ANZCVS SCIENCE WEEK 2018

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Contents

Stem cell therapy in cats: What's the evidence?

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Introduction to Stem Cells

Stem cells are unspecialized cells that have the potential to self-propagate and differentiate to multiple cell types. In veterinary medicine, these are predominantly adipose-derived mesenchymal stem cells (aMSCs). MSCs can be autologous or allogenic. Autologous MSCs are derived from the patient's own tissue. Allogenic MSCs are derived from a healthy tissue donor.

Most of the beneficial properties of stem cells are due to paracrine effects, rather than specifically stimulating tissue regeneration. They have been shown to possess anti-inflammatory, anti-oxidant, anti-fibrotic, anti-apoptotic and angiogenic effects, and tend to home to damaged tissue.

How are they harvested?

Adipose-derived MSCs are generally harvested from the feline abdominal fat pad. MSCs can also be derived from bone marrow or amniotic fluids.

There are two main methods of obtaining aMSCs. Most research has centered around culture-expanded MSCs, where MSCs are derived from fat, isolated and expanded in pure culture. This results in a pure population of MSCs. MSCs can also be derived from the stromal vascular fraction (SVF) where adipose tissue is subject to enzymatic processing.

Most commercial stem cells available (including point-of-care derived products) are SVF. Most of the feline (and rodent model) studies to date have utilized culture-expanded MSCs, but there is some research currently underway investigating the utility of SVF-derived MSCs.

MSCs can be expanded from cryogenically-preserved cells, however one study found that allogenic MSCs culture-expanded from cryo-preserved cells were associated with adverse effects (vomiting and respiratory signs), especially when administered intravenously at higher concentrations. These effects were not seen when the cryo-preserved cells were culture-expanded and then administered.

It is thought that approximately 20-50% of MSCs (excepting those derived from specific pathogen-free cats) may be infected with Feline Foamy Virus (FFV) which does not result in clinical disease but may hinder culture expansion of autologous MSCs.

How are they administered?

The majority of feline studies have utilized MSCs administered intravenously, however they have also been administered locally (e.g. intra-renal, intra-renal artery, intraperitoneal, retroperitoneally, intra-articular etc). A number of studies have demonstrated that in the majority of cases, there are no adverse effects via administration of autologous or allogenic MSCs in cats (with the exception of the cases of high doses of MSCs culture expanded cells from cryo-preserved cells as mentioned above).

What studies have been done?

Chronic kidney disease

Several studies have been performed evaluating the use of stem cell therapy in cats with Chronic Kidney Disease (CKD). These studies built on a number of successful rodent model studies. However, it must be noted that the rodent models were not 'naturally occurring' forms of CKD, and MSCs were administered shortly after nephrectomies were performed (compared with slow progressive decline in CKD), and so the results cannot be directly extrapolated.

A pilot study showed modest improvements in GFR and serum creatinine following intra-renal injection of autologous culture-expanded MSCs (Quimby *et al* 2011), however no significant improvements were seen in cats receiving allogenic MSCs intravenously (Quimby *et al* 2013, Quimby *et al* 2016). It is possible that local administration may be more effective, as intravenous administration may result in trapping of MSCs in the extensive network of pulmonary capillaries.

Intra-arterial administration of autologous SVF is currently being investigated. This involves autologous SVF directly administered into the renal artery using interventional radiology techniques. This has the benefit of allowing MSCs to act locally and bypass the pulmonary vasculature. Although results of this study have yet to be published, reports indicate that initial results look promising.

Acute kidney injury
One study has evaluated the use of allogenic MSCs in an experimental model of acute ischaemic kidney injury, which did not show a significant benefit to administration of MSCs (Rosselli *et al* 2016).

Gingivostomatitis
Administration of MSCs has shown promising results in cases of refractory Feline Chronic Gingivostomatitis (FCGS), a frustrating condition thought to be largely immune-mediated. In two subsequent studies, cats with refractory FCGS (that had not responded to full mouth extractions) were administered allogenic MSCs (Arzi *et al* 2017) and autologous MSCs (Arzi *et al* 2016) resulting in a 64% (allogenic) - 71% (autologous) response rate. No significant adverse effects were noted. In these studies, autologous MSCs were more effective more rapidly (especially for very severe cases). Further work is being undertaken at UC Davis.

Chronic enteropathy
One randomized, single-blinded placebo-controlled evaluated the use of allogenic mesenchymal stem cells resulted in owner-reported improvements in the majority of cats (5 out of 7) 1-2 months after administration. (Webb & Webb 2014). One cat that was euthanased for other reasons had no evidence of GIT disease on post-mortem.

Conversely, improvements were not seen in placebo-treated cats (treated with saline) at the same time point. Although this initial study is promising, further evaluation in cats with chronic enteropathy is required.

Feline asthma
Allogenic MSCs have been evaluated in both acute (Tzril *et al* 2016), and chronic (Trzil *et al* 2014) experimental models of feline asthma. In the acute model, treated cats were found to have reduced eosinophil percentages on BAL, improvements in functional testing, and improved appearance of airway remodeling on CT scans at month 9 following treatment (Tzril *et al* 2016).

In contrast, the chronic model (where aMSCs were administered twice a month for a total of 6 doses) did not show improvements in BAL findings or functional testing. Partial improvement of remodeling on CT scan at month 8, but this effect was not sustained (Trzil *et al* 2014).

Osteoarthritis
At the time of writing, no feline studies have been published as yet. Two canine studies using autologous SVF cells injected intra-articularly showed clinical improvements in dogs with osteoarthritis.

Conclusion

At this stage, while stem cell therapy looks to be a promising treatment option for certain disease conditions in the future, there is still much unknown about optimal doses, methods of processing and administration, as well as potential risks. There is a huge body of research currently underway and clinicians should be cautious until more is known regarding safety, efficacy and optimal use of stem cell therapy.

Further reading

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Treatment guidelines for respiratory disease in cats

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The International Society for Companion Animal Infectious Diseases (ISCAID) Antimicrobial Guidelines Working Group was formed to develop guidelines for antimicrobial drug use in dogs and cats, because of concerns that antimicrobial drug resistance has dramatically increased in prevalence among isolates from dogs and cats in the last decade. The founding members of the ISCAID Working Group are Scott Weese, Joseph Blondeau, Dawn Boothe, Edward Breitschwerdt, Luca Guardabassi, Andrew Hillier, Michael Lappin, David Lloyd, Mark Papich, Shelley Rankin, Jane Sykes, and John Turnidge. It should be noted that members of the working group receive support from a variety of industry groups that provide funding for honoraria and research.

Guidelines for treatment of respiratory disease in dogs and cats were published in 2017 in the *Journal of Veterinary Internal Medicine* as an open access document (www.iscaid.org). Recommendations have been based on available data, whenever present, along with expert opinion, considering principles of infectious diseases, antimicrobial treatment, antimicrobial resistance, pharmacology, and internal medicine. The guidelines were subsequently reviewed by a group of peer experts in the field. Recommendations were voted on and published together with the level of agreement with each statement. Throughout the document, an emphasis has been placed on early identification of the underlying cause, and shortening duration of therapy as much as possible. The information below provides an update for practitioners on the current perspective of the author as part of the Guidelines Working Group. Case examples will be used to illustrate the principles outlined.

Guidelines for the treatment of respiratory disease

Acute upper Respiratory tract disease (URTD)
Clinicians should consider an observation period of up to 10 days without antimicrobial treatment for cats and dogs with acute URTD. Antimicrobial therapy should be prescribed if a mucopurulent nasal discharge is accompanied by fever, lethargy or anorexia. In the latter case, appropriate empiric therapy would be doxycycline (first choice) followed by amoxicillin (the latter is not active against *Mycoplasma* spp.). The recommended duration of therapy should be 7-10 days. Veterinarians should avoid performing culture and susceptibility (C&S) on nasal discharge from cats with acute URTD, as it usually yields commensal bacteria or secondary invaders. If 10 days of empiric antimicrobial therapy is ineffective, the need for a diagnostic work-up as described for chronic upper respiratory disease should be emphasized to the client.

Chronic upper respiratory tract disease in cats
A diagnostic work-up is recommended for cats with chronic upper respiratory signs, including imaging of the nasal cavity (preferably advanced imaging such as computed tomography) and rhinoscopy. If treatable causes of nasal discharge such as a foreign body, fungal infection, polyp, or neoplasia are not identified, then nasal lavage or brushings could be submitted for C&S testing, and a nasal biopsy could be submitted for histopathology. Treatment should be based on these results.
Should nasal discharge recur, it is recommended that the last effective antimicrobial drug be used for a minimum of 48 hours; if this is ineffective, then switching to a different class of antimicrobials should be considered, provided a diagnostic work-up to rule out other causes of nasal discharge (tumors, fungal infection, foreign bodies etc) has been performed. Continued switching of antimicrobial drugs in the absence of addressing the underlying cause typically only selects for resistant bacterial species.

Bacterial bronchitis
Airway lavage with cytologic examination and C&S testing is usually indicated if bacterial bronchitis is suspected. Endotracheal lavage should be considered. Non-bacterial causes of bronchitis should always be considered. While awaiting results of the above tests, empiric treatment is recommended with doxycycline for 7

to 10 days. If this results in clinical improvement, treatment should be continued for 1 week past resolution of clinical signs.

Pneumonia

Antimicrobial therapy for pneumonia should be initiated as soon as possible and within 1-2 hours if signs of sepsis exist. Antimicrobial therapy should be parenteral while patients with pneumonia are hospitalized. If there is no evidence of systemic sepsis, parenteral administration of a beta-lactam such as ampicillin is recommended for empiric therapy; if signs of sepsis are present, then a combination of a fluoroquinolone and a drug that targets gram-positive bacteria and anaerobes (e.g., ampicillin or clindamycin) is recommended pending the results of C&S if possible. Animals should be re-evaluated for possible discontinuation of antimicrobials no later than 10 to 14 days after starting treatment.

