probably) disseminated intravascular coagulation which is also really uncommon in cats. I have the following comments and suggestions:-

The only cases of congenital hyper acute Toxo that I recall were in kittens less than 4 weeks of age (usually 2-3) and these (like this one) were all in pure-bred cats with mothers fed on raw kangaroo meat.

Characteristic clinical signs included acute neurological/ pain signs (crying out continuously) and acute necrotising hepatitis with necrotic/yellow foci spread throughout the liver and acute death – all kittens in the litters died.

The only other time I experienced this type of syndrome (once) when the cat (1-year-old) was given immunosuppressive doses of cyclosporin and prednisolone on a transplant donor (acute pneumonia).

According to the history and clinical signs (not symptoms), this kitten also probably had clinical herpes / calici (almost mandatory in breeding catteries) which may have played a significant immunosuppressive role.

Breeders that have endemic viral problems often have a stash of antibiotics which they use on kittens they are selling to mask clinical signs and one of the favourites is Delta Albaplex® which is a combination of prednisolone, novobiocin and tetracycline.

Having ruled out other immunosuppressive viral causes it would have been useful to know: at what age the kitten was acquired; what happened to the litter mates; did the breeder give any drugs; and what were the mother/ kittens fed by the breeder and the owner; and have there been any other similar cases in the cattery? The significant zoonotic risk to the breeder should maybe also be explained to them. A urine sample may have added some more info (e.g. haemoglobinuria) for little extra cost.

All in all, a really interesting article with good info gained from bloods. ■

Comment courtesy of:

Sue Foster

BVSc, MVetClinStud, FANZCVS

e sfoster01@bigpond.com

Vetnostics (Sydney), ASAP Laboratory (Melbourne), QML Vetnostics (Brisbane)

Firstly, well done to Emma and Bruce for coming up with the provisional diagnosis of toxoplasmosis which proved to be correct.

The haematology on this kitten was really interesting and concurrent FeLV, whilst not demonstrated here, would not have been surprising. Risk of clinical toxoplasmosis in cats is increased with immunocompromise caused by infection (e.g., feline immunodeficiency virus, feline leukemia virus, feline infectious peritonitis, hemoplasmosis) or drug therapy. Reported haematology results in toxoplasmosis can be very variable. There may be anemia, leukocytosis or leukopenia. In severely affected cats, leukopenia can persist until death. A left shift, sometimes degenerative as in this kitten, has been reported in a number of cases.

Kittens with toxoplasmosis may be stillborn or die before weaning. Clinical signs usually pertain to involvement of the liver, lungs, and CNS. Lethargy, depression, hypothermia, and sudden death can occur with some kittens suckling until death and it was interesting that 'Lucien' would still eat initially when food placed in his mouth, despite his advanced and progressive disease. Kittens may have abdominal distension due to an enlarged liver and ascites, and hepatomegaly was easily palpable in Lucien. Encephalitic kittens may sleep most of the time or cry continuously. It is not known how frequently kittens develop toxoplasmosis as many kitten deaths and illnesses are not investigated or reported.

The most common serum biochemical abnormalities in clinical feline toxoplasmosis are increased bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) as were present in this kitten. Increases in AST or ALT activity may reflect liver or muscle necrosis. Creatine kinase (CK) can be increased if there is muscle necrosis. It would be interesting to know what the ALP and CK were in this kitten. Lung involvement is also extremely common in all cases, especially those where there is immunocompromise.

Finally, it is always a pleasure helping veterinarians such as Emma who themselves are going the 'extra mile', caring for their patients at home and interested enough to follow up cases with necropsies when the outcomes have been unfavourable. It helps us all learn for next time. ■

WINNER

SMALL ANIMAL

Treatment of Herpetic Keratitis with Topical Aciclovir in a Persian Cat

Wye Li Chong

BVSc, MANZCVS (Small Animal Surgery)

p +612 6287 8100

e wyeli.chong@rspca-act.org.au

RSPCA

12 Kirkpatrick Street

Weston Creek ACT 2611

C&T No. 5531

***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 crusting. Upon clinical examination, it was noted that the right eye was miotic 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.

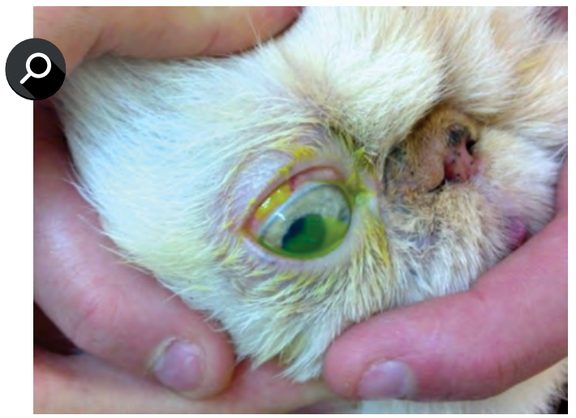

Figure 1. Geographic ulcer with small superficial neovascularisation coursing from 12 o'clock of cornea to most dorsal aspect of ulcer.

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 polymycin 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 resolution. Oral lysine 500mg BID was recommended but declined.

Figure 2. 1 week later showing marked reduction in size of ulcer. Clinical resolution was noted a week after.

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, 2006). 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 called geographic ulcers (Nasisse, 1990; Andrew, 2001) In the present case, the presumptive diagnosis of FHV-1 was made based on the characteristic appearance of the ulcer,

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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 acyclovir 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. However, in a clinical trial performed by Williams et al (2005) in vivo, 4 cats with confirmed FHV-1 with geographic ulcers were treated with frequent (5 times daily) topical application of 0.5% acyclovir 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 virutoxic 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 this clinical trial with resolution of clinical signs within 2 weeks of commencing anti-viral therapy. The use of oral famciclovir 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 acyclovir in this case was largely due to the owner being a retiree and hence able to commit to frequent medication.

idoxuridine ≈ ganciclovir >> cidofovir ≈ penciclovir >> acyclovir ≈ foscarnet

Figure 3. Relative in vitro efficacy against FHV-1 (Maggs & Clarke, 2004).

The presentation of the present case was interesting in that the owners had not perceived the cat to have ocular discomfort. This could largely be due to reduced ocular sensitivity in brachycephalic animals; Corneal touch thresholds in brachycephalic compared to domestic short haired cats measured by Blocker & Woerdt (2001) showed reduced corneal sensitivity particularly in the central cornea in the former compared to the latter. The authors concluded that the combination of prominent eyes and decreased corneal sensitivity seen in brachycephalic cats make them more susceptible to chronic keratitis, corneal ulceration, and sequestration. Furthermore, the mild severity of clinical signs perceived by the owner could also be due to the present case's up-to-date vaccination status; Vaccinated cats can still develop latent FHV-1 infections with periodic reactivations however with less clinical signs and viral shedding (Field *et al,* 2006).

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 (2008)'s 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. ■

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SMALL ANIMAL

Book Review: Radiology of Australian Mammals

Reviewed by

Zoe Lenard

BVSc (Hons), FANZCVS (Radiology)

C&T No. 5532

Have you ever been faced with an injured possum, lame wombat or wallaby 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?

Take it back a step: 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 lumpy jaw 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 provides the first detailed reference for normal anatomy of Australian mammals and thankfully it is an outstanding resource in a concise format. The text focusses on the more common species of echidna, platypus, macropods (kangaroos and wallabies), koala, wombats, dasyurids (carnivorous marsupials like quolls and Tasmanian devils), possums and gliders, bandicoots and the bilby, and bats. Studies were obtained on animals primarily presented to Taronga Zoo in Sydney and all animals were anaesthetised for examination. Authors Larry Vogelnest and Graeme Allan are eminently qualified to present such a text; Vogelnest is the senior zoo veterinarian at Taronga Zoo with over 25 years' experience, and Allan remains Australia's most experienced small animal radiologist with over 40 years' experience. Other authors make small but important contributions, including veterinarians Susan Hemsley (koalas), Nadine Fiani (dental radiology), Frances Hulst (pathology case studies) and taxonomic advisor and curator Paul Andrew (Appendix 1).

Most of the book documents the normal radiographic anatomy of the aforementioned species. The first chapter, Radiographic Technique, reinforces standard radiographic principles including obtaining orthogonal views, appropriate labelling and obtaining comparison views. Tables featuring suggestions for sedative doses for the different species are an excellent inclusion.

The text follows a logical format easily accessible for the casual user who needs to brush up on radiographic anatomy for a single species. I was expecting each of the nine chapters on different species to be dry and uninteresting to read in a cover-to-cover format; how wrong I was! Did you know that echidnas share skeletal similarities to crocodilians, and their keratinised spines (quills) make the most fantastic shadows across their image (Figure 1)? Or that ghost bats have a simple carpus with fused bones that are capable of extreme hyperextension during flight or hyperflexion during wing 'storage' (Figure 2)? Or that kangaroo mandibular condyles are convex, allowing free rotation of the mandible in every direction (Figure 3)?

Chapters on echidnas and platypuses (monotremes – Figure 4) are fascinating as these species have highly unusual radiographic anatomy. The monotremes' unique pectoral

Figure 1. Short-beaked echidna.

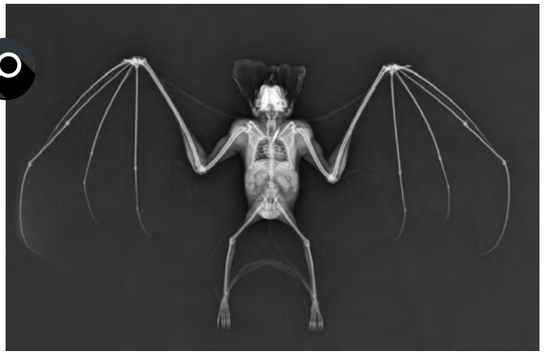

Figure 2. VD ghost bat.

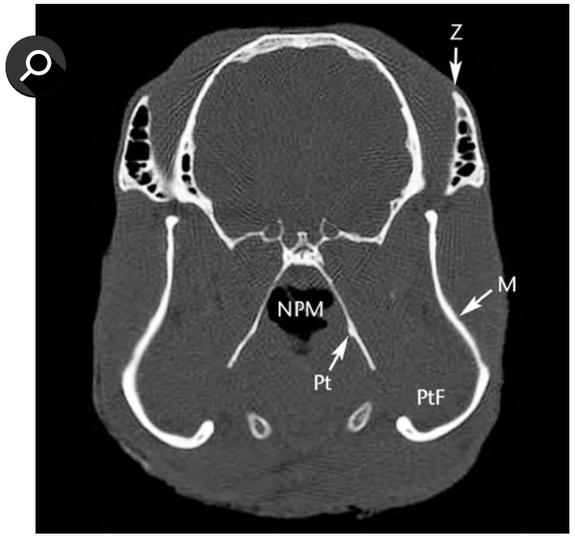

Figure 3. Transverse skull kangaroo.

girdle results in the formation of a box-like bony structure which is firmly connected to the body, not dissimilar to typical mammalian pelvis. The result is vastly different to other mammals with the pectoral girdle firmly anchoring the thoracic limbs of these 'digging machines' to the spine. To the eye of a practitioner familiar with companion mammals (dogs, cats, horses) these creatures have a bizarre anatomy and had I been faced with a platypus radiograph to interpret without this book, it may have been hard to work out up from down.

The bulk of each chapter focusses on demonstrating normal skeletal anatomy of each species; sections on the appendicular and axial skeleton identify all of the bones in the body regardless of the size of the animal. Important distinctions between species are included when relevant. Less detail is presented of the radiographic anatomy of the soft tissues like thorax and abdomen, although references to genital anatomy are included. Details about pouch anatomy will help in marsupial radiographs. The chapter on dental radiography provides the dental formulae for the major species with high quality dental radiographic images.

The final chapter presenting pathological case studies is terrific in a text of normative anatomy, with cases included from practitioners around the country to create a 'sampler' or 'vignette' of disease without trying to be all encompassing. Fractures feature heavily with examples of typical fractures (for example, fracture of cervical vertebra 2 is common in macropods after collision with fences), each with an explanation for why the pathology may be relevant (fractures of the echidna beak, although subtle, can affecting breathing and eating). References to metabolic disease are included (important in any captive population) as well as examples of osteoarthritis and osteomyelitis in several species. Cryptococcosis is more

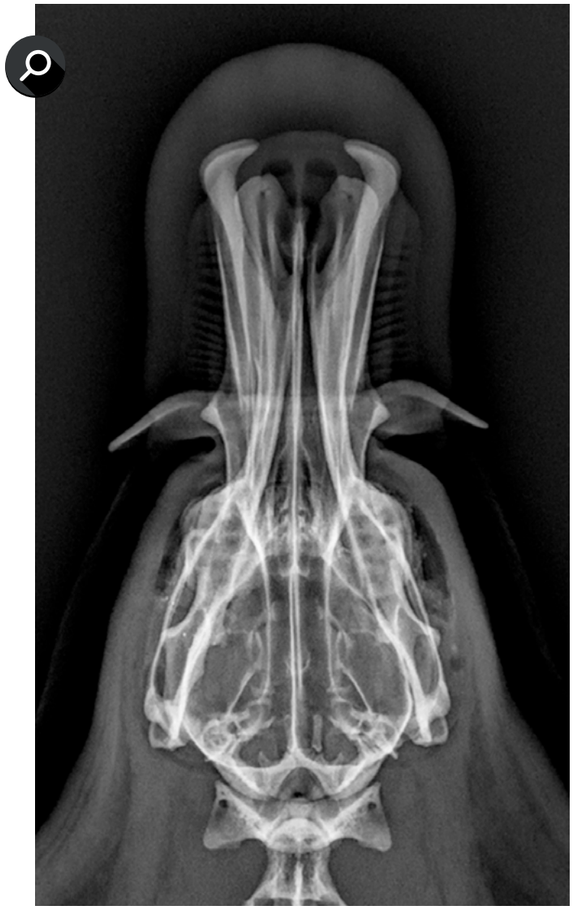

Figure 4. Platypus.

common in koalas than in domestic mammals, presenting as aggressive bone lesions with several excellent examples pictured. Necrobacilliosis (lumpy jaw) of macropods, dental malocclusion in wombats, aspiration pneumonia, and several examples of gastrointestinal ileus are presented across many species. The final appendix provides a taxonomic checklist of Australian mammals using both common and scientific names.

This publication is outstanding for its high-quality images and concise text. Given many of the species included are small (sometimes weighing much less than 1 kg), every image is beautiful. Images typically are large with simple labels and perfectly positioned arrows demonstrating bony and other anatomy. This book is an essential reference for anyone (practitioner, radiologist, nurse, and student) who might deal with Australian mammals. If you don't think you are likely to radiograph a wombat or possum soon, buy a copy anyway and leave it on your coffee table next time you have guests. It is fun to read (yes, I am a radiology-nerd), it will be a great conversation-starter and you will look like a most sophisticated practitioner! ■

SMALL ANIMAL

Real-time PCR Testing for Enteric Pathogens in Dogs & Cats

Jacqui Norris
Assoc Prof in Veterinary Microbiology & Animal Disease
e jacqui.norris@sydney.edu.au

Mark Krockenberger
Assoc Prof Veterinary Pathology
e mark.krockenberger@sydney.edu.au

Damien Higgins
Assoc Prof Pathobiology & Wildlife
e damien.higgins@sydney.edu.au

Jan Šlapeta
Assoc Prof in Veterinary Parasitology
e jan.slapeta@sydney.edu.au

Richard Malik
Valentine Charlton Specialist, Centre for Veterinary Education
e richard.malik@sydney.edu.au

Gary Muscatello
Lecturer in Applied Animal Microbiology
e gary.muscatello@sydney.edu.au

C&T No. 5533

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 or severe diarrhoea. Routine faecal flotation remains an important first step and can be easily done in practice. Wet preparations of fresh faecal mucous can also be useful in animals with signs of large bowel diarrhoea, and can easily be 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), Tritrichomonas 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* cuticular 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, but no evidence of helminths. The multiplex PCR panel is positive for *Giardia, Tritrichomonas* and Feline Enteric Coronavirus, although the C_t 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 tritrichomoniasis being the most likely enterpathogens responsible for the clinical signs being observed. The high C_t 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 coccidian, then after a few days to start a 2-week course of ronidazole (from BOVA Compounding) to address the *Tritrichomonas*. 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 microbiome, 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 faecal 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 Bornavirus, Avian Polyomavirus, *Chlamydia psittaci,* Psittcine 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:

Method	Pathogen
Immunofluorescence	Feline Coronavirus (FIPV) (cytospin/effusion)
Immunohistochemistry (FFPE tissue)	*Chlamydia* genus Feline Coronavirus (FIPV) Feline Calicivirus including the virulent form Feline Herpes Virus-type 1 Canine Distemper Virus *Toxoplasma gondi*
Real-time PCR (swab/ tissue)	*Chlamydia* genus, *C. pecorum, C. pneumoniae* *Toxoplasma* Canine parvovirus Feline coronavirus
PCR and sequencing*	Bacterial ID 16s Fungal ID 18s
Serology (serum)	*Cryptococcus* Ag LCAT *Cryptococcus* Ag lateral flow ELISA (IMMY)
Faecal protozoal detection*	*Cryptosporidium, Giardia, Tritrichomonas fetus* (culture and immunofluorescence) *T. fetus* (culture)

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.

Rollover names below:

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

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

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REPLIES

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Reply to C&T No. 5517 The Debate About Feral Cats

(Issue 281 Dec 2015)

C&T No. 5534

Reply No. 1

Michael Banyard

BVSc (Hons), PhD, HDA, FAVA

e mbanyard@netspeed.com.au

Any discussion of the factors that have caused the decimation 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 terminally ill 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 part of our civilisation. The ethical obligation to minimise the killing of native animals and try and prevent extinctions is as valid as our concern for Cecil. The outcome needs to be a balanced and an integrated plan and not *laissez-faire*. We didn't accept pleuropneumonia or brucellosis but used our scientific and technical knowledge to manage a humane long term strategy albeit one that involved the cull and slaughter of many animals. Similarly, choices about the slaughter of at-risk animals in exotic disease outbreaks.

With regard to the impact of feral cats, foxes, introduced herbivores and fire regimes in the Australian landscape, they all collectively have a significant impact on the abundance and, ultimately, the survival of many native mammals. The scientific management work of the Australian Wildlife Conservancy (AWC) as described in their regular newsletter, *Wildlife Matters,* is encouraging reading for their whole-of-system approach and scientific rigor to manage the best possible outcome with the knowledge currently available. AWC estimate that cats are killing 75 million native animals every night (*Wildlife Matters,* Summer 2013/14 p2). There have now been many trials where cats and foxes have been excluded from area on islands and the mainland to demonstrate the return of native mammals or the survival of reintroduced ones. AWC's Newhaven Wildlife Sanctuary which will provide a 650 sq kilometre sanctuary appears to be based on good science. Certainly, killing feral cats should not be the objective but managing our environments for native species where possible should be. ■

Reply No. 2

James M. Harris

OAM, BS, DVM, FRSPH

Mayfair Veterinary Clinic
2 Russell Crescent, Sandy Bay TAS 7005

e jmhdvm34@gmail.com

Humans tend to prioritise other species. The cat folk put cats on top of the list. Dogs for dog people. Most put rats low down except for the rat people and lawyers are on the bottom of the list. :)

Let us look at the whole picture. The domestic cat, *Felis domesticus* 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 panleucopenia 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. ■

Reply No. 3

Mario Viscardi

DVM & Associates

Ballantrae Drive Veterinary Clinic
Shop 10/91 Ballantrae Drive, St. Andrews NSW 2566

t +612 9820-2711
e drviscardi@bigpond.com

I thought Dr Harvey's article was spot on. In Italy, prior to the 1970s, the country was rabies-free. Rabies then appeared in the Alpine regions of Italy's border with Yugoslavia. Blanket poisoning of foxes in Italy was commenced; the resulting vacuum that was created by this policy was promptly filled by foxes from Yugoslavia.

In my experience, farmers don't desex female cats. If feral cats are poisoned, that vacuum will quickly be filled by non-desexed cats which currently keep down mice and rats for farmers.

There is also a population of semiferal cats around outer suburbs of all Australian towns and cities which would readily occupy vacated territories left by the 'culled' feral cats. So, if any, it would be a very temporary relief to the native fauna. ■

COMMENTS

SMALL ANIMAL

Comment On C&T No. 5521 Ag Gag Legislation Introduced in NSW

(Issue 281 Dec 2015)

C&T No. 5535

Mario Viscardi

DVM & Associates

Ballantrae Drive Veterinary Clinic
Shop 10/91 Ballantrae Drive, St. Andrews NSW 2566

t +612 9820 2711
e drviscardi@bigpond.com

Letter to The Editor

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.

Has this edition of C&T been reviewed?* I feel that extreme views, if not challenged, do more harm than good in the long term. Animals destined for food must be treated humanely, definitely not what we have seen in the ABC footage from Indonesia. There is a lot more that needs to be done to improve the situation, and by distorting the truth we work against this. Animals and humans deserve better.

Mario Viscardi DVM.

***Note:** The C&T Series is a forum and the CVE very rarely censors articles. Rather, it encourages veterinarians to contribute to the debate by taking advantage of the 'right of reply' and the opportunity for a counter viewpoint to be published in the following issue.*

Comment in response to Dr Viscardi's letter to the editor RE C&T NO. 5521 Ag Gag Legislation Introduced in NSW

Sue Foster

Spokesperson for Vets Against Live Export (VALE)

w www.vale.org.au
e info@vale.org.au

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 'Stunning was observed to deliver the single biggest animal welfare benefit and the general 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

Figure 1.

Figure 2.

undoubtedly was, resulted in an increase of stunned slaughter from 15% to 85% in two years i.e. something that could have easily been achieved (and painlessly for pastoralists) over the previous 20 years had there been any real commitment to animal welfare in the live export industry. There is no doubt that it would still have been 'aspirational' if the trade suspension had not occurred.

Dr Viscardi's comment re starvation of cattle due to the trade suspension is not referenced, simply because there is no reference. There were many comments made in the media about this and it was certainly unfortunate that the trade suspension was followed by a prolonged drought in Queensland but there is no hard data on how many cattle may have starved due to the trade suspension itself. It should be pointed out that in some areas of the Kimberley, for example, it is reported that 1000 cattle are mustered for every 50 that are exported, only a 5% turnoff (Joint Select Committee on Northern Australia 2014). The remaining cattle largely have no commercial value (NCV) (Joint Select Committee on Northern Australia 2014) i.e. they are there whether the live export trade is active or suspended. In WA at least, this situation has implications for pastoral and environmental degradation of the rangelands (Novelly and Thomas 2013, Joint Select Committee on Northern Australia 2014) and also for animal welfare (cattle of NCV are worth less than the fuel and the bullets that may be required to shoot them).

Figure 3.

Figures 1-3. Photos taken in early September 2014 (still months away from the wet season and any feed) show significant degradation of the rangelands with its resultant effect on animal welfare.