Pyothorax

Pyothorax in cats should be treated with IV fluids and usually requires drainage of pus after placement of chest tubes. Surgical debridement may be required. Empiric antimicrobial therapy pending the results of C&S on material obtained by thoracocentesis should be with a parenteral combination of a fluoroquinolone and a penicillin or clindamycin. It has been recommended that treatment continue for at least 3 weeks and ideally 4-6 weeks, but the optimum duration is unknown. Animals should be re-evaluated 10 to 14 days after starting treatment.

"Take home" points

Consideration should be given to withholding antimicrobial therapy for cats with acute upper respiratory tract disease without mucopurulent nasal discharges or systemic signs of illness.

1. Doxycycline is an appropriate empirical choice for cats with acute upper respiratory tract disease that is accompanied by mucopurulent ocular and nasal discharges.
2. The need for a diagnostic work-up, including imaging of the nasal cavity and rhinoscopy, should be emphasized to clients that have dogs and cats with chronic upper respiratory tract disease.
3. Appropriate empirical therapy for pneumonia is either a beta-lactam or a combination of a beta-lactam or clindamycin and a fluoroquinolone. Shorter courses of antimicrobial therapy (10-14 days) should be considered when compared with those recommended previously (4-6 weeks), based on clinical reassessment.
4. Cats with pyothorax should be treated with drainage and possibly surgical debridement, in combination with parenteral antimicrobial therapy that targets gram-negative bacteria, gram-positive bacteria, mycoplasmas and anaerobes pending the results of culture and susceptibility testing. The presence of anaerobes should always be assumed, even if they are not cultured.

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Diagnostic approach to fever in cats

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Introduction

A high rectal temperature may result from true fever or hyperthermia. Hyperthermia can result from seizures or anxiety, especially in hot weather. It can also occur as a result of drug therapy. Cats are especially susceptible to drug-induced hyperthermia, which can result from drugs such as narcotics or ketamine. Hyperthermia, rather than fever, should be suspected in animals that appear otherwise systemically well and active. The rectal temperature should be retaken after 30 minutes of being indoors in a cool, quiet environment to determine if the high rectal temperature is clinically relevant.

True fever occurs when endogenous pyrogens such as Il-1 are released by white blood cells, primarily macrophages. It can result from infection, immune-mediated disease, inflammatory disease (e.g., pancreatitis), and neoplasia. *Bacterial infections are not the most common cause of fever in cats.* In cats, fever often results from viral infection (FeLV, FIV, feline respiratory viruses, feline coronavirus). (In dogs, neoplasia and immune-mediated disease are more common causes of fever than infectious causes). Perhaps the most common causes of persistent fever in the author's hospital population are FIP or lymphoma in cats, but the prevalence of different causes of fever likely varies considerably in different geographic locations and whether the hospital is a primary or referral hospital.

Diagnostic approach to true fever

Once it is established that fever is present, because fever is a non-specific sign, the problem must then be localized. The approach is similar to an animal that evaluated for inappetence. Typically, disease that occurs in organs that drain to the exterior (the lower urinary tract, nasal cavity, oral cavity, and skin and ears) are not associated with fever, although acute upper respiratory tract infections may be associated with fever in cats. Rule outs for fever are numerous but these should be considered whenever fever is present in the absence of localizable disease (Table 1). In animals with acute illness, fever may develop a day or two before the appearance of lesions.

Table 1. Differential diagnosis for fever

Category	Causes
Infectious	Viral – FeLV, FIV, feline coronavirus, feline respiratory viruses, feline panleukopenia virus (cats). Bacterial –soft tissue cellulitis, peritonitis, pyometra, pyothorax, pneumonia, pyelonephritis, osteomyelitis, endocarditis, mycobacteriosis, ehrlichiosis, bartonellosis, plague, tularemia, leptospirosis, hemoplasmosis, and certain virulent GI pathogens, such as *Salmonella*. Foreign bodies (such as migrating plant awns) should also be considered. Fungal/Algal – especially cryptococcosis, sporotrichosis Protozoal – leishmaniosis, toxoplasmosis, cytauxzoonosis
Immune-mediated (rare in cats)	Polyarthritis, IMHA, ITP, drug reactions
Neoplasia	Leukemia, lymphoma, multiple myeloma, histiocytic sarcoma, carcinoma, sarcoma

Inflammatory	Pancreatitis, cholangitis

Localization of the underlying cause

A thorough history and physical examination is key to localizing the underlying cause. The history should include questions about exposure to the outdoors, daily activities, exposure to other animals, the health of in-contact animals, and geographic locations in which the animal spends time. Medication history (e.g., immunosuppressive drug use, response to antimicrobials in the recent past) is also important. Attention should be paid to all surfaces and parts of the body on physical examination. A thorough oral cavity examination may reveal lingual ulceration with upper respiratory viral infection. A thorough ocular (including fundoscopic) examination should be performed. Serial physical examinations (every 12 hours) may also be helpful to localize the cause of fever, because as noted above, lesions may develop over time. This is especially true for cat bite abscesses and viral respiratory infections.
If disease cannot be localized based on physical examination, a CBC, full biochemistry panel, UA and urine culture should be offered. FeLV and FIV testing should be performed in all cats with fever.

The CBC may reveal evidence of anaemia or thrombocytopenia, such as might occur with infectious diseases like hemoplasmosis, or immune-mediated diseases such as IMHA. The presence of neutropenia may be either 1) secondary to bacterial infection, in which case it is usually accompanied by toxic changes and bandemia 2) an underlying cause of secondary bacterial infection and fever (e.g., because of marrow disease), or 3) an immune-mediated problem (i.e., immune-mediated neutropenia, which is rare in cats). Findings on the biochemistry panel may help to localize the problem (e.g., azotemia or increased liver enzyme activities). If hyperglobulinemia is present, serum protein electrophoresis could be considered to evaluate for underlying hemic neoplasia (i.e., monoclonal gammopathy with multiple myeloma or lymphoma). Feline infectious peritonitis should also be considered. Bartonellosis has also been associated with hyperglobulinemia in cats. Urinalysis and urine culture are important, because they may reveal bacteriuria. Bacteriuria could indicate pyelonephritis or be a sign of bacteremia.

If pyrexia is persistent but the CBC, biochemistry panel, UA and urine culture are normal (or show non-specific changes like leukocytosis or mild hypoalbuminemia), essentially "pyrexia of unknown origin (PUO)" exists. However, in veterinary medicine, some practitioners are quick to assign the term "PUO".

In contrast, in human medicine, the following criteria are used to define PUO:

1. Prolonged fever of > 3 weeks' duration associated with vague, non-specific signs of illness

2. Temperature more than 1.5°F above normal on several occasions

3. Diagnosis uncertain after 1 week of hospitalization involving repeated physical exams and routine laboratory tests.

True PUO can be expensive and frustrating for pet owners, because the goal of further diagnostics is to "find" an abnormality. Thoracic radiographs, abdominal radiographs and abdominal ultrasound are indicated. Blood cultures (3 over a 24-hour period), and culture of bile collected by ultrasound-guided gall bladder aspiration (if liver enzymes suggest cholangitis) should also be considered. Serology and special culture for *Bartonella* and tick-borne disease could be considered, but the results may be difficult to interpret; PCR for vector-borne pathogens may be more useful if doxycycline treatment has not been administered. Other diagnostics that could be considered are fungal antigen assays (depending on regional prevalence), an echocardiogram (endocarditis), CSF analysis, and bone marrow aspiration. In the author's experience, the most common cause of true PUO in cats is viral upper respiratory disease and feline coronavirus infection. Usually pyrexia in cats either resolves spontaneously or an underlying cause is identified.

Funny feline syndromes

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Feline hyperaesthesia syndrome

Known by several names (rolling skin disease, neurodermatitis, neuritis, psychomotor epilepsy etc), feline hyperesthesia syndrome is a poorly characterized, uncommon disease of cats. In truth, any syndrome which is known by so many different names is bound to be poorly characterized and, true to form, the aetiology of FHS is not well understood. Diagnosis is achieved by exclusion of other causes of the clinical signs, which means excluding dermatological, neurological and behavioural problems.
FHS may occur in any cat, but typically affects young cats (1-5 years of age). There is no sex predilection, however Siamese, Burmese, Persian and Abyssinian cats are reportedly more commonly affected. Clinical signs include a rippling or rolling of the skin along the lumbar spine, with palpation of the lumbar musculature usually eliciting signs of pain. Mydriasis often occurs during episodes of FHS, and cats often stare at their tail then attack the tail and/or flanks. Frequently, affected cats will bite at the tail base, forelegs and paws, run wildly around and vocalize as they run. Normally placid cats may display aggression. Episodes may be induced by patting or stroking the cat's fur, or grooming.

Differential diagnoses include dermatological conditions (flea allergy dermatitis, allergic skin disease, infectious dermatitis), neurological disease (epilepsy, brain tumours, spinal cord disease e.g. disk disease, neoplasia, infectious disease), musculoskeletal disease (myositis, myopathy) and behavioural disease (compulsive disorder, displacement behaviours). Diagnosis of FHS requires exclusion of the above disorders.