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) are just some of many recording gross mismanagement of pastoral properties and 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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A general comment on the replies in this issue

Richard Malik

CVE Valentine Charlton Consultant

e richard.malik@sydney.edu.au

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 Barnyard (who was a research immunologist early in his career, and later a companion animal clinician in Canberra), Mario Viscardi (well known outspoken veterinarian and raconteur) 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 rainy England. I mean the Asian seafarers that released the dingo-precursor 5 to 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 brumbies 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 human beings. 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 Gambit* (www.stephendandocollins.com/australian-history/pasteur's-gambit) about early attempts to develop biological control of this pest, and groups 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 saddle when they build the Ghan railway line).

4. In recent times, 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 normal grant cycle of 3 years does not allow for investment in a problem that might take 10-20 years to crack. Immunocontraception 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 Banyard is completely correct in terms of the value of eradicating feral pests from islands, or to create fenced-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 to create 'island like sanctuaries' using expensive fencing is also laudable and worth doing in key areas, perhaps with eco-tourism as a means of paying for or recouping the capital investment, as occurs in an establishment in the Adelaide Hills that was once a dairy farm. The publication *Wildlife Matters* is, however, a promotional magazine aimed at obtaining funding from the public; it targets the cat for the worst attention, but if you read carefully what they are on about, it is getting rid of all non-domestic animals – wild dogs (dingo hybrids), foxes, feral cats and rabbits. That is the easy part, the hard part is finding money to pay for the fences, the upkeep of the fences, and making sure that fire-prevention measures ensure such natural zoos are protected from bushfires. **But, as an overall strategy, it can never work in a country the size of Australia.** It is interesting

C&T No. 4901 A Life Well-lived... The Life of an Aussie Vet *C&T Series* 250, April 2008.

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 feeds, 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 (methylene 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 suburbia into the Australian bush. Most of the young cats that are not taken in by well-meaning people

Figure 1. Mother goose and goslings. Foxes beware – go near these and you will die! These are family.

Figure 2. Happy small ruminants and a horse with short-man syndrome. Could you eat these guys?

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 immunocontraception or the use of a contagious infectious disease for which a vaccine can be made to protect owned cats (as was the case for Calicivirus and rabbits).

14. Now, the really hard question: **Can a vet who loves all animals condone owning 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 a 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* fit 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 frames for dogs. Unfortunately, my present crew of cats prefers 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. ■

Figure 3. Pets or dinner? Which one will make these a winner?

Figure 4. 'Hattie' in the kitchen. Are herbivores ethically sounder than carnivores as pets? This sheep thinks so. So do her babies.

PERSPECTIVE

NO. 122

Veterinarians Taking the Lead on Animal Welfare & Ethics: A Summary & Reflection of the Day

Andrea Harvey

BVSc, DSAM (Feline), Diplomate ECVIM-CA, MANZCVS (Associate), MRCVS

e andreaharvey.cat@gmail.com

Centre for Compassionate Conservation
The University of Technology Sydney

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 the lawyers were far ahead of the veterinary profession in leading the way in animal welfare and ethics, with particularly impressive presentations from philanthropist Philip Wollen, Shatha Hamade (Legal Counsel for Animals Australia), and Emmanuel Gluffre (Legal Counsel for Voiceless), all of whose courage, moral principles and determination for animal justice simply shone through, and left me feeling ashamed that our profession was not doing more. The organisation and atmosphere of that conference was also impressive with the audience being left in no doubt of the dedication of these young lawyers to animal welfare – from the power of the speakers, to the passion of the chair person, to the all-vegan catering sending out a powerful message. I was keen now to see if this CVE/Sydney University symposium would change my mind and if I would finish the day agreeing with the title, 'Veterinarians taking the lead on animal welfare and ethics'.

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 OWN perception of its physical and emotional state (which may be different to OUR perception) and is independent of where it lives, the cultural and economic status of its owners/handlers/managers, and what WE think the animal's purpose is;**
- **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.**

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Dr Rosemary Elliott gave, in my opinion, the most thought-provoking and inspiring lecture of the day. She described how 'vets are uniquely qualified to take the lead on animal welfare and ethics, and that the public certainly expect us to, and our professional codes of conduct highlight that the welfare of animals should be our primary concern.' **But are we all upholding the integrity of our profession by abiding by these codes, and are we really taking the lead on animal welfare and ethics?**

Dr Elliott went on to eloquently critique why our profession remains ethically compromised in our contribution to national animal welfare issues. What she went on to describe cannot be argued with, and is a sad situation for our profession, and one which I believe we must be strong and united in addressing. She explained, 'the AVA holds positions on a number of animal use practices with proven negative welfare outcomes that are clearly biased in favour of industry, and at odds with public opinion. They are also inconsistent with the AVA's Code of Practice. The AVA's position statement on live animal export does not question the ethics of the trade, but rather naively insists upon working towards humane slaughter, placing faith in World Organisation for Animal Health (OIE) guidelines and in legislative commitments for enforcing animal welfare standards that are non-existent in most importing countries. It makes no mention of the Exporter Supply Chain Assurance System (ESCAS) or its abysmal failures to safeguard animal welfare. The position statement on poultry supports conventional battery cages for laying hens and upholds The Model Code of Practice for the Welfare of Animals – Domestic Poultry, 4th edition 2002, which also supports beak trimming and stocking densities for meat chickens that would equate to 2 birds on an A3 page. Then there is the position statement on hunting (2004), which states that: 'Within the AVA (as within the wider community), there are diverse opinions on the moral aspects of hunting. The AVA's prime concern is for the welfare of 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 unregulated cruelty involved in hunting and appearing to condone the use of other animals in the process, such as pig dogs.'

Expecting a different stance from the AVA special interest group in animal welfare and ethics, I was shocked when later in the day Dr Alex Burleigh, President of the AVA group 'Australian Veterinarians for Animal Welfare and Ethics', seemed to confirm these concerns, when he stated that 'Ethics is not used in developing AVA policies'. Dr Burleigh went on to explain how the AVA uses an 'evidence-based approach' and only considered ethics when no evidence was available. This seems particularly strange coming from the 'welfare and ETHICS group'. This begs the question that if the AVA special interest group in welfare and ethics is unable to come to the obvious conclusion that 'any reasonable person' would come to on national animal welfare issues such as the unethical and inhumane nature of the live export trade then, quite seriously, what hope does the profession have in 'taking the lead on animal welfare and ethics'? If the AVA can take on board Prof John Webster's simple words of wisdom outlined earlier when writing policies, that in itself would be a huge leap forward for the profession.

Dr Elliott went on to raise the issue of 'the competing priorities juggled by all professional veterinary associations, whose main aim is to serve the needs of thousands of

Figures 1-4. Animals have the capacity to experience pleasure, joy and friendship, as well as suffering. Veterinarians should advocate for animals using sentience as the foundation of ethical decisions.

Figure 1.

Figure 2.

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 importance of 'conflicts of interest' in difficult scenarios where there is conflict between the interests of the animal and the client, and discussed some particularly complex situations such as animal hoarders and breeders of brachycephalic dogs.

Conflicts of interest became a topic raised repeatedly throughout the day. Dr Susan Hazel discussed the role of veterinarians in the racing industry, another industry that has been under close public and media scrutiny. Dr Teresa Collins discussed the use of animals in veterinary teaching, perhaps a small area compared with livestock and sporting industries, but a critically important area as this contributes to shaping the morality of future generations of veterinarians in the ethics of animal use. Dr Janice Lloyd discussed the emotionally elaborate lives of large marine mammals and the ethical and welfare issues surrounding keeping these mammals in captivity, another area where rewards for humans in the form of entertainment and financial rewards significantly conflict with the best interests of the animals. Dr Raf Freire discussed how we measure welfare and highlighted how practitioners often omit to consider broader aspects of welfare such as mental state, social behaviour and mortality. Dr Clive Phillips discussed how Australia is lagging behind in animal protection and that, despite some achievements, there are significant animal welfare challenges ahead particularly with increasingly intensive farming, and wild animal welfare issues related to urbanisation and global warming. He explained how, **in order to achieve moral use of animals, we need moral sensitivity, moral reasoning, moral courage and moral action, again highlighting that science alone isn't enough.**

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

Figure 3.

Figure 4.

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**

Figures 5-8. Knowing that animals have the capacity to experience pleasure, friendship, joy, pain, frustration and distress – do we find these images acceptable? Is this what the profession is striving for? Are we doing enough to advocate for the higher order needs of animals, and are we even managing to prevent pain and suffering?

Figure 6.

Figure 5.

Figure 7.

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.

Figure 8.

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 crammed 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 sentience will take us to some uncomfortable and confronting positions, and how we deal with this will determine the view 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 sadly the veterinary profession is going to be left behind on animal welfare and ethics, and not leading the way. Do we really want this to happen? Or do we follow the concluding words of Dr Sue Foster: **'Vets must take a stronger lead and stand up for the animals that we graduated to look after'.** ■

View lectures from the 2015 Welfare & Ethics Symposium here

Figure 9. Prof John Webster speaking at the Welfare and Ethics Symposium in December, 2015. (Photo courtesy of Anne Fawcett)

ISFM FORUM

SMALL ANIMAL

CRD Patient Anaemia

Pete Coleshaw

Jaffa's Health Centre for Cats
52 St Francis Road Salisbury, UK

t 01722 414298
e jaffa@jaffavets.com
w jaffavets.com

C&T No. 5536

I have a renal case with an asymptomatic packed cell volume (PCV) of 21. The September 2013 *JFSM* article lists criteria for darbopoietin 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
e scaney@catprofessional.com

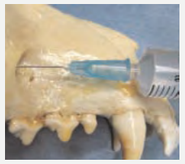
I don't think that there is currently much published evidence to support decision making for you or answer the questions you pose.

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 Beckman



cve.edu.au/files/brett-beckman_regional-nerve-blocks.pdf

PERSPECTIVE / WINNER

NO. 123

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

Winners: Kathryn Stalder,[1] Caroline Marschner[1] & Richard Malik,[2] Damien Higgins,[1] Larry Vogelnest,[3] Graeme Allan[1,4] & Mark Krockenberger[1]

1. Faculty of Veterinary Science, School of Environmental and Life Sciences, The University of Sydney, NSW — kath.stalder@gmail.com, cmar3346@uni.sydney.edu.au
2. Centre for Veterinary Education, The University of Sydney, NSW — richard.malik@sydney.edu.au, damien.higgins@sydney.edu.au
3. Taronga Zoological Park, Mosman, NSW — LVogelnest@zoo.nsw.gov.au
4. Veterinary Imaging Associates, PO Box 300, St. Leonards NSW — graeme.allan@sydney.edu.au, mark.krockenberger@sydney.edu.au

Abstract

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 ultrasonography 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, uteri, 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.