Treatment of FHS may involve both behavioural modification therapy and pharmacological therapy. Behaviour modification might include identifying the trigger of the events and redirecting the cat's activity to a more appropriate behavior such as play and increasing play activity. Pharmacological therapies may include anticonvulsant therapy such as phenobarbital which is anecdotally beneficial in some cases. In others, gabapentin may be of benefit. Gabapentin is not only an anticonvulsant therapy (which, incidentally is not particularly effective in controlling seizures in cats) but also an analgesic treatment. It has been proposed that cats suffering from FHS may experience bouts of neuropathic pain leading to allodynia (sensation of pain resulting from non-noxious stimuli such as light touch). Gabapentin is frequently used for conditions associated with neuropathic pain in people (as are tricyclic antidepressants and selective serotonin reuptake inhibitors) and thus may be of use in cats with FHS. Other treatments include the SSRIs fluoxetine (0.5-2.0mg/kg PO q 24hours) and tricyclic antidepressants (clomipramine 0.5-1.0mg/kg PO q24h) and occasionally benzodiazepines (however care should be used with diazepam due to its potential to cause idiosyncratic hepatic necrosis). Randomised controlled trials evaluating treatment of FHS are lacking.

Feline orofacial pain syndrome

Described predominantly in the Burmese cat, FOPS is a condition characterized by signs of acute oral discomfort and mutilation. Affected cats usually present with exaggerated licking and chewing movements, and pawing at the mouth with severe cases developing mutilation of the tongue, lips and buccal mucosa. Most often the condition is unilateral, though it may be bilateral. Inappetence or anorexia may develop secondary to pain. Neurological examination is usually unremarkable with no apparent trigeminal nerve deficits. In cats it is thought that the disease is most likely a neuropathic pain disorder analogous to trigeminal neuralgia and/or glossodynia in humans. In most cases, discomfort appears to be relieved by anti-epileptic drugs (usually gabapentin), most likely because of their anti-allodynic rather than their anti-epileptic properties.
In a large, retrospective study of FOPS, 113 cases were reviewed. Of these, 100 were Burmese cats and the mean age of cats at time of first FOPS event was 7 years (range 0.1-19years). Nineteen of the cats presented at less than 6 months of age with their first event and all were Burmese cats, and it is thought that perhaps eruption of dentition contributes to the development of disease. The majority of cats had multiple episodes of FOPS, and mouth movements (e.g. eating, drinking, grooming) appeared to precipitate the events. In approximately one third of cases, no underlying cause (oral disease or stress) could be identified. Interestingly, two Burmese cats

with FOPS also had FHS. Review of pedigree information suggested a hereditary component to disease which is currently the subject of further investigation.

In the study discussed above, 53 cats had dental treatment performed and of these, 35 had resolution of their FOPS. It is my opinion that these cats probably did not have "true" FOPS but rather dental-related pain/discomfort. Other treatments used included NSAIDs, corticosteroids, antibiotics, opioid therapy, anti-epileptic drugs and amitriptyline. All of these had variable effect. On broad evaluation of the results, anti-epileptic drugs appeared to have greater benefit than opioids, corticosteroids and NSAIDs, suggesting that neuropathic pain plays a role in the development of disease. The authors hypothesise that as the clinical signs relate to areas receiving sensory innervation from the trigeminal nerve, trigeminal neuralgia may be the underlying cause of disease.

Cutaneous asthenia (Ehlers-Danlos syndrome; Dermatosparaxis)

Cutaneous asthenia is a rare hereditary disorder that results in altered strength and/or extensibility of the connective tissues. Most commonly, cats with EDS present with hyperextensible skin and lacerations or skin wounds that occur due to cutaneous fragility. Clinical signs usually develop within a few weeks of birth, with normal scratching and play behavior resulting in skin tears which often heal rapidly and leave scars. Other presentations include development of non-traumatic hernias, especially ventral, perineal and inguinal hernias. Rarely, joint hyperextensibility may be present. A tentative diagnosis can be reached based on clinical findings using the skin extensibility index.
Skin extensibility index = (vertical height of skin fold) ÷ (body length from occipital crest to tail base) x 100. Reference limit <19%.

Confirmation requires light and electron microscopic evaluation of affected tissue and, potentially, biochemical studies.

In humans, molecular and biochemical studies have identified 9 types of EDS. Most are caused by mutations in genes encoding fibrillar collagen or enzymes that catalyze the modification of collagen fibrils. Cutaneous asthenia has been described in DSH, DLH and Himalayan cats. Two forms have been identified – an autosomal recessive form in Himalayan and mixed breed cats caused by a deficiency of type I procollagen-N-peptidase which leads to accumulation of partially processed type I procollagen; and an autosomal dominant form believed to be fatal when homozygous. Collagen fibrils are abnormal in size and with excessive space between them, resulting in thinning of the dermis.

Interestingly, EDS has also been reported also in Burmese cats, in which a distinct clinical presentation exists. In this breed, lesions consist of necrotic eschars or atrophic alopecia which develop in the absence of skin lacerations.

EDS is not curable. Treatment may include declawing of cats to prevent injury (or use of "Catcaps" to cover the cats claws), minimizing injury by desexing affected animals (prevent injury during mating) and housing affected cats indoors (minimize risk of trauma). If skin tears occur, the wound should be sutured if possible.

Differential diagnoses for cutaneous asthenia include acquired skin fragility syndromes (e.g. that associated with hyperadrenocorticism, diabetes mellitus, hepatic disease, administration of corticosteroids, hepatic disease). These can usually be excluded with relative ease.

Hypokalaemic polymyopathy of Burmese cats

Hypokalaemic polymyopathy is a genetic disease of Burmese cats which has been reported in Australasia, Europe and South Africa. Whilst affected cats usually display signs of muscle weakness and muscle pain within the first 12 months of life, only some will show characteristic signs of hypokalaemia, such as ventroflexion of the head and neck. Recently, molecular genetic research has identified a single nonsense mutation in the gene encoding for the enzyme lysine-deficiency 4 protein kinase (WNK4) as the cause of the syndrome. This enzyme is predominantly located in the distal nephron, thus the pathomechanism of disease in affected cats is likely to be a potassium-wasting nephropathy. The disease is inherited as a highly penetrant autosomal recessive trait.

The identification of the genetic basis for disease has allowed simplification of what was once a problematic diagnosis.

Clinical signs are usually periodic or episodic, but may be incessant. Signs reflect muscle weakness due to hyperpolarization of the muscle cell membrane and myalgia, however the actual presentation varies tremendously. Cervical ventroflexion, head bobbing or nodding, adoption of a "Meerkcat-like" posture, inability to jump, hind limb paresis, a "swaying" or "crouching" gait, tremor (especially when fatigued), shifting lameness, stiff stilted gait, and pain on palpation of the muscles have all been described. With time, cats can develop a severe incapacitating weakness resulting in inability to walk or ability to walk only a short distance. Handling of cats during an attack is reported to result in pupillary dilation and claw protrusion. On evaluation, serum potassium levels of less than 3mmol/L are detected if samples are collected during the event but in some individuals, serum potassium levels have normalized by the time of sample collection.

Treatment of hypokalaemic polymyopathy of Burmese cats involves oral supplementation of potassium. Whilst potassium gluconate may be used, respected feline clinicians recommend potassium chloride tablets 300mg PO BID (of the enteric coated sustained-release formulation) as a more practical alternative to the large potassium gluconate tablets. When oral supplementation is not successful in normalizing serum potassium levels, oral spironolactone may be of benefit.

In some cats, the condition appears to resolve at around 2 years of age, while others require life-long supplementation.

Feline hippocampal necrosis

Feline Hippocampal Necrosis is a seizure disorder which has clinical signs and MRI features similar to temporal lobe epilepsy in humans. Clinical signs are characterized by complex partial seizures with orofacial involvement (salivation, facial twitching, lip smacking, chewing, licking or swallowing), motionless staring, and behavioural changes (usually development of severe aggression).
MRI characteristics of FHN include bilateral symmetrical T2- and FLAIR-hyperintensity of the pyriform lobes and both hippocampi. Histologically, hippocampal necrosis is seen, the cause of which remains unknown.

Historically, the prognosis for FHN has been considered poor however a large study of cats with FHN suggested that if treatment with anticonvulsant therapy (primarily phenobarbitone and/or levetiracetam) is pursued for sufficient time, clinical recovery may occur. In one study of 17 cases, cats only became well- controlled after 4-11 days of treatment, and normal behavior only gradually returned over 7-90days. Thus, long-term therapy is required.

Feline audiogenic reflex seizures (FARS)

FARS represent a collection of seizure patterns including myoclonic seizures, generalized tonic-clonic seizures and absence seizures. Myoclonus is a sudden involuntary jerking of a muscle or group of muscles. The defining feature of FARS include a geriatric onset (>10 years) of auditory-induced myoclonic seizures. The disease is recognized in both pedigree (predominantly Birmans) and non-pedigree breeds. In most cats with FARS, myoclonic seizures predominate with generalized tonic-clonic seizures are less frequent. Rarely, cats with FARS may have only myoclonic seizures. Absence seizures occur in less than 10% of case. Whilst avoiding certain sounds can reduce the seizures, this is often challenging for owners and thus anti-seizure medication is often required. It is proposed that frequent sounds induce myoclonic seizures that progress to generalized tonic-clonic seizures. In a recent prospective study, levetiracetam was found to be more effective than phenobarbitone in the control of clinical signs associated with FARS when pharmacological therapy is deemed necessary.

Gastrointestinal eosinophilic sclerosing fibroplasia

Feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) is a relatively recently described inflammatory disease affecting the stomach or intestines and draining regional lymph nodes. The aetiology of the condition is unknown, however lesions are defined by the presence of eosinophilic masses, largely confined to the GIT and associated lymph nodes. Grossly, lesions may be confused with neoplasia, and

histopathologically they may be confused with sclerosing mast cell neoplasia, fibrosarcoma or extra-skeletal osteosarcoma. Long-haired cats, specifically the Ragdoll, appear to be over-represented, with a possible predisposition in male cats. Most commonly cats present with chronic vomiting and/or diarrhea and weight loss, with an abdominal mass being detected on physical examination. A peripheral eosinophilia is usually present. Cats are usually middle-aged (median age 7yrs, range 2-11years).