Key words: Koala; *Phascolarctos cinereus;* protocol; ultrasonography; ultrasound; urinary; genital; *Chlamydia*

Background

Several structural and functional changes can occur in the urogenital tract of female and male koalas *(Phascolarctos cinereus)* as a result of infection with *Chlamydia pecorum* and, to a lesser extent, *C. pneumoniae*. Pathological lesions seen in the urinary tract include bladder wall thickening and mucosal ulceration.[1-3] Cystitis may ascend to involve the upper urinary tract, provoking thickening and subsequent dilatation of the ureters, and focal or generalised pyelonephritis with interstitial fibrosis.[1,3,4] Gross anatomical changes observed in the reproductive tract of koalas with chlamydial disease include oedema, fibrosis and occlusion of uteri, urogenital sinus or oviducts, resulting in mural thickening and distension by inflammatory exudate in the lumen[3,5,6] and the formation of para-ovarian cysts involving the ovarian bursa.[3] Chlamydiosis reduces the reproductive capacity of individual animals as well as koala populations[7] and its high prevalence in the wild[1,6,7]

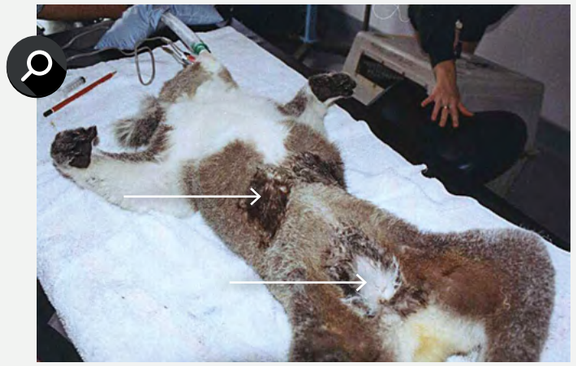


Figure 1. Anaesthetised koala positioned for ultrasonographic scanning in dorsal recumbency. *The opening of the pouch and the sites for examination of the kidneys are indicated by arrows.*

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is also reflected by the rate at which koalas with chlamydial disease are admitted to care.[8]

Many of the severe structural changes associated with chlamydial disease in koalas can occur without clinical signs,[9] yet detecting these is crucial to therapeutic and prognostic decision making. Abdominal radiology is a poor diagnostic tool in the koala as there is minimal intra-abdominal fat to provide radiological contrast[10] and the voluminous caecum obscures visualisation of most of the organs. Ultrasonography has become a common diagnostic tool for characterising urinary and reproductive tract diseases in small animal medicine[11] 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[12] and was recently demonstrated to be an accurate tool to evaluate the urogenital tract in this species.[13] 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.

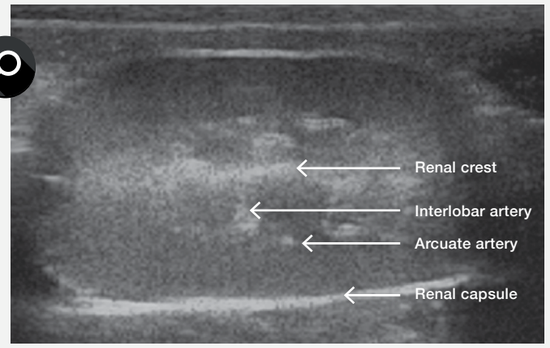

Figure 2. Dorsal scan of the right kidney from a healthy koala.

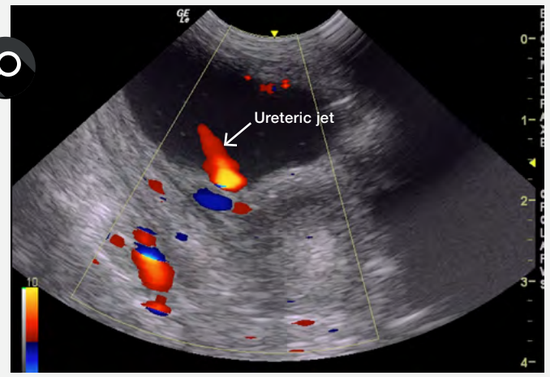

Figure 3. Sagittal scan of the urinary bladder, *using colour flow Doppler to highlight the ureteric jet (colour coded red/yellow).*

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

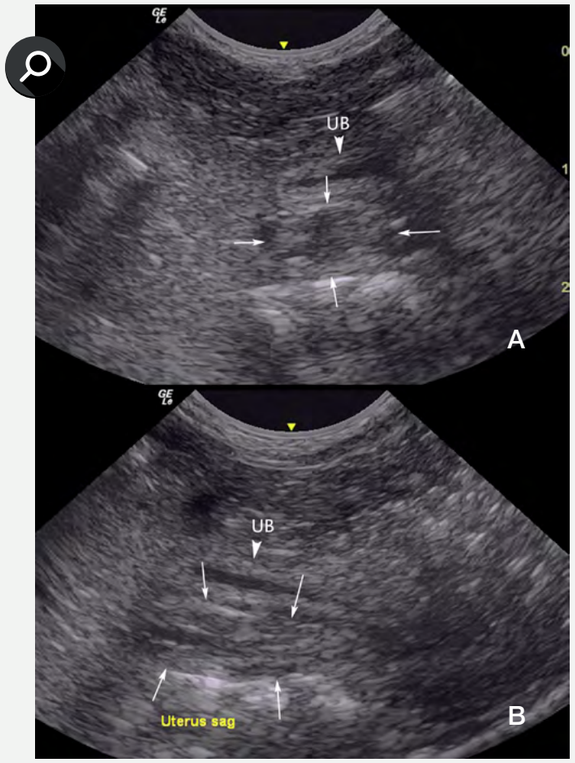

Figure 4. Sonographic images of the uteri and urinary bladder in healthy koalas. *In A (transverse view of the pelvic contents), the urinary bladder (UB) is in the near field, while the right uterus is evident as a circular structure delineated by arrows. In B (sagittal view of the pelvic contents),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

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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 (Midget 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 ultrasonography 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 ultrasonography machine (Aloka 500) with 5 MHz linear transducer (model UST-974-5) 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 UPP-110S 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 Esaot 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 uteri were visualised consistently. The transducer head was then gradually rotated to trace each uterus cranially along its sagittal plane. Measurements of uterine luminal diameter and wall thickness were obtained and described in terms of size, shape, position and echogenicity.

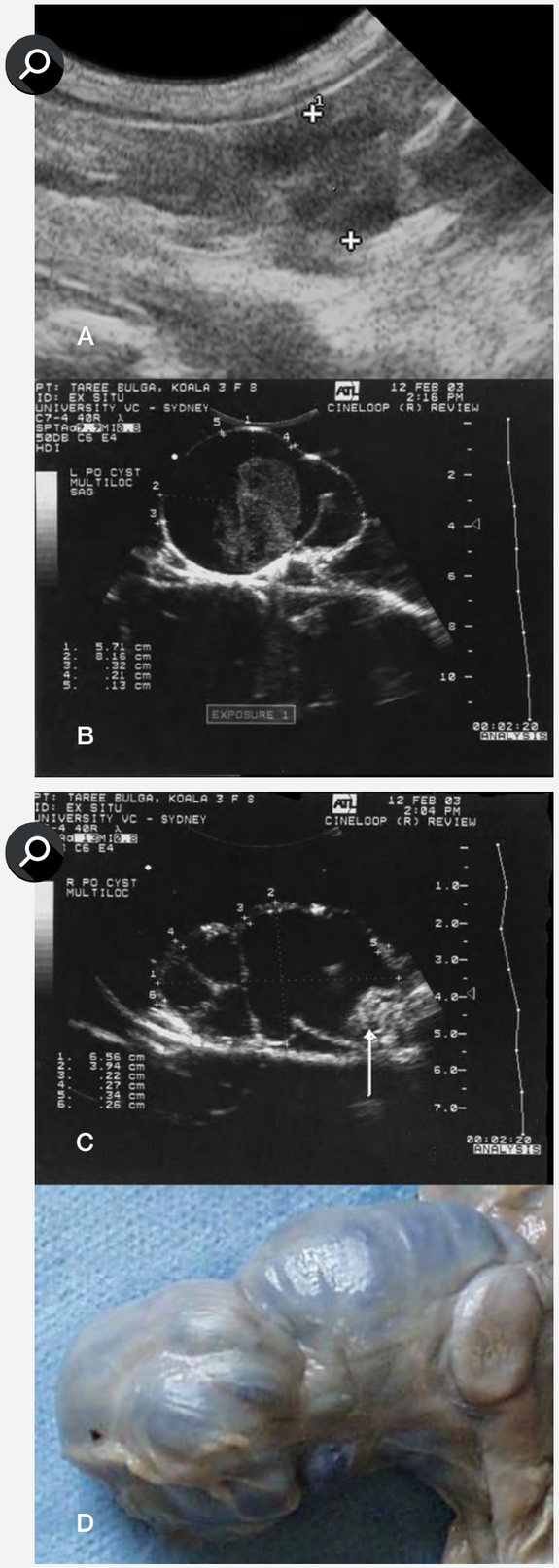

Figure 5 (Right). Multilobulated paraovarian cysts from a koala PCR-positive for **Chlamydias.** *The right ovary from a healthy koala is illustrated in A (defined by 2 marking points). In B, image of a Chlamydia diseased animal was obtained* in situ *after euthanasia, while C was taken prior to necropsy, but* ex situ *(in organ bath). D demonstrates the gross appearance of the lesions at necropsy.*

Organ/- structure	Ultrasonographic Appearance	Organ Size (mm)
Urinary Bladder (n=10)		
Bladder	The urinary bladder appeared as a distinctive oval to round shaped organ, with a thin wall and an anechoic lumen. An outer hyperechoic serosa and inner hyperechoic 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.	Bladder wall thickness: 1-5.5mm, depending on the degree of distension
Kidney (3 males; 6 females)		
Renal capsule	The fibrous renal capsule appeared as a thin hyperechoic structure adjacent to the peri-renal fat surrounding the kidney in both the transverse and sagittal planes.	**Left** Length: 32-47mm Width: 20-31mm Height: 20-33mm **Right** Length: 38-40mm Width: 15 -35mm Height: 18 -33mm
Renal cortex	The renal cortex was uniform in texture with a finely mottled appearance. It was less echogenic than the renal capsule, but hyperechoic relative to the renal medulla.	
Vasculature	Arcuate arteries were visible as circular and linear hyperechoic areas at the cortico-medullary junction. Interlobular arteries and their associated adipose tissue appeared as circular areas of increased echogenicity between adjacent renal pyramids.	
Uteri (n=6)		
Transverse section of uteri	The right and left uteri appeared as round, homogenously hypoechoic organs located dorsal or dorsolateral to the urinary bladder and ventral to the colon.	Left uterus: 4-15mm Right uterus: 6-11mm Wall thickness: 2-4mm
Longitudinal section of uteri	The tubular nature of the uterus could be discerned, although imaging in this plane was more difficult as the epipubic bones precluded obtaining an adequate acoustic window in many instances.	
Sagittal section of uteri	Uteri appeared as homogenous hypoechoic structure	
Uterine Lumens (n=2)	The uterine lumen was seen in longitudinal plane and was narrow, anechoic to hypoechoic and bounded dorsally and ventrally by echogenic uterine walls.	
Testis/Epididymis (n=2)		
Testes	Round to ovoid homogenous structures of medium echogenicity. The Tunica albuginea is visible as a clear hyperechoic line surrounding the testis and epididymis.	**Adult** Length: 27mm Width/Depth: 17mm **Juvenile** Length: 20mm Width: 14mm Depth: 10mm
Epidydimis	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.	
Prostate (n=1)		
Sagittal section of prostate	The prostate was round to ovoid, relatively homogenous and of intermediate echogenicity. Difficulties were encountered in this plane and measurements are unlikely midsagittal and in maximal dimension.	Length: ~12mm
Transverse section of prostate	The prostate was heart shaped, echogenicity and texture varied from hypoechoic peripherally (within glandular tissue) to hyperechoic centrally (fibromuscular tissue).	Width: 24mm Height: 16mm
Prostatic urethra	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.	
Lateral Bulbourethral Glands (n=2)		
Only most cranially pair of lateral bulbourethral glands visualisable	The bulbourethral glands were oval in shape and typically glandular in appearance, with hypoechoic parenchyma of medium to fine echo-texture.	Aged Adult: 15 x 16mm Subadult: 11 x 12mm

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

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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.[14-16] 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.