A bacterial cause for the condition has been investigated. A recent study used cytology, FISH, special staining techniques, and/or culture and identified bacteria in 9/13 cases. However, there was no consistency of bacterial species involved amongst cases.

Historically FGESF has been given a poor prognosis, however the majority of cases were euthanased at the time of surgery (on suspicion of neoplasia) or due to incorrect diagnosis. A recent study identified prolonged survival time after complete surgical resection +/- prednisolone therapy. The authors of that study recommend routine use of antibiotics given the high percentage of lesions where bacteria were detected, with amoxicillin-clavulanate and metronidazole being appropriate choices given the bacteria identified.

Plasma cell pododermatitis

Plasma cell pododermatitis ("spongy footpad disease") is a rare skin disease affecting cats. Although the aetiopathogenesis is unknown, persistent hypergammaglobulinemia, marked plasma cell infiltrates and a beneficial response to glucocorticoid therapy suggests an immune-mediated basis. Affected cats usually present with soft, non-painful spongy swelling of multiple footpads which may become ulcerated causing pain and lameness. Histologically, a severe inflammatory infiltrate composed of mature plasma cells in a perivascular pattern is seen. When ulceration is present, large numbers of neutrophils and macrophages, as well as vasculitis, may be seen. No sex, age or breed predisposition has been described for plasma cell pododermatitis. Whilst spontaneous remission can occur, some cases appear seasonal and others respond well to medical or surgical therapy. Traditionally treatment involved use of immunosuppressive doses of corticosteroids or wide surgical excision. However, more recently doxycycline therapy has been reported to produce partial or complete remission of lesions in more than 50% of cases. This may be due to the immunomodulatory effects of doxycycline or may indicate an underlying infectious aetiology. One study evaluated the possibility of an infectious cause of disease using PCR and/or immunohistochemistry to detect a range of pathogens (*Mycobacterium bovis, Bartonella* spp, *Erhlichia* spp, *Anaplasma phagocytophilum, Chlamydia felis, Mycoplasma* spp, *Toxoplasma gondii* and FHV-1), but found no evidence of infectious disease in any of the tissue samples from 14 cases. It is my preference to perform a treatment trial with doxycycline and only commence prednisolone therapy if the lesions do not resolve. Surgical excision is reserved for only the most severe lesions which do not respond to medical management.

Mosquito-bite hypersensitivity

Mosquito-bite hypersensitivity is one of those conditions where pattern-recognition is your friend. As would be expected, affected cats usually present during the summer months in warmer, moist climates. Lesions usually affect the nose and ears, however the pads of the feet may also be involved. Lesions are of crusting, scaling and ulceration. When the pads are affected they are usually thickened and swollen, tender and red and may have fissures present. Occasionally, pyrexia and lymphadenomegaly may also be present. Histopathologically, inflammatory infiltrates (eosinophilic and neutrophilic) predominate. A diagnosis is reached by exclusion of other causes such as dermatophytosis, FHV-1 ulcerative dermatitis, SCC, bacterial infection, eosinophilic plaques and by improvement of the lesions when the cat is confined to a mosquito-free environment. Lesions will usually resolve within 7-10days of confining cats to a mosquito-free environment or use of repellents. However, in severe cases, or where exposure to mosquitos will occur, corticosteroids may be required.

Lung-digit syndrome

Lung-digit syndrome is the name given to the unusual metastatic pattern of primary lung tumours in the cat (notably bronchogenic carcinoma). Cats are the only domestic species in which lung-digit syndrome, i.e. metastatic spread of a primary pulmonary neoplasm to the digit, has been described. However, a similar

syndrome of subungual metastasis of primary lung tumours occurs in humans. Studies have shown that approximately 75% of amputated feline digits submitted for histopathology are affected by neoplasia, and approximately 20% by metastatic lung tumours. Most commonly, tumour metastases occur in the distal phalanx of the weight-bearing digits, with multiple-digit and multiple-limb involvement common. Importantly, cats with primary lung tumours often present NOT with clinical signs related to the pulmonary disease, but due to signs referable to metastases, namely lameness.

As would be expected, cats with lung-digit syndrome are usually middle-aged (mean age at presentation 12yo). There is no sex or breed predilection. The affected digit usually appears red and swollen, there is often a purulent discharge from the nail bed, and dysplasia or exsheathment of the associated nail is common. Lesions are often mistaken for paronychial infection. However pattern recognition should alert the clinician to the fact that any middle-aged to older cat presenting with such a lesion should have investigation for primary lung tumour performed.

Radiographs of the affected digit reveal bony lysis of the distal phalanx, and the lysis may "cross the joint", raising the index of suspicion for metastatic disease. In any case, thoracic radiographs are indicated prior to amputation of the affected digit. Prognosis for cats with lung-digit syndrome is poor, with mean survival time approximately 2 months. Amputation may be palliative.

Cats with primary lung tumours infrequently present with clinical signs referable to the primary lesion. Other sites of metastasis include skin, eyes, skeletal muscle, bone, and even the aortic trifurcation resulting in clinical signs mimicking aortic thromboembolism. A recent retrospective study of feline lung-digit syndrome performed by Australian colleagues identified a number of cats with metastasis to the skeletal muscle, suggesting this is perhaps more common than the existing literature would suggest.

Feline nutrition: A clinician's perspective

Sue Foster

Introduction

Recent research into instinctual diets using nutritional geometry in a controlled environment has shown cats select 52% of their metabolisable energy (ME) from protein, 36% from fat and 12% from carbohydrates. The natural diet of feral cats consists primarily of small animals birds, fish, reptiles and invertebrates with a macronutrient profile of 52% ME from protein, 46% ME from fat and 2% ME from carbohydrate (Plantinga et al 2011). Thus the instinctual dietary preference of domestic cats closely resembles the nutrient composition of cats in the wild (Hewson-Hughes et al 2011).

Whether cats have evolved from their ancestral requirements is not clearly established thus it is unknown whether this distribution of macronutrients provides optimal nutrition. However, it is interesting that the preferred macronutrient levels are substantially different than minimal requirements recommended by the NRC (Rutherfurd-Markwick et al 2013). As it would appear that cats have retained much of the dietary preference, behavioural attributes and physiological digestive function as the wild species (Buff et al 2014), this does pose the questions:

How do we formulate processed diets to provide an adequate diet for cats?

Have we got it right?

Proponents of commercial cat food usually point to a decrease in diseases of nutritional excess (hypervitaminosis A) or deficiency (secondary nutritional hyperparathyroidism) to support use of commercial foods. Watson (1994) searched hospital records for dogs and cats and calculated a 75% decline in nutritional secondary hyperparathyroidism, hypervitaminosis A and thiamine deficiency between 1975-1993. This reflects a substantial improvement in these diet-related medical conditions. However, it is important to note that diets resulting in those conditions would not have fitted the profile of an ancestral diet: meat diets without bone for nutritional secondary hyperparathyroidism, addition of sulphites to meat or a diet comprised of mainly fish for thiamine deficiency and feeding liver in high quantities or exclusively for hypervitaminosis A. It is also important to note that it is easy to assess improvement in known straightforward dietary-related conditions but far more difficult to detect increased or decreased incidence of medical diseases where the contribution of diet is more subtle. Any improvement in medical diseases due to commercial diets needs to be balanced against the emergence of potential adverse conditions but this data is rarely present.

Proponents of a raw meaty bones diet (RMBD) try and provide a diet that mimics an ancestral diet. They claim numerous health benefits, none of which, apart from reduced dental calculus (Clarke and Cameron 1998, Marx et al 2016) seem to have been conclusively demonstrated. They can also be reluctant to accept that adverse effects such as pathogenic bacterial contamination of these diets are possible (Freeman et al 2013). In addition, to decrease potential issues with toxoplasmosis, RMBD for cats should be frozen for 48h or more before feeding (Foster 2016), which undoubtedly alters some components of those diets.

Whilst some practitioners have been vocal advocates for RMBDs, the profession has, by and large, tended to trust the nutritionists and the commercial food companies who invariably employ them and/or fund their research. There is little doubt that in most first world countries and many developing countries also, that commercial pet food is now the mainstay of nutrition for cats. The major veterinary associations of the world have endorsed this and in the United States, American Animal Hospital Association, American Veterinary Medical Association (AVMA) and the Food and Drug Administration (FDA) have all issued statements on the avoidance and safe handling practices of raw foods. AVMA and the American College of Veterinary Nutrition also endorsed a publication on the potential risks versus benefits of pets consuming raw meat based diets (Freeman et al 2013). In addition, a recent Australian study (Martinez-Anton et al 2018) that concluded raw chicken is associated with acute polyradiculoneuritis in dogs, is now being used as further evidence of the risks

of raw food despite the fact that 30% of the enrolled dogs in that study did not meet the study's own inclusion criteria.

Feline nutrition is a complex and controversial topic and one that needs more than a cursory review or single lecture but finding an independent person with the adequate nutritional knowledge and science to review the enormous current and historic body of literature is difficult. There has been much written on the disadvantages of raw diets (Freeman et al 2013) but there are seemingly few reviews on proven or potential adverse effects of commercial cat food on feline medical conditions and health. This is just one internist's incomplete overview of potential issues with commercial cat food.