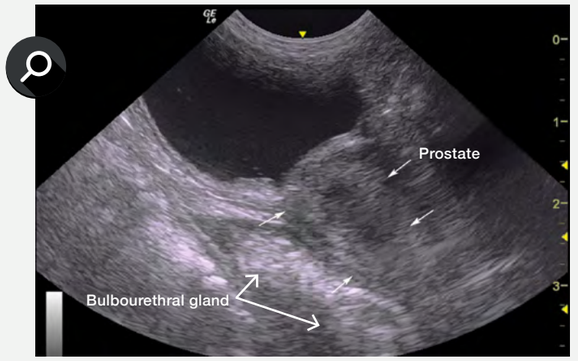

Figure 6. Sagittal scan through the bladder neck. *At this view the prostate and the bulbourethral gland are shown.*

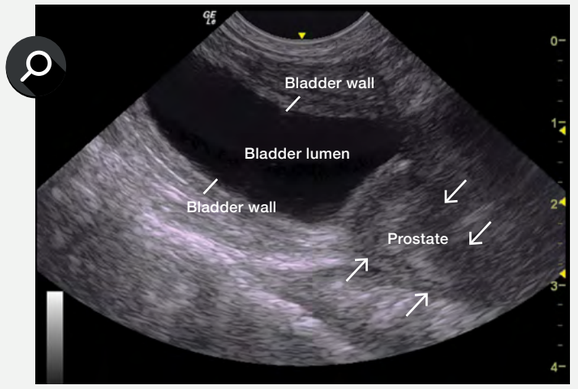

Figure 7. Normal urinary bladder with prostate. *Measurement points for the bladder wall thickness indicated with bars. The prostate is indicated by arrows.*

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-UM9-HDI 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 evaluation 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.[17] 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.

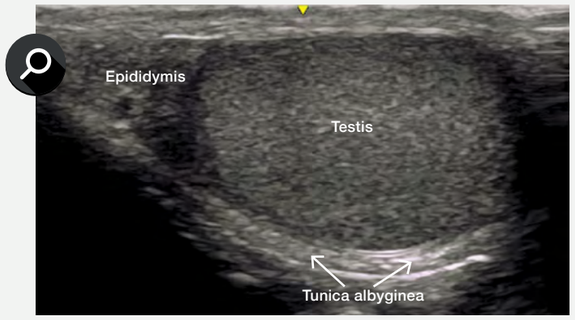

Figure 8. Ultrasound image of a koala testis, sagittal view. *Smooth surface and homogenous parenchyma of the testis are shown here.*

No.	Ultrasonographic Appearance	Necropsy Findings	Histology Findings
	Urinary Bladder (n=3)		
1	Bladder lumen contained flocculent echogenic material and the bladder wall was thickened (>5.5mm using electronic callipers).	The bladder wall confirmed to be >5.5mm using manual callipers.	Severe chronic active, predominantly plasmacytic cystitis in association with smooth muscle hyperplasia.
2	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. Hyperechoic foci were evident within the bladder wall in both planes.	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 dilatation of the right ureter.	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.
3	A thickened urinary bladder wall (4-9mm) with very little lumen visible with a heterogeneous mass lesion of moderately increased echogenicity was visible between the hyperechoic submucosa and the hypoechoic muscularis layers. The echo-texture in this region was more granular than that of the neighbouring bladder wall.	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.	The mass lesion was situated between the submucosa and the muscularis, consisting of spindle shaped cells of morphology and arrangement consistent with leiomyoma. A similar tumour was observed in the walls of the urogenital sinus.
	Kidney (n=3)		
1	Right kidney appeared misshapen and of increased echogenicity compared to the apparently normal left kidney.	A misshapen right kidney, with small indentations evident on the capsular surface.	Evidence of infarction and segmental cortical necrosis.
2	**No abnormalities**	One kidney misshapen	Multifocal pyogranulomatous interstitial pyelonephritis. The presence of inflammation in the renal medulla and the wedge-shaped pattern of inflammation suggested an ascending infection.
3	No abnormalities	**No abnormalities**	Mild chronic pyelonephritis

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

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 chlamydiosis 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 chlamydiosis. The concept of utilising ultrasonography as a diagnostic tool in koalas is not novel[13,19] 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 at identifying urinary tract changes and the presence of paraovarian cysts.[13] 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 chlamydial 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.

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No.	Ultrasonographic Appearance	Necropsy Findings	Histology Findings
	Uteri (n=2)		
1	Uteri exhibited mural thickening (>4mm) and an irregular mucosal surface. The walls were anechoic to hyperechoic and varied in thickness from 4-8mm. Uterine lumens appeared anechoic due to the presence of intraluminal fluid.	Uteri were enlarged, with turgid, thickened walls surrounding fluid-filled lumens.	Moderate to severe chronic lymphocytic and histiocytic inflammation was observed histologically, with scattered plasma cells present in the muscularis.
2	Hypoechoic and homogenous mass could be appreciated at the caudodorsal aspect next to the urinary bladder.	No abnormalities	Lymphosarcoma
	Para-Ovarian Cysts (n=6)		
1-6	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 paraovarian 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.	Five out of the six animals were affected bilaterally. 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-loculated or compartmentalised. The fluid within the cysts varied in appearance from colourless to yellow or brown and from clear to turbid.	Ovarian bursitis was present in two cases. This was characterised by a mild to moderate plasmacytic infiltration with varying degrees of fibrosis.
	Prostate (n=1)		
1	The prostate ranged from an anechoic to hyperechoic echogenicity. The glandular portion was mottled in appearance. The outer fibromuscular capsule, more evident *ex situ* than *in vivo,* was discernable as a hyperechoic band surrounding both prostatic lobes. A focus of increased echogenicity was also present centrally, surrounding the prostatic urethra.	No abnormalities	Mild chronic prostatitis and fibrosis

Table 2. Continued.

Anecdotally, koala fur is slow to regrow, and its thermo-regulatory function is likely of particular importance in this species, which exists on a limited energy budget.[20] 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 uteri. 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 by 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 uteri. Further studies at different stages of the reproductive cycle are therefore required.

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. ■

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SMALL ANIMAL

Possible Dysautonomia – Has Anyone Seen a Case Like This?

Christopher Simpson

e simpson_christo@bigpond.com

Victoria Veterinary Clinics, Hong Kong

C&T No. 5537

Question

Just wondering if you have seen dysautonomia in dogs before?

We had a dog in that we discharged recently after 10 days in hospital. It had a syndrome with some characteristics of dysautonomia. Initially, it was brought in for ptyalism and inappetence. The dog is a street-roaming village mongrel (20kg). Good body condition score and bright. Full bloods were unremarkable.

It was spectacularly distressed – drooling severely with ropy strands of malodorous brown material and severe halitosis. It compulsively swallowed to the point of frothing and would NOT allow handling of the mouth at all while conscious. It was very, very sensitive round the head. All other physical examination findings normal.

We took radiographs, and there was a large amount of bone material in the stomach. We told the owners that there was possible gastrointestinal (GI) obstruction, and advised repeat radiographs to rule this out.

The following day, the bone had moved out of the stomach and somewhat into the small intestine (SI) but there was now generalised gastrointestinal dilation with gas. The dog was still very distressed. I performed an ultrasound but couldn't see a small intestine foreign body obstruction.

We then anaesthetised the dog and upper GI scoped him. His mouth was full of ulcers. Caudal to the pharynx, everything was normal, oesophagus and stomach 100% normal. Couldn't get out the pylorus in this dog, however.

We put in an E-tube to bypass the mouth, and woke him up.

The next day he was worse; regurgitating, drooling profoundly, no gut sounds, and now couldn't urinate. Pollakiuria with distress. An ultrasound showed a large bladder which was readily manually expressible, but the dog couldn't empty it at all.

We gave him barium in case we had missed an obstruction which stayed in the stomach for > 24hrs, with small amounts moving out into the small intestines. Radiographs repeatedly showed severe small intestine gas accumulation and ileus/query obstruction.

We took him to exploratory laparotomy and ran the bowel.

Structurally, nothing abnormal was detected; no foreign body, but NO gastrointestinal motility at all at surgery. We passed a urinary catheter – no mechanical obstruction – and his bladder was easily expressible. We biopsied the gastrointestine in 6 places, then woke him up again.

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.

We started him on pro-kinetic – initially metoclopramide continuous rate infusion and cisapride (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 reported hypomotility in the GIT and urinary tract. There are no significant microscopic changes in the submucosal and intermuscular ganglia within the examined tissue sections.

Provisionally, I have called this a dysautonomia-like syndrome, which appears to be coincident with ingestion of some foreign matter, which may have been bone, meat, garbage, or some other intoxicant. I wonder if it had been caused by some bacterial toxin, or is immune-mediated/molecular mimicry? Or is the foreign material a red herring? I would be prepared to accept it was just a case of severe gastroenteritis, except for the bladder signs, which were totally convincing. ■

HK Diagnosis Report

Download here

SMALL ANIMAL

CALL FOR CASES! Cat Paws Hyperkeratosis

C&T No. 5538

A major benefit of participating in our Feline Medicine Distance Education program is the opportunity to network with like-minded colleagues around the world through the listserves. See below a question posted by Emir Chaher from Portugal on the ISFM listserve and the instantaneous responses from vets in the UK, Australia and NZ, all voluntarily assisting Emir and his patient. This is veterinary collaboration at its best!

We would be very interested to hear from CVE Members and C&T Series readers if they have seen similar cases, or care to comment on this article.
Email: elisabeth.churchward@sydney.edu.au

Question posted to ISFM Listserve

The CVE & ISFM jointly run the Feline Medicine Distance Education program

Emir Chaher

DVM, FEI Vet

m +351 912511550
p +351 212342423
f +351 212342636
e chaher@iberovet.com

Rua do Bocage
Lote 32, R/C Dto.
2890-052 Alcochete
Portugal

Hi colleagues

The owner of this cat came to me for a second opinion. She sent me these pictures by email. The 7-month-old male is castrated and has not been tested for FIV/FELV or other conditions yet. I haven't seen the cat yet but he will come tomorrow for an exam. The owner says he's difficult to handle so I will probably sedate him. It seems as all finger paws are affected by these 'cutaneous horns' and he is starting to have locomotion problems.

Have you seen something similar? Any advice on management?

I'm planning to examine it thoroughly, take blood samples and maybe X-ray and try to cut/remove the 'horns'.

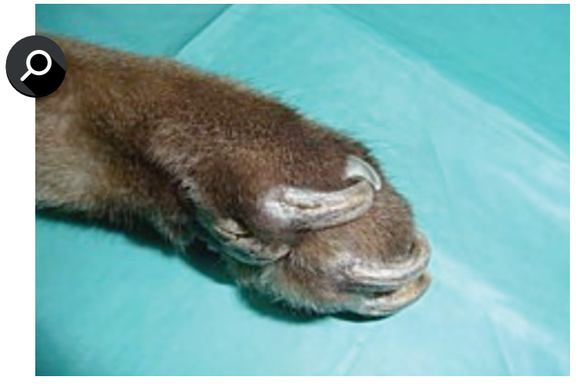

Figure 1a.

Figure 1b. Two representative photographs of the affected pads.

Reply 1a

Elise Robertson

BS, BVetMed, MACVSc (Feline), DipABVP (Feline), AFHEA, MRCVS, ABVP (Feline Practice), Feline Medicine DE Tutor

e e.robertson@felinevet.net

You can see this in FIV positive cats...I can't recall why though...