Periodontal disease

In response to major controversy in the 1990s in Australia, with RMBD lobbyists claiming that commercial diets result in periodontal disease, a university academic with no affiliations with the pet food industry undertook a review of the literature. Watson (1994) found reasonable evidence that soft diets were associated with increased frequency and severity of periodontal disease and that harder foods requiring vigorous prehension and mastication are preferable for dogs and cats. He recommended that pet owners should pay attention to the physical qualities (texture, abrasiveness, chewiness) of foods they provided for dogs and cats as well as to their nutrient content. One of his recommendations was to include raw bones in diets.
Despite the claims that periodontal disease was causally linked to various diseases (Lonsdale 1995, Lonsdale 2001), evidence for a causal association between periodontal disease and other disorders was found to be limited (Watson 1994). However, Watson (1994) commented that "as periodontal diseases may be the most common disease seen in small animal practice and its effects on gums and teeth can significantly affect a pet's health and well-being, there is already reason for concern and ….proof of additional systemic effects is not needed to justify action." He went on to comment that "The author believes a prima facie case exists for implicating soft food diets in the aetiology of periodontal disease."

Four years later, an Australia study funded by the Pet Food Industry Association of Australia found that dental calculus scores were significantly higher in domestic cats than in feral cats but there was no statistical difference in the prevalence of periodontal disease between the two groups (Clarke and Cameron 1998). The criteria for the calculus score and the scores for each cat were tabulated but there were no criteria provided for the periodontal disease scores and no raw data provided. There was no data on age apart from study cats being adult. There was also no data on general health status for the domestic or feral cats and it was likely that the cats were sourced from two different locations in Australia. Regardless, neither this study nor Watson's study were referenced in the American College of Veterinary Nutrition-endorsed paper discussing risks and benefits of raw meat-based diets for dogs and cats (Freeman et al 2013).

Immunology

It has been claimed that commercial food is linked to immunologic dysfunction. Lonsdale's study, published in the *Journal of Small Animal Practice* (1995) using a small number of cats was significantly flawed and attracted widespread and justified criticism. However, a rigorous study that was conducted to examine the potential benefits of dietary supplementation on the feline immune system (Rutherfurd-Markwick et al 2013) had interesting findings that did not receive much attention or discussion in the paper itself. Forty three cats (8 or 9 per group) were fed a low protein control diet (22.7% DM basis), the same diet supplemented with yeast-derived nucleotides, salmon oil or l-arginine or a commercial moist high protein diet (53.0% DM basis) for a period of five weeks. The low protein diets were formulated using a commercial moist diet base with added fat and starch and fed ad libitum, along with water. Supplementation with arginine caused a significant enhancement of lymphocyte proliferative responses to the T-cell mitogen, phytohaemagglutinin, after 35 days, while supplementation with either nucleotides or salmon oil resulted in significant enhancement after both 14 and 35 days. Dietary supplementation with arginine, nucleotides or salmon oil each led to significant increases in blood leucocyte phagocytic activity after both 14 and 35 days. The study concluded that a number of dietary ingredients have the ability to modulate the immune system of healthy cats possibly resulting in a greater ability to fight infection and disease.

The low protein diet which had 22.7% crude protein on a DM basis was made by adding additional fat, pre-gelatinized starch, minerals and vitamins to a highly palatable moist commercial diet (which had passed a minimum feeding protocol for proving an adult maintenance claim for a cat food (AAFCO, 2012)). However, the low protein diet still contained 51.7g crude protein per kcal ME which is higher than the minimum recommended crude protein allowance for a maintenance diet (50.0g/kcal ME) as specified by the NRC (2006). Thus, cats on a diet that actually met NRC requirements for protein, were shown to have much poorer immunity than supplemented cats. The high protein diet fared better but the changes observed were not as large as those observed for cats fed the supplemented low protein diets. The authors concluded that for future experiments, there is no need to use a low protein diet for a baseline control as the high protein diet (53.0% protein DM basis) would be suitable. The obvious conclusion from this is that a high protein, complete commercial diet is not optimal for immunity and any improvements should be assessed against what is essentially a "baseline" diet. Many of the improvements that were seen with supplementation were discussed in light of favourable comparisons to feral diet composition. The authors made no comment on the adequacy of commercial diets but it is clear from this research that routine commercial diets may not provide for optimal immune function in cats.

Hyperthyroidism

Feeding of commercially prepared cat foods is considered a major potential risk factor for development of thyroid pathology and hyperthyroidism in cats (Peterson 2012). There are a number of diet-related candidates that could have a role including soy isoflavones, dietary iodine and thyroid disruptors. All epidemiologic studies reported to date have identified that feeding an increased proportion of canned cat food in the diet is a risk factor for developing hyperthyroidism (Peterson 2012).
Soy isoflavones, well known goitrogens, may be present in cat food and short-term feeding of soy has a measureable effect on thyroid hormone homeostasis. It has been proposed that cats might develop goiter on soy-based diets if those diets also have low iodine content (Peterson 2012). However, as van Hoek et al (2014) commented, measurable isoflavones are present in more dry diets than wet diets, thus if soy isoflavones are a risk factor, it would contradict the proposed increased risk of hyperthyroidism in cats fed canned foods.

Dietary iodine is another candidate. Iodine content of cat food is extremely variable inter- and intra-manufacturer (Peterson 2012). In the mid-1980s, shortly after hyperthyroidism was first reported, most commercial cat diets were found to contain very high amounts of iodine. Subsequently, the recommendations for iodine supplementation of cat food diets were lowered because of the concern that iodine may contribute to the development of hyperthyroidism. Between the 1980s and early 2000s, iodine concentrations appear to have ranged between non-detectable and extremely high levels in a variety of canned foods (Peterson 2012). The most recent study to address this issue (Edinboro et al 2013) compared American cat foods from 2008 to 2009 and found dramatic variation among canned foods (resulting in ingestion of approximately 49–9639 μg iodine/day). The authors suggested that the disparity in iodine concentrations may lead to development of nodular hyperplasia and, later, clinical hyperthyroidism, if cats consume diets that are at first iodine-deficient and later contain excessive iodine (Edinboro et al 2013). Consistently low iodine diets could also predispose to thyroid hyperplasia and goiter (Peterson 2012).

Thyroids disruptors in diets such as bisphenol A (BPA) have also been considered to have a potential role. BPA is a key component of the epoxy resins commonly used for lining the interior of metal cans. This thin epoxy coating helps prevent corrosion of the can and makes it possible for food products to maintain their quality and taste, while extending shelf life. Cat foods have been found to contain measurable levels of BPA and in one study, it was confirmed that the BPA in the food had originated from the can coating (Peterson 2012). Thus Peterson (2012) surmised that feeding canned cat food may pose a greater risk than feeding food from pouches or sachets especially if using larger cans that have more BPA than small cans. One of Peterson's suggestions to reduce risk of hyperthyroidism in cats was to use home-cooked food (Peterson 2013).

Seemingly, in response to the review by Peterson (2013), the European pet food industry federation (FEDIAF) requested a review of food-associated factors proposed in the aetiology of feline hyperthyroidism (van Hoek et al 2014). Two of the three authors were employees of Royal Canin at the time of writing. Whilst their "critical review" was meticulous with respect to analysing flaws in previous studies, their own study was not without

flaws. For example, the statement that "70% of European owners follow a mixed feeding regime for their cats and this applies for all life stages" was unreferenced despite being used to discuss reliability of retrospective studies assessing canned food causality and association. In addition, a subtle but significant bias was detectable. For example, whilst all five epidemiologic studies on hyperthyroidism may have had limitations, the fact that five separate studies (with different limitations) found an association between hyperthyroidism and canned food was downplayed and, in fact, not discussed.

It is worth noting that raw meat diets have been recently implicated in causing reversible hyperthyroidism in dogs (Zeugswetter et al 2013, Köhler et al 2012) due to high thyroid content in the diets.

Obesity and Diabetes mellitus

The role of commercial cat foods in diabetes and/or any protective function of more natural diets, in the development of feline diabetes has not been addressed. Whilst it is likely that obligate carnivores do not thrive on the high carbohydrate content of commercial diets, there have not been rigorous studies of dietary risk factors for feline diabetes. A recent systematic review of diabetic remission (Gostelow et al 2014) found that dietary carbohydrate reduction might be beneficial, but required further study. Significant flaws were found in all the studies on diabetic remission and it was concluded that a lack of well-designed trials prevents reliable remission rate comparisons (Gostelow et al 2014).
What is known, is that one of the major risk factors for the development of diabetes in cats is obesity (Nelson and Reusch 2014). It has been shown that obese cats are 3.9 times more likely to develop diabetes mellitus compared with cats with an optimal body weight (Scarlett and Donoghue 1998). In addition, a number of experimental studies in healthy cats have demonstrated decreased insulin sensitivity with weight gain, for example in one study, each kilogram increase in weight led to approximately 30% loss in insulin sensitivity (Nelson and Reusch 2014).

In analysing obesity, Zoran and Buffington (2011) wrote that the decrease in energy expenditure after gonadectomy when promoted by environments with reduced opportunities for physical activity may increase the risk for development of obesity. When these events are combined with the ready availability of highly palatable, energy dense diets and free-choice feeding, obesity in a large number of indoor cats is the predictable result. They concluded that it is clear that the current approach to diet and feeding management of cats is not optimal. New feeding approaches (diet composition as well as feeding strategies) may help stop the epidemic of obesity, but such new approaches currently are unknown.

Feline Lower Urinary Tract Disease

This is far too complex an issue to discuss in detail. Suffice to make two comments:

a) Reputable commercial diets that were strongly recommended by veterinary lecturers in the late 1980s are now condemned for their role in feline lower urinary tract disease (Adams L, FASAVA 2013)

b) Calcium oxalate uroliths and ureteroliths of any composition were very uncommon in Australia in the 1980s and 1990s. Both seem to be increasingly diagnosed in Australia in the last ten years. Whilst failure to diagnose ureteroliths may reflect availability of high quality diagnostic imaging, it is significant that even with high quality diagnostic imaging in the mid to late 1990s, there were very few cases reported. In addition, in referral centres, necropsies were commonly performed routinely throughout the 1980s and 1990s so ureteroliths and any resultant hydronephrosis should have been evident. Diets formulated to contain higher protein, sodium, potassium, moisture, calcium, phosphorus, and magnesium contents and with decreased urine acidifying potential may minimize formation of calcium oxalate uroliths in cats. Diets formulated to contain higher fat content and lower protein and potassium contents and with increased urine acidifying potential may minimise formation of magnesium ammonium phosphate uroliths (Lekcharoensuk et al 2001). One has to question whether the change from the "80s diets" to reduce struvite issues has predisposed to the current increased incidence of ureteroliths and calcium oxalate uroliths in cats. It is concerning that in a study of chronic kidney disease using a colony of over 400 cats historically used in palatability studies by Hill's Pet Nutrition Inc (Topeka, KS), all of which were on complete diets (some commercial, some non-commercial), 5 cats (greater than 1%) had calcium oxalate stones (Hall et al 2014).

One thing for sure is that this is a constantly researched and evolving area of feline nutrition. It is very possible that lecturers 20 years on again, will be as critical of our efforts in 2018 as experts in 2018 are of the efforts of the 1980s and 1990s.

Inflammatory Bowel Disease

Protein intake appears to play an important role in gastrointestinal tract disease. Dietary protein can result in gastrointestinal disease in cats due to food intolerance or food allergy. However, the situation appears to be far more complex. For example, analysis of evidence suggests that inflammatory bowel disease results from an immune-mediated disorder initiated by alterations in the intestinal microbiota. Evidence from studies of the intestinal microbiota of cats has identified a significant influence of diet (specific nutrients) on the number and species of bacteria present in the gastrointestinal tract and demonstrated that these are altered in inflammatory bowel disease (Zoran and Buffington 2011). It has been suggested that both dry food diets (moderate amounts of protein and moderate to high amounts of carbohydrate) and canned diets (increase and qualitative difference in the immunogenicity for certain proteins compared with those of unprocessed diets) may be associated with promotion of a less-than-ideal microbiota in the cat (Zoran and Buffington 2011).
The digestibility of the nutrients (and particularly protein and carbohydrate) in the diet may also be a key issue because undigested foods can become nutrients for pathogenic bacteria in the gastrointestinal tract as well as serving as antigens (Zoran and Buffington 2011). One cross-over study in kittens showed that there was significantly higher digestibility of dry matter in two commercial raw diets compared to one commercially available canned heat-processed diet (Hamper et al 2015). The commercial diet and one of the commercial raw diets (pre-frozen) were formulated to meet nutritional profile levels for cats at all life stages. The diets differed in macronutrients with both raw diets having significantly higher organic matter, crude protein and gross energy than the commercial canned diet. Whilst it is not known whether the difference in digestibility was due to the different macronutrient proportions, different ingredients, a processing effect or alterations in gut flora due to the any of these factors, the finding itself is most concerning, namely two commercial raw diets had improved nutrient levels and better digestibility than a complete processed diet.

At this stage, the exact role of commercial diets in the development of inflammatory bowel disease is unknown and considerably more research is required.

Nutritional Deficiency

The incidence of obvious nutritional deficiencies on poorly formulated home-prepared diets has declined since the onset of feeding commercial cat foods (Watson 1994). However, commercial diets are also not immune from dietary deficiency issues, with the most serious historical deficiency from commercial diets, being that of taurine. Cats are obligate carnivores with an absolute dietary requirement of taurine. In a study reported in 1987, all cases of dilated cardiomyopathy (DCM) in cats presented to one hospital were taurine-deficient (Pion et al 1987). In addition, echocardiographic measures of cardiac function returned either to normal or near-normal following 2-3 months of taurine supplementation. It was discovered that the cause of the taurine deficiency was nutritional.[1] Dry cat foods contained too little taurine while the taurine in canned foods was not biologically available in adequate amounts. This was quickly remedied by the cat food manufacturing industry adding more taurine to their dietary formulations. Subsequently the prevalence of feline DCM decreased to <10% of pre-1987 levels.[2] Whilst only some cats are susceptible, thus other factors likely to be involved, this dietary deficiency on supposedly "complete" commercial foods highlighted the fact that diets are only as "complete" as the knowledge available at the time. It was also a very obvious deficiency and Zoran and Buffington (2011) note that protein deficiency issues, unless extreme and causing specific illness, may develop insidiously.

[1] Dilated cardiomyopathy. See: http://www.vetmed.ucdavis.edu/vmth/small_animal/cardio_kittleson/cases/case32/text.htm Accessed 20 May 2018

[2] Dilated cardiomyopathy. See: http://www.vetmed.ucdavis.edu/vmth/small_animal/cardio_kittleson/cases/case32/text.htm Accessed 20 May 2018

The argument that commercial foods are nutritionally complete and that deficiencies only occur on home-prepared diets is also not always true with respect to thiamine either. Thiamine deficiency has traditionally been associated with non-commercial diets including pet meat. However, a relatively recent study found that thiamine concentrations in 13% of the 90 canned diets assessed were below the minimum level required by AAFCO and 15.6% were below the NRC recommended allowance (Markovich et al 2014). In 2017, thiamine deficiency was also detected in a commercial cat food line sold in Australia. Iodine deficient commercial diets have also been demonstrated (Edinboro et al 2013) as previously discussed. Additionally, an Australian study that showed 9 of 20 cat foods did not adhere to their 'guaranteed analysis', eight did not adhere to the standards for nutrient composition, and that various deficiencies and excesses of crude protein, crude fat, fatty acids and amino acids were observed in the majority of the cat foods (Gosper et al 2015). These contemporary reports of absolute nutritional deficiency occurring in "complete" commercial diets indicate that cats on commercial diets are not immune from deficiency issues even today. It may also be, that in future years, further research into feline immunology (eg such as supplementing to formulate diets closer to that of feral and ancestral cats), diet digestibility or geriatric health, will demonstrate that "today's diets" are deficient for optimal or even perhaps normal feline health and function.

Nutritional excess

Classically, nutritional excess syndromes in cats are restricted to those occurring on poorly formulated home-prepared food eg hypervitaminosis A. However, in both Australia and United Kingdom, hypervitaminosis D resulting in various signs such as muscle weakness and renal azotaemia, is being increasingly reported from "commercially complete diets" (Foster 2012, Wehner et al 2013, Simpson and Agnew 2017). It is even possible that some of the cases of hypercalcaemia that have been attributed to idiopathic hypercalcaemia may be due to hypervitaminosis D as testing for Vitamin D is often not performed by general practitioners in such cases.

Obesity, as previously discussed with diabetes, is another issue of nutritional excess. Obesity has also been related to disease other than diabetes so is a health risk in its own right (Scarlett and Donoghue 1998). Specifically, there is a link between obesity and lameness, presumably due to osteoarthritis.

Toxicity

Small animals are particularly susceptible to any diet-related toxicity issues as they are often restricted to a small number of food sources. Numerous pet food-associated diseases have been reported worldwide including proximal renal tubulopathy, aflatoxicosis, ionophor toxicity and melamine-cyanuric acid toxicity. Some of these have affected large numbers of pets. For example, in the 1990s in the Netherlands, more than 800 cats were paralysed by ionophor-contaminated cat food (van der Linde-Sipman et al 1999). This number was relatively insignificant when compared to the number of dogs and cats that developed pet food-associated renal failure due to melamine and cyanuric acid: more than 6,000 dogs and cats in Asia in 2004 and an estimated 39,000 dogs and cats in North America in 2007 (Osborne et al 2009, Yamka 2018 pers comm). The same toxicity was ultimately responsible for considerable human mortality in Asia, particularly Chinese infants fed adulterated milk replacement products.

In Australia, acquired proximal renal tubulopathy was reported in dogs fed jerky treats (Thompson et al 2013) and a cat which ate a large quantity of a dried fish treat also developed acquired and reversible proximal tubulopathy (Foster, unpublished). However, the most serious dietary toxicites in cats in Australia occurred in cats fed Orijen cat-food (Champion Petfood, Canada) and cats fed Best Feline Friends cat food (Weruva, USA).

Between June 2008 and March 2009, 87 cats in Australia developed symmetrical hindlimb ataxia, paraparesis, tetraparesis, paraplegia or tetraplegia in association with eating Orijen cat-food, a seemingly reputable imported, dry pet food (Child et al 2009). Diffuse leucoencephalopathy was found at necropsy, similar to that reported in a group of cats fed irradiated food. Diligent investigation by Child, identified that irradiation performed by the Australian Quarantine and Inspection Service was the likely cause of this serious toxicity.

Between March and May 2017, hundreds of cats in Australia developed unusual clinical signs. An association

with the consumption of some lines of Weruva cat-food was identified. The extent of the epidemic was halted by the quick actions of the retailer to recall all food after only three cases. The cats exhibited unusual neurologic signs predominantly cerebellar (first syndrome), pyrexia, body cavity and joint effusions (a second "syndrome") and/or gastrointestinal signs (a third "syndrome"). Numerous cats died or were euthanased, due to the illness itself or owner finances. The cause of the various clinical syndromes has not been identified but many of the affected cats had significant blood and hair mercury concentrations. Some of the foods were found to exceed the European Union and US FDA acceptable limits for mercury. Many of the foods were also found to be deficient in thiamine. Investigation into this complex and multifactorial toxicity is continuing.