Response from Emir

Thanks Elise. Yes, I know. He will be tested tomorrow and it will be interesting to know if that's the cause or is associated.

But my main concern is about the **management and prognosis** because the cat is so young and the condition is so extreme.

Any advice?

Reply 1b

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 FIV/FeLV 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.

Reply 2

Andy Sparkes

ISFM Veterinary Director
e 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.

Figure 2a.

Figure 2b.

Reply 3

Richard Malik

CVE Valentine Charlton Consultant
e richard.malik@sydney.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?

Reply 1c

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 3b

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.

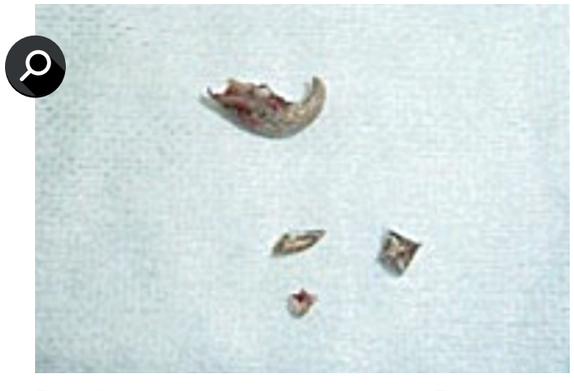

Figure 2c. Before (a) and after (b) debridement. The resected, cornified tissues, cutaneous horns (c).

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Response from Emir

The cat came in today. Really impressive lesions. Never saw something like that before. I even took X-rays to document the case. The cat was sedated and tolerated the procedure very well.

Some of the horns where detached at the base and came off relatively easily. Others were quite vascularized and bled when cut too short. I used some aluminum chloride homeostatic solution and it stopped relatively quickly.

I removed the ones from the front leg's 5th digit directly from the paw and cauterised the base to see if this would have an additional therapeutic benefit.

I cut as much as possible from digit and palmar/plantar paws. I kept some of them in ethanol, Richard. Where should I send them? I would be very grateful.

Tested for FIV / FELV (rapid test) >> NEG / NEG. Kept a serum sample for other test if needed.

Body and Clinical Exam: Nothing abnormal detected.

I think I'll wait for the PCR result before using Aldara. Thank you all for the interest and advice.

Best wishes, Emir.

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

John Munday

BVSc, PhD, Diplomate ACVP
IVABS Massey University, Tennent Drive
Palmerston North New Zealand 4410
e 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.

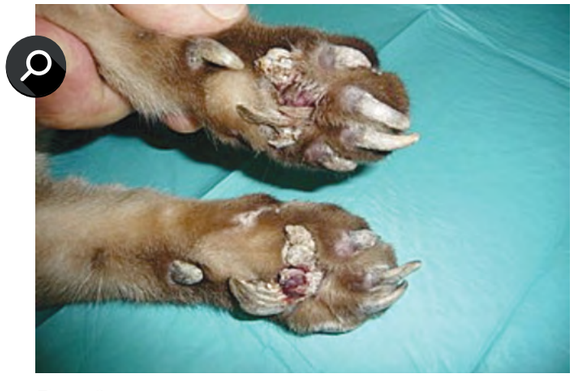

Figure 3a.

Richard to John

THANK YOU. Emir would love to send stuff to you. I hoped the ethanol would be DNA friendly.

I suspect it will not be hard to get more material – and for histology, I thought you would be interested in perhaps a slightly deeper biopsy.

The older literature talks about FeLV, but apparently the discussion forum consensus is that this is no longer the case.

I have seen the odd cutaneous horn on old cats, but not on the pads, let alone ALL the pads.

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.

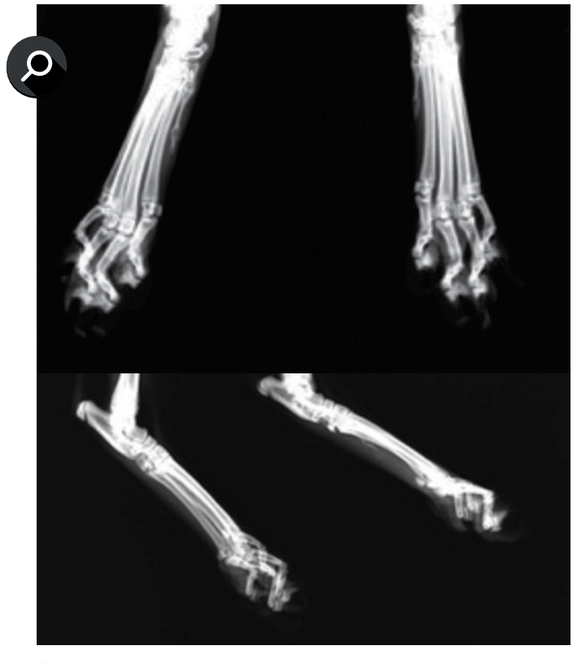

Figure 3b. Another photograph of the patient's forelimb and radiographs to show there is no bony involvement.

Pathology Results

John Munday

BVSc, PhD, Diplomate ACVP

IVABS Massey University, Tennent Drive
Palmerston North New Zealand 4410

e J.Munday@massey.ac.nz

A histological section was cut from the sent paraffin block. Examination revealed large quantities of keratin that contained faint pyknotic remnants of cell nuclei. No underlying epidermis was visible within the sections. There were no histological changes that were considered suggestive of a papillomaviral etiology to the cutaneous horns.

DNA was extracted from shavings of the tissue block using a commercially-available kit. As the potential causative papillomavirus was unknown, three sets of PCR primers that were all designed to detect a wide range of different papillomavirus types were used. These included the FAP59/64, MY09/11, and CP4/5 primer sets. As *Felis catus* papillomavirus type 2 (FcaPV-2) currently appears to be the most common cause of papillomaviral skin disease in cats, the JMPF/R primers that are specific for this virus were also used. However, none of the primers amplified papillomavirus DNA from the samples.

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 detectible papillomaviral 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 j.munday@massey.ac.nz

Comment from Emir

Meanwhile the 'horns' have grown again, so we could get new samples. ■

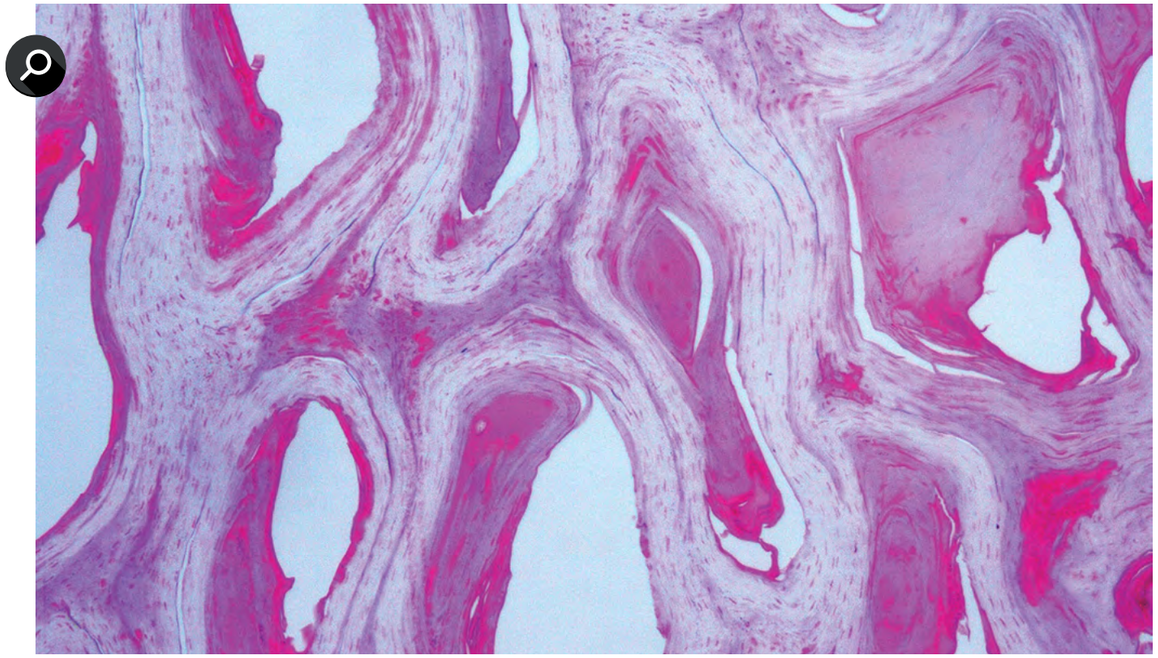

Figure 4.

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PERSPECTIVE

NO. 124

Understanding PCR & its Uses in the Veterinary Lab

Ángeles Sánchez-Pérez

Valentine Charlton Postdoctoral Research Associate in Feline Virology

e angeles.sanchez-perez@sydney.edu.au

Faculty of Veterinary Science
The University of Sydney

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 Charlton 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

Angeles pictured with her 'grandkit' Zorro.

Angeles 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 (NJ, 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, Angeles 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. Angeles currently combines her position at the Faculty of Veterinary Sciences with her role as the Bosch Live Cell Analysis Facility Officer.

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 important variations 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 Mg^{2+}.

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

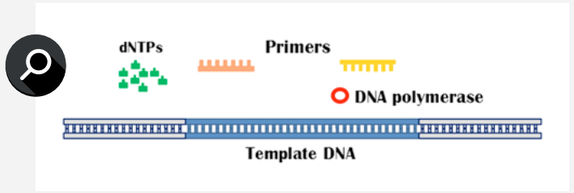

Figure 1A.

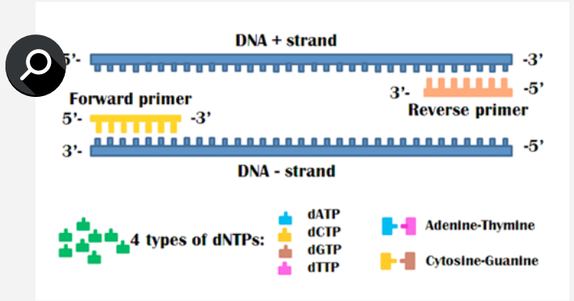

Figure 1B.

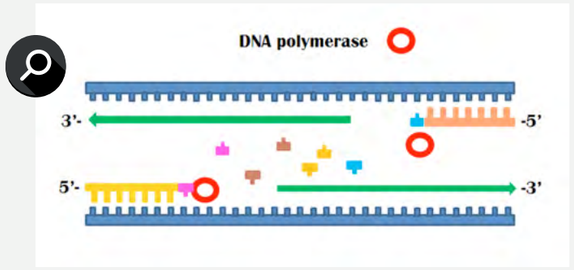

Figure 1C.