Conclusion

It is simplistic to suggest that because commercial diets have reduced a few obvious nutritional disorders that occurred on home-prepared diets, that commercial diets are necessarily better for cats. Analysis of the true impact of commercial diets on feline health is far from simple and is probably not currently possible. There are likely complex genetic, epigenetic and environmental factors that interact with diet to result in adverse effects. However, analysis of the available data suggests that although meeting nutritional needs is necessary for maintenance of the health and well-being of cats, it alone is not sufficient to assure a healthy existence. As suggested by Zoran and Buffington (2011), researchers and clinicians should be urged to take a broader view to provide the best solution possible for this carnivore we have moved indoors.

Many "complete" diets from the 1980s would be considered inadequate today. In addition, many of the recommended complete diets from the 1980s and early 1990s are now highly criticised for their role in feline diseases. It behoves all veterinarians to realise the "complete" and "recommended" today may well not be "complete" and "recommended" in future decades. It is probably a sensible recommendation that cats fed commercial diets be provided with a wide range of different brands and flavours to avoid unforeseen nutritional or toxicity issues that could occur as a result of feeding a single food type. It is also more important than ever before that veterinarians record any potential adverse effects of any diet on the AVA website PetFAST (http://www.ava.com.au/petfast).

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Computed tomography of the liver

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Hepatic computed tomography (CT) is very useful when looking for primary or metastatic neoplasia, but there are a number of other indications for hepatic CT. Identification and characterization of portosystemic shunts is readily achievable with CT. Reports of CT evaluation of other disease states such as diffuse hepatopathy and biliary disease are less common, but CT may also have utility in these cases.

The canine liver is normally isoattenuating to the spleen, with individual variation ranging from 65-85 HU. Hepatic contrast enhancement should be uniform, although it may be mildly patchy in the arterial phase. The gallbladder is hypoattenuating to the liver (fluid attenuating) and has uniform contents. Because the liver has a dual blood supply, with 20% of flow coming from the hepatic artery and 80% from the portal vein, multiphase angiography is useful for characterizing hepatic disease.

Portosystemic shunts

Portosystemic shunts are anomalous communications between the portal venous system and the systemic venous circulation. Because the caudal vena cava has lower pressure than the intrahepatic capillary beds normally fed by the portal vein, blood will flow through the shunting vessel toward the vena cava and away from the liver (hepatofugal flow). Portosystemic shunts may be congenital or acquired. Congenital shunts may be intrahepatic or extrahepatic and are usually single communicating vessels. Acquired portosystemic shunts occur secondary to portal hypertension and are seen as numerous small extrahepatic vessels.

Prior to more common usage of CT for diagnosis of portosystemic shunts, ultrasound was often used for the diagnosis. Reported accuracy of ultrasound is highly variable, with 47-95% sensitivity and 67-100% specificity – sonographer experience certainly plays a role. One study directly comparing CT angiography to ultrasound for detection and characterization of portosystemic shunts found that CT was considerably more sensitive than ultrasound for detecting shunts (96% vs. 68% sensitivity) and was also better for characterization of the origin and insertion of the shunting vessel.[1] CT angiography has also been found equivalent to mesenteric portovenography for identification of extrahepatic shunts but better for characterization of the regional anatomy.[2] Advantages of CT angiography relative to other modalities include fast scan times; good spatial, contrast, and temporal resolution; and lack of anatomic superimposition. Use of multiphase CT angiography is helpful for evaluation of the various vascular beds, though there are different means of achieving this.[3]

Extrahepatic portosystemic shunts are large vessels which are generally readily seen with CT angiography. The portal vein diameter decreases immediately cranial to the origin of the aberrant vessel. The sites of origin and insertion of congenital shunts are highly variable, with the most common morphologies including left gastro-caval (previously known as splenocaval), left gastro-phrenic, and left gastro-azygos.[4-6] Intrahepatic portosystemic shunts are similarly large and usually solitary, and these are generally characterized as right-, central-, or left divisional based on the location within the liver. Urate nephroliths or cystic calculi are commonly identified with CT in patients with congenital shunts.

Acquired portosystemic shunts are often identified as collections of numerous small tortuous vessels which, if small enough, may look more like an ill-defined blush of contrast medium rather than discrete vessels. Shunting through a gonadal vein directly to the caudal vena cava or via the left renal vein is common, as is development of other varices,[7,8] and many patients with acquired shunts have ascites.

Diffuse hepatic disease

Numerous disease processes diffusely involve the liver, with frequently seen processes ranging from hepatic lipidosis and steroid hepatopathy to cholangiohepatitis and round cell neoplasia. Unfortunately, diagnosing these conditions with imaging has historically been challenging. Although ultrasound is commonly used to examine the liver, ultrasonography has very poor accuracy (to put it nicely) when it comes to trying to differentiate diffuse hepatic diseases and conditions, or even normal livers from diffusely abnormal livers.[9-11] While CT may

be able to provide information about the liver, there are currently no studies assessing use of CT for characterization of diffuse hepatic disease in veterinary medicine. Similar to what is described in human medicine, CT can be used to identify hepatic lipidosis, as fat deposition in the liver will cause a reduction in parenchymal attenuation.[12] However, CT was not found to be useful in identifying patients "at risk" for ultimately developing hepatic lipidosis.[13] Thus, further work would be necessary to determine if CT may be useful for identification of other diffuse processes.

Biliary disease

A gallbladder mucocele is an accumulation of mucus and inspissated bile within the gallbladder leading to gallbladder distension, which may lead to biliary obstruction or gallbladder rupture. Ultrasound is commonly used to diagnose gallbladder mucoceles, with multiple different patterns described.[14] Patients with a mucocele and gallbladder rupture are nearly 3x more likely to die than those without gallbladder rupture, but ultrasound had only 56% sensitivity for identification of gallbladder rupture.[15] Thus, CT may provide some benefit in identifying patients with gallbladder rupture: one report of two cases states that CT could identify a gallbladder rupture as a wall defect could be seen and fluid extended from the gallbladder directly to the perihepatic region.[16] Data are limited, though, and accuracy of CT for diagnosis of gallbladder mucocele or biliary rupture has not been evaluated in veterinary medicine.

Final thoughts

CT is very useful for identification and characterization of both congenital and acquired portosystemic shunts. While CT could also be beneficial for examination of diffuse hepatic disease and biliary disease, especially in light of the limitations of ultrasound, data in support of this are quite limited at this point.

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Computed tomography of the thorax

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Thoracic computed tomography (CT) is used for a number of different indications, including assessment of patients with respiratory signs, further characterization of pathology identified on survey radiography for surgical planning and prognosis, and guidance of sampling of pulmonary, mediastinal, or pleural lesions. However, while CT can be helpful for identifying pathology in any of the compartments and organs of the thorax, it is not uniformly helpful in differentiating disease processes and narrowing lists of differential diagnoses.

Compared to radiography and ultrasound, CT has fast scan times (especially with use of multi-detector row scanners), good spatial and temporal resolution, and very good contrast resolution. CT also exhibits excellent "structural resolution," as there is no superimposition of structures, there is good penetration of all thoracic structures, and multiplanar and 3D reformatted images can be produced. The primary disadvantages of CT for evaluation of the thorax are cost, availability, and need for sedation or anesthesia.

When performing thoracic CT, sternal recumbency is generally best, as this maximizes the volume of non-dependent aerated lung tissue (i.e., the larger caudal lung lobes). If there is a large volume of pleural fluid or gas, thoracocentesis prior to CT should be considered to improve pulmonary inflation. During the scan it is important to minimize breathing motion – since most veterinary patients will not voluntarily hold their breath on command, a positive-pressure breath hold or induction of brief apnea under anesthesia will be beneficial.

Indeterminate lung infiltrates and airway disease

A common situation for consideration of thoracic CT is to attempt to better characterize indeterminate pulmonary pathology identified on conventional radiography. Unfortunately, there is little veterinary literature to assist with providing meaning to the CT findings in these cases. One paper examining CT performed after radiography found that CT provided additional information in 88% of cases, with additional suggestion of etiology and further information of disease extent in most of those cases.[1] There was also a change in diagnosis made in approximately half of the cases, although many of these were a change to neoplasia, so there was no evaluation of one's ability to discriminate neoplastic and non-neoplastic etiologies with CT.[1] The tree-in-bud CT pattern has been associated with bronchial disease in cats, although some patients with this pattern also had concurrent neoplasia.[2] Thus, specificity of this finding may be limited, and use of CT to differentiate diffuse pulmonary diseases remains of unknown utility.

Pulmonary neoplasia

Evaluation of pulmonary masses and the search for pulmonary metastases are common indications for thoracic CT. Computed tomography is more sensitive than radiography for detection of pulmonary nodules, and CT may also be more specific for etiology in some situations. However, CT is not always necessary to provide a ranked differential list for a pulmonary mass – based on studies of thoracic radiography, histiocytic sarcomas are more common in the left cranial and right middle lung lobes, while adenocarcinomas are more likely to be located in the left caudal lung lobe.[3] Additionally, histiocytic sarcomas are generally larger than carcinomas, and 57% of histiocytic sarcomas had internal air bronchograms versus 16% in carcinomas.[3] CT evaluation of histiocytic sarcomas found similar results, with the right middle lung lobe identified as the most common site.[4] Intrathoracic lymphadenopathy, moderate enhancement, a bronchocentric distribution, and poorly defined margins were also common CT findings of histiocytic sarcoma.[4] However, some CT characteristics may not be specific to tumor type, as primary pulmonary carcinomas are also described as bronchocentric with mild to moderate heterogeneous contrast enhancement.[5] Nonetheless, sharp margination and location in a cranial or caudal lobe (not middle) may be useful markers of carcinomas.[5]

Pneumothorax and pleural effusion

Thoracic CT is very sensitive for identification of pleural gas, and it can be useful for determining the cause of that gas. CT has been used to identify pleural bullae and blebs as the cause of pneumothorax in some cases, but

it may be less useful at defining other causes of pneumothorax such as inflammatory disease.[6] Additionally, there are limits to the sensitivity and specificity of CT for identification of bulla rupture as a cause of pneumothorax, as ruptured bullae may look like normal lung, atelectasis, or pleural thickening; and bullae may leak even with an apparently intact wall.[7] In light of this, CT findings in terms of bulla identification should not be used to make a surgical decision, and surgical exploration would be indicated in pneumothorax regardless. However, CT isn't completely worthless, as it can be useful in identification of foreign bodies or trauma as a cause of pneumothorax and can guide the surgical plan, as has been described in cases of pneumothorax resulting from bronchopleural fistulas.[8]

Pleural effusion can also be readily detected with thoracic CT. As with causes of pneumothorax, the underlying etiology for pleural fluid can sometimes be identified with CT, although sensitivity depends on the causative agent. In studies evaluating CT findings in patients with thoracic plant awn foreign objects, the object itself is found 25-33% of the time, although other markers such as soft tissue tracking are seen more often and can help guide surgery.[9,10] In general evaluation of canine pleural effusion, CT attenuation values of the fluid can be used to suggest the composition of the fluid, as chylous effusion and transudate (approximately 6 HU) reportedly have significantly lower density than exudate and hemorrhage (20-21 HU).[11] Or you could do a thoracocentesis and perform fluid analysis, which is more specific for fluid type and is also therapeutic. Your call.