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 strenght) and cofactors (Mg^{2+}).*

(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.*

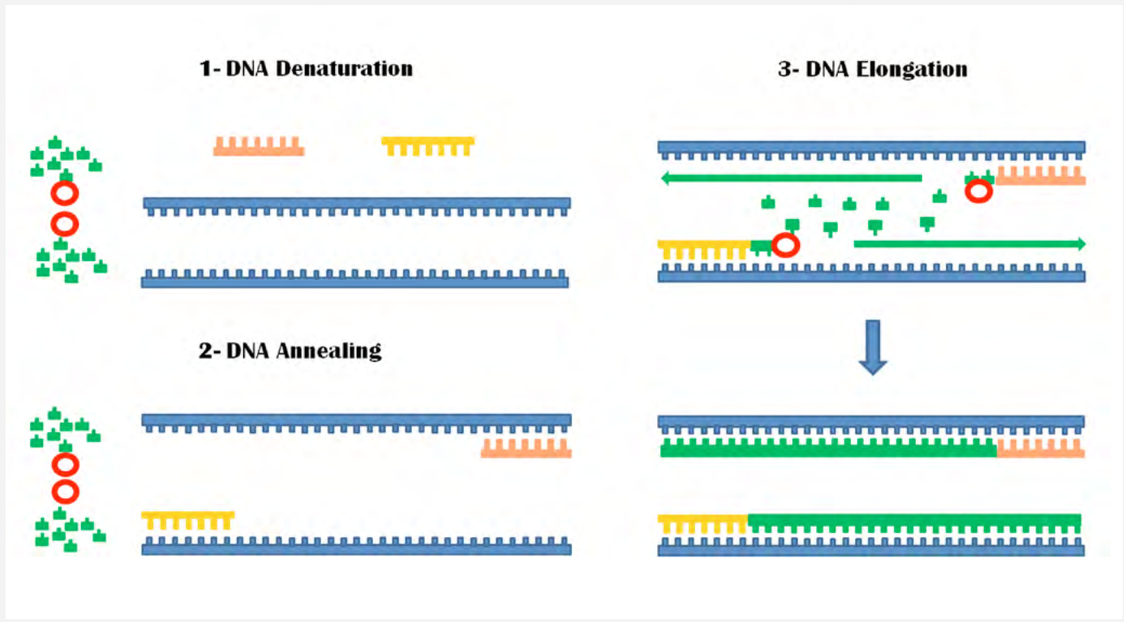

Figure 2.

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 primers 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.

provided by the nucleotide bases. Adenine can only pair with Thymine (they share two hydrogen bonds) and Cytosine with Guanine (they share three hydrogen bonds). This is why one DNA strand can be used as template to make the complementary strand and, coupled with the cellular proofreading and error-checking mechanisms, also explains the DNA replication fidelity.

DNA primers: These are very small pieces (usually 18 to 24 nucleotides long) of synthetic oligonucleotides (single-stranded DNA), called primers because they are used as a starting point for the DNA polymerase. Primers are designed according to the DNA sequence at the very end of the DNA to be amplified (see Fig. 1B). The Reverse primer must be complementary to the sequence flanking the 3'-end of the DNA Positive (+) strand (Fig. 1B), whereas the Forward primer must be complementary to the sequence at the 5'-end of the DNA Negative (-) strand (Fig. 1B).

Deoxynucleotides (dNTPs): They are the monomers, the basic building blocks, that are joined together to form the polynucleotide chains. DNA has four types of dNTPs (Fig. 2B): Deoxyadenosine triphosphate (dATP), deoxycitidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP). Each nucleotide is composed of a nitrogen-containing base (A for adenine, C for cytosine, G for guanine, and T for thymine), a sugar (2-deoxyribose) and three phosphate groups.

DNA polymerase: DNA polymerases are enzymes capable of synthesizing DNA. But they need DNA primers, as they can only add new nucleotides to an existing strand of DNA. DNA polymerases always move in the 5'- to 3'-direction (Fig. 1C), they start at the 3'-end of the primer and add new dNTPs to the nascent DNA chain by using the opposite DNA strand as template. The DNA polymerase used in PCR is Taq, originally isolated from the bacterium *Thermus aquaticus*, a microorganism found in hot springs at Yellowstone National Park (USA). Taq is ideally suited for PCR, as it has a high optimal temperature (around 75°C) and can survive repeated incubations at 95°C. As it is the case for other biological enzymes (molecules, mostly proteins that accelerate, catalyse, chemical reactions), to work properly Taq polymerase requires a particular pH and ionic strength (provided by the **buffer**) and cofactors (Mg^{2+}).

Three Stages in the PCR Cycle

As shown in Fig. 2, PCR amplification of DNA occurs in cycles that include three steps: Denaturation, Annealing and Elongation.

1- Denaturation: DNA is denatured to uncoil and separate the two DNA strands, producing the single-stranded DNA template necessary for amplification. DNA denaturation is usually carried out by briefly heating the sample to 94-96°C (Fig. 2-1).

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 1 024 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: $\mathbf{y = 2^x}$, where **y** 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

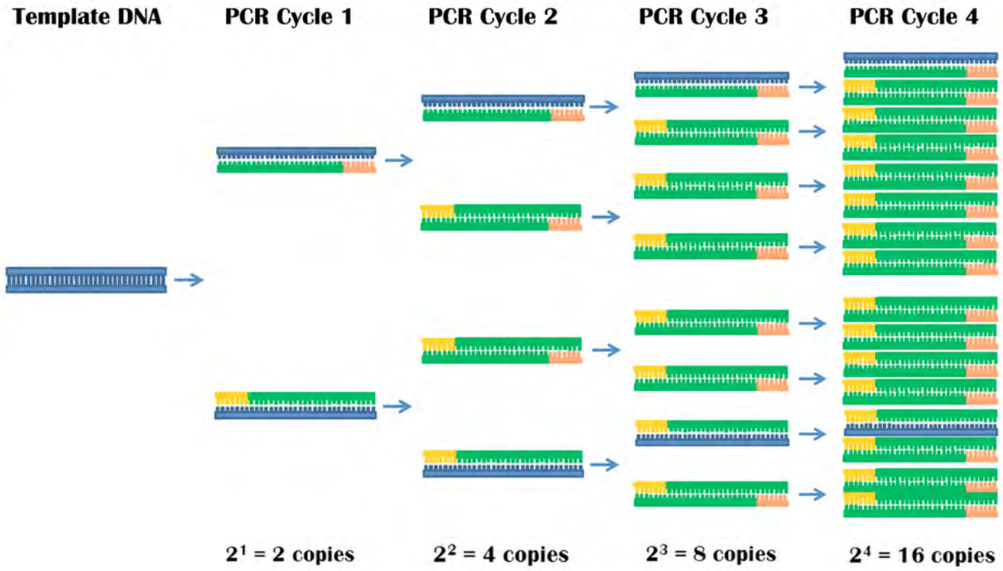

Figure 3. Exponential DNA amplification by the Polymerase Chain Reaction.

If we assume that the PCR process is 100% efficient, every PCR cycle will produce one copy of each template DNA present at the start of the cycle. If we start with 1 double-stranded DNA fragment, cycle 1 will produce a second double-stranded DNA fragment, hence there are 2 DNA fragments available to be amplified in cycle 2. This leads to an exponential increase in the number of the target DNA fragments present in the reaction: 1 before the start of PCR, 2 in cycle 1, 4 in cycle 2, 8 in cycle 3, 16 in cycle 4 and so on. The number of DNA fragments present in the PCR sample is given by the formula $y = 2^x$, where y is the number of DNA fragments and x is the mumber of PCR cycles.

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longer denaturation step (2 to 5 min), to ensure complete denaturation of the template DNA, as the template DNA is usually much larger that the PCR product and requires a longer denaturation time. In addition, the elongation step in the last PCR cycle is usually longer (5 to 10 min) to ensure that any remaining single stranded DNA is completely copied. This is particularly important when amplifying long target sequences.

A Brief History

In 1971, Kleppe[1] 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 1993). In his 1990 publication in Scientific American[2], 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[3]. 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[4] 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 ground breaking 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

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be added to each PCR cycle. Moreover, the extension cycle had to be performed at a relatively low temperature (37°C), thus allowing partially matched primers to anneal to the template DNA. As pointed out in the PCR Collection Science Magazine[4], the PCR technique did not flourished until the heat stable Taq polymerase was used in the procedure.

Chien, Edgar and Trela discovered Taq Polymerase in 1976[5]. 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 preform and also considerably simplified PCR automation.

Finally, on November 1987, both the Taq enzyme (AmpliTaq® 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' in NCBI PubMed[6]. 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[7]. 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.*[8] 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 quantitate the PCR products: fluorescent DNA-binding dyes and oligonucleotide probes (summarised in Fig. 7), and this

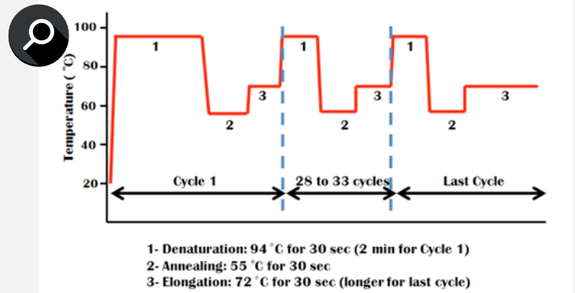

Figure 4. A typical PCR profile using Taq polymerase.

The first cycle includes a longer denaturation step, to ensure complete denaturation of the template DNA.

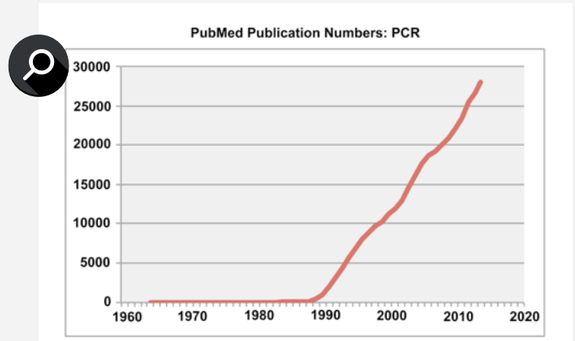

Figure 5. Number of PCR publications per year, as listed in PubMed

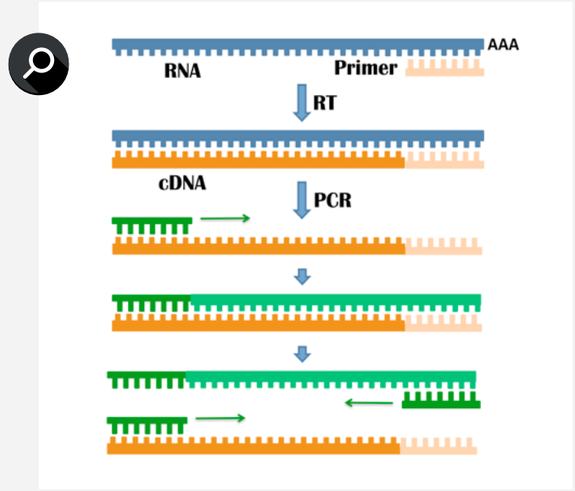

Figure 6. RT-PCR.

RT (Reverse Transcription) refers to the reaction required to copy RNA into DNA (cDNA). This reaction requires an oligo nucleotide primer complementary to the RNA sequence (shaded orange). The RNA depicted here is a messenger RNA; as mRNAs span a polyA tail, oligo dT can be used as the RT primer. The cDNA obtained is then amplified by PCR.

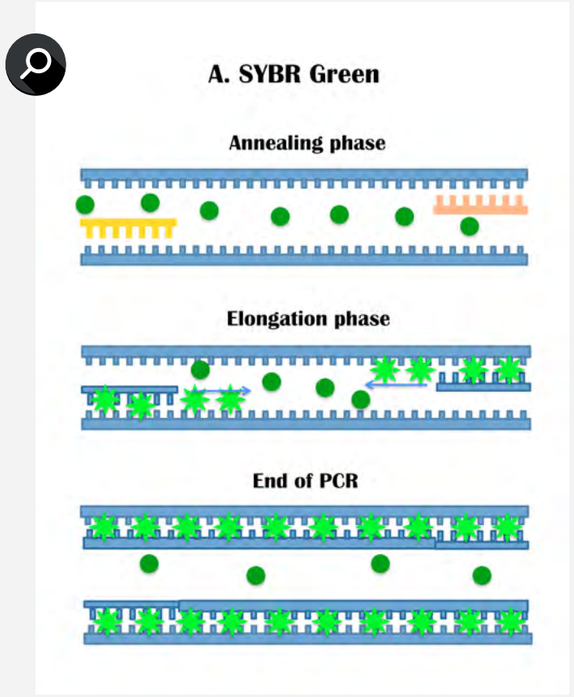

Figure 7A.

Figure 7. Current qPCR approaches:

(A) Fluorescent DNA-binding dyes

SYBR Green is the most popular DNA-binding dye currently used. This dye emits very low fluorescence when it is free in the PCR solution. On the other hand, the DNA-bound compound is highly fluorescence, 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.

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requires thermocyclers capable of exciting, detecting and recording fluorescence.

Fluorescent DNA-binding dyes, such as SYBR® Green (Molecular Probes, Fig. 7A), are included in the PCR buffer. This dye, when free in solution, emits very low fluorescence (too low to be detected). However, as the DNA is amplified by PCR, the dye binds to the DNA product and this DNA-bound dye emits most of its energy as fluorescence.

An alternative qPCR approach includes additional oligo probes situated between the two amplification primers. The most popular are the Dual-Labeled probes, such as TaqMan® (Roche Molecular Diagnostics & Applied Biosynthesis).

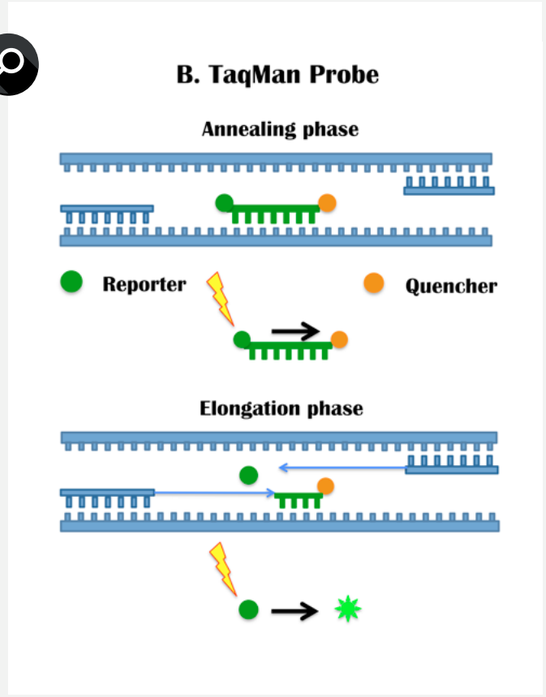

Figure 7B.

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 at same wavelength (488 nm), but emit light at different wavelengths, the peak is at 518 nm (yellow-green) for FAM and 556 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 recorded and analysed individually by the PCR instrument, and hence the three reactions can be carried out in the same PCR tube.

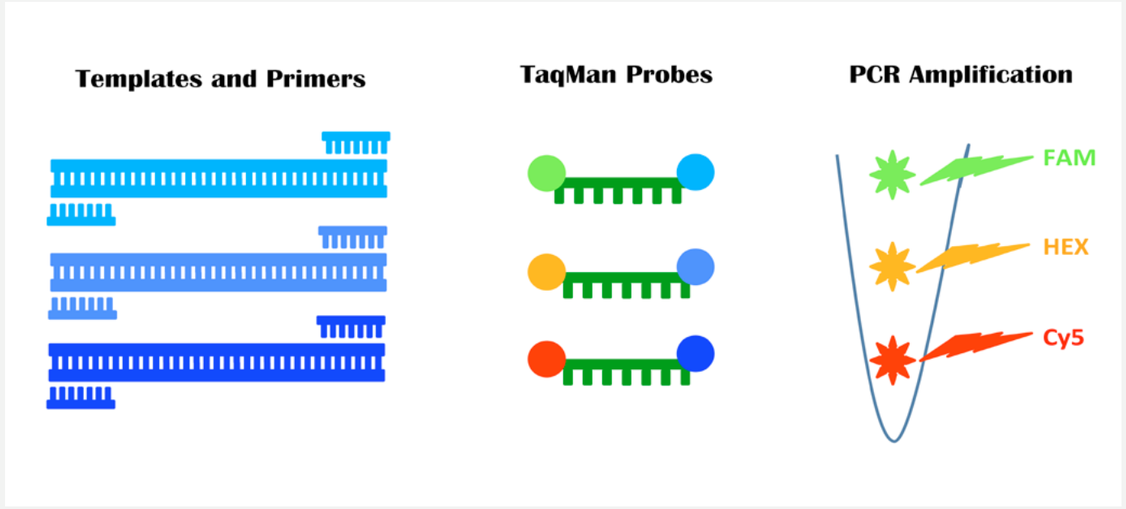

Figure 8. Multiplex PCR using TaqMan®.

This figure shows the amplification of three different DNA sequences simultaneously. It requires three sets of DNA primers and three separate TaqMan® probes, each labelled with a different reporter (FAM, HEX or Cy5).

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. 9 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[9]. 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[10]. 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 diarrhoea 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.

Real-Time PCR Test Available

DETECT PROTOZOAN INFECTION

- Giardia
- Babesia
- Leishmania
- Tritrichomonas
- Toxoplasma

DETECT BACTERIAL INFECTION

- Haemotrophic Mycoplasma
- Salmonella
- Leptospira
- Bordetella bronchiseptica

DETECT VIRAL INFECTION

DNA Viruses:
- Canine Parvovirus
- Feline Leukemia Virus
- Canine Adenovirus

RNA Viruses:
- Feline Coronavirus
- Canine Coronavirus
- Canine Distemper Virus
- Canine Parainfluenza Virus
- Canine Influenza Virus
- Canine Distemper Virus
- Canine Herpesvirus
- Feline Immunodeficiency Virus
- Feline Calicivirus

GENETIC TESTING

Genetic Disease:
- Polycystic Kidney Disease in Cats
- Degenerative Myelopathy in Dogs

Genetic Traits:
- Blood Typing
- Feline coat length

Figure 9.

Avoiding false negatives: The right type of sample must be provided for testing; for example, EDTA-treated blood is good for analysing haemotrophic 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

Figure 9. Examples of commecially 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.

The main characteristic of PCR, its sensitivity, is also responsible for the main caveats of this technique.

ADVANTAGES OF PCR

Sensitivity: A few DNA copies are usually enough for detection.
Kwon & Higuchi[9] 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 fulfill 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.

Figure 10.

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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 11A and B. Understanding C_t values.

Graphical representation of real-time PCR data in an Amplification Plot (fluorescence signal versus cycle number). The blue line represents a sample lacking the target DNA, while red lines represent samples containing different amounts of the target DNA.

(A) Shows the main parameters in the amplification plot.

(B) Defines the C_t value (the PCR cycle number at which the fluorescence intersects the threshold).

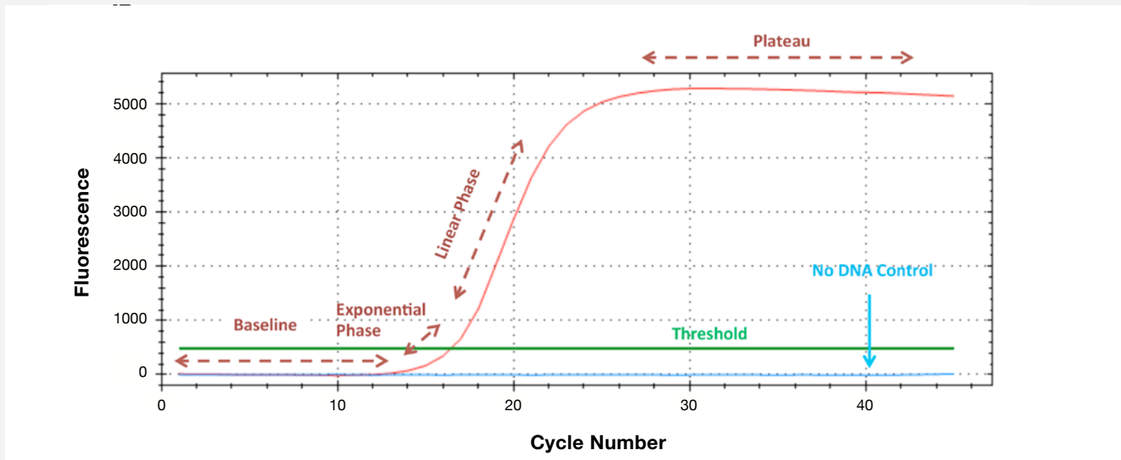

Figure 11A.

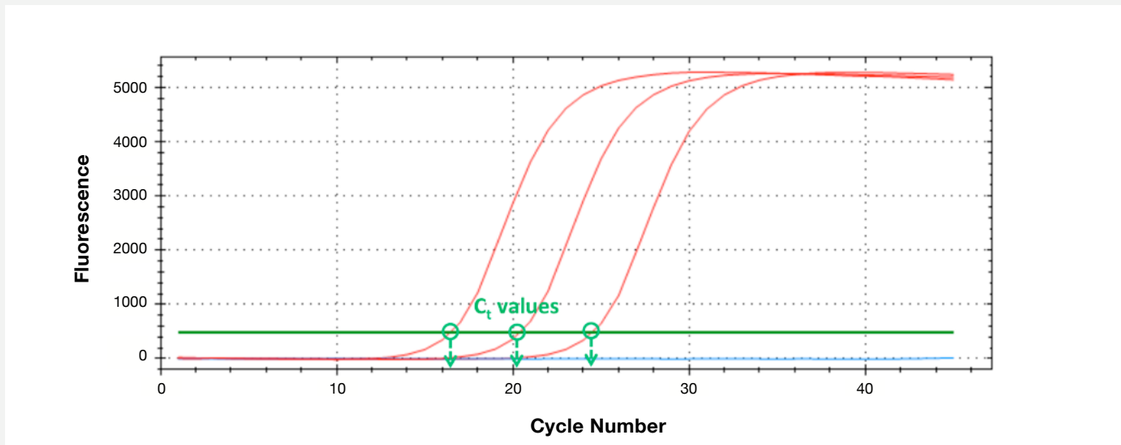

Figure 11B.

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_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_t value decreases with an increasing amount of template. In this way, the sample with the smallest C_t value (**Fig. 11B**) contains the highest concentration of target DNA. It must be noted that C_t values are relative values, and depend on the instrument and reaction conditions used. Hence, the C_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_t value of 20 contains twice as much original DNA material as a sample with a C_t value of 21. This same sample would have 4 times as much DNA as a third sample with a C_t value of 22, and so on. So in other words a lower C_t value denotes a larger amount of target DNA contained in the original sample. The samples shown in Fig. 11 are different amounts of positive controls (of known DNA concentrations) amplified in a qPCR assay that uses SYBR® Green and in this assay they are strong positives. However, the C_t values vary depending on the assay, as the background fluorescence will determine the position of the threshold. Many diagnostic tests use a PCR probe, such as TaqMan®, and these assays usually require a C_t cut-off value above which a sample is considered negative (false positive). This cut-off value represents the limit of detection of the assay, as higher C_t values are considered cross-contaminations, non-specific amplification or fluorescent artifacts. Hence, the C_t cut-off value for a particular assay is essential to assess these PCR results.

The C_t values can be used to quantitate the amount of DNA present in the sample by comparison with the C_t values of samples containing known amounts of DNA. The logarithm of the DNA concentration has a linear relationship with the C_t value. The standard curve obtained when plotting those two values can be used to calculate the concentration of DNA in samples with unknown amounts. ■

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