Pulmonary thromboembolism and lung lobe torsion

A final primary indication for CT evaluation of the thorax is to identify potential vascular disease. Pulmonary thromboembolism (PTE) is obstruction of pulmonary arteries due to direct vascular insult (as can be seen with neoplasia or parasitism), hypercoagulability, or other systemic/distant disease. Little veterinary literature describing CT for assessment of PTE is available, but CT is commonly used in human medicine for evaluation of pulmonary embolic disease. In the era of single-detector row scanners, results of CT for detection of PTE were mixed, with 45-100% sensitivity and 78-100% specificity depending on the study cited; pulmonary angiography or ventilation-perfusion scans were still considered the gold standard for identification of pulmonary embolism.[12,13] However, the advent of multi-detector row scanners brought an overall improvement in sensitivity (83%) and specificity (96%), and use of a venous phase scan in addition to an arterial scan improved sensitivity further (90%).[14] No veterinary studies have evaluated the accuracy of CT for identification of PTE, but one study describes CT findings in presumed cases (total occlusion of vessels by filling defects, irregularity of vessel lumens).[15]

Lung lobe torsion is rotation of a lung lobe about its bronchus and vessels, resulting in occlusion of those structures and congestion and decreased aeration of the affected lobe. This is a life-threatening condition, so rapid and accurate diagnosis is critical. Radiography can be used to make the diagnosis in some cases but can be equivocal; because of this, CT is often considered the diagnostic standard. Key CT features of a complete lung lobe torsion include lack of enhancement of the affected lobe (including an abrupt end to the lobar vessels), an abrupt end to the associated bronchus, and enlargement and emphysema of the lobe.[16] Pleural effusion is almost invariably present,[16] although this may be either a cause or an effect of the torsion. CT has been shown to be more sensitive than radiography for identification of defining features such as narrowing or collapse of the affected bronchus and presence of vesicular emphysema.[17]

Final thoughts

CT is very good for identification of some intrathoracic diseases, such as pulmonary neoplasia, pulmonary thromboembolism, and lung lobe torsion. CT is also very sensitive for identification of pneumothorax and pleural effusion, although ability to identify the cause of pleural pathology is variable. For diffuse disease of the lungs, CT may be less useful for differentiating various disease processes, although the available literature is limited.

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Imaging in Oncology

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Possibly the most common use of computed tomography (CT) in veterinary medicine is for evaluation of neoplasia. CT is tremendously useful in oncology, as it is more sensitive and specific than many other diagnostic modalities for identification of a number of different pathologies. Additionally, CT is helpful for evaluating extent of disease, aiding in surgical planning and other therapeutics, and prognosticating.

General considerations and small organ tumors

For most oncologic applications, both pre-contrast and post-contrast scans are important. (You don't need to give contrast medium if your only question is whether or not there are pulmonary metastases, but otherwise you should plan on a contrast medium injection.) While single post-contrast scans provide useful information, use of multi-phase post-contrast scans is often more beneficial. Scans performed in the arterial, venous, and delayed phases can aid in identification of some lesions, as not all lesions enhance at the same time. Further, multi-phase post-contrast CT can potentially provide more detailed characterization of lesions, which may help determine if a nodule is benign or malignant.

CT is generally more sensitive than ultrasound for identification of insulinomas and their metastases, although there is a risk of large numbers of false positive identifications.[1] When looking for insulinomas, the arterial phase is optimal for identification, as insulinomas tend to be markedly enhancing during this phase but later appear very similar to the normal surrounding pancreas.[2,3] However, in humans insulinoma enhancement is variable, and some are more persistently hyperenhancing while others remain isoattenuating to the surrounding parenchyma,[4,5] so there may be a wider range of enhancement patterns in canine insulinomas than is currently reported.

Some attempts have been made to use CT for differentiation of adrenal tumor etiologies, but there is considerable overlap in imaging findings among various tumor types. Two studies have found that pheochromocytomas are more associated with vascular invasion than adrenocortical adenocarcinomas or adenomas.[6,7] A contrast-enhancing rim is more suggestive of an adenoma,[6] while pheochromocytomas tend to have the highest attenuation values in pre-contrast, arterial-phase, and venous-phase scans.[7] However, given the wide ranges of imaging findings, it would be a fallacy to presume that one could confidently discriminate among these etiologies with CT alone.

There is no primary literature on use of CT for evaluation of parathyroid nodules in veterinary medicine, likely because of the reliance on ultrasound in our profession. However, while an older study provides convenient cut-points for diagnosis of parathyroid nodules (a nodule $\geq$ 4mm is likely an adenoma or carcinoma, while a nodule < 4mm is likely hyperplastic),[8] other studies demonstrate that incidental thyroid nodules are commonly found in hypercalcemic dogs,[9] and parathyroid glands and thyroid nodules cannot always be differentiated with ultrasound.[10] Thus, CT may be helpful in more accurately evaluating parathyroid disease in veterinary medicine. In humans, many parathyroid adenomas are easily visualized with CT, and most are hyperenhancing, especially in the early phase,[11,12] providing an argument for use of multi-phase post-contrast CT when examining the parathyroid glands. Early experiences in veterinary medicine at the University of Minnesota support this premise, as parathyroid adenomas have been identified as nodules which are specifically hyperenhancing in the arterial phase. However, this finding is not specific, as C cell hyperplasia secondary to hypercalcemia from other causes may have the same appearance.

Hepatic neoplasia and other focal / multifocal disease

Primary hepatic neoplasia has been reported as relatively uncommon in dogs and cats, and hepatic metastases from other tumors are reasonably common, although at least one relatively recent report found more primary hepatic neoplasms than metastatic ones.[13] Hepatic abscesses are much more rare, and in many areas of the world, an abscessed neoplasm is more likely than a primary abscess. However, nodular hyperplasia and other benign processes in the liver are very common, so it is important to differentiate these nodules from

malignancies. Ultrasound seems to have limited ability to do this, as one veterinary study found that the only predictors of malignancy when evaluating hepatic masses were greater size and presence of peritoneal effusion.[13]

In both human and veterinary medicine, CT may provide a fairly accurate means of determining if a hepatic nodule or mass is benign or malignant. In the human literature, characteristics such as abnormal internal vessels and strong arterial enhancement with relative hypoenhancement in the portal venous phase have been associated with hepatocellular carcinoma, and complete ring enhancement is commonly seen in metastatic neoplasia.[14,15] Conversely, benign lesions such as focal nodular hyperplasia and hepatocellular adenomas are described as having strong arterial enhancement and relative isoenhancement on the portal venous phase.[15]

Multiple veterinary studies have reported similar findings when examining hepatic nodules and masses in dogs. Generally, benign masses such as adenomas and nodular hyperplasia tend to exhibit hyperenhancement on the arterial phase and isoenhancement on the delayed phase, while malignant lesions are often hyperenhancing on the arterial phase and relatively hypoenhancing on the venous and delayed phases.[16,17] However, this is a gross oversimplification of real life, as many lesions were heterogeneously enhancing on some phases and did not cleanly fit any of the categories. And one study found absolutely no associations between CT findings and etiology of hepatic masses.[18]

Nonetheless, a recent study in dogs found a relatively straightforward CT method for classifying hepatic masses as benign or malignant with a reported 91% accuracy. In this study, evaluation of the degree of enhancement of the most hypoattenuating region of a mass was found to be most useful in categorizing the mass. When subtracting the pre-contrast density from the delayed-phase density of the lowest-attenuation region of the mass, a mass with enhancement (attenuation change) of < +37 HU was highly likely to be malignant, and a mass with enhancement of > +37 HU was highly likely to be benign.[19]

Splenic neoplasia and other focal / multifocal disease

Due to the common nature of both benign splenic masses (nodular and lymphoid hyperplasia, extramedullary hematopoiesis) and malignancies (such as hemangiosarcoma), classification of splenic masses as benign or malignant is important in veterinary medicine. Unfortunately, while ultrasound is quite sensitive for identification of splenic masses, ultrasound's specificity for determining the etiology of those masses is truly terrible. Thus, characterization of splenic masses might be another opportunity for CT to demonstrate value.

Of course, life isn't going to be that easy, and reports on the utility of CT in examination of splenic masses have mixed results. One study using pre-contrast and single-phase post-contrast CT found that malignant masses had a post-contrast attenuation of < 55 HU and benign masses had post-contrast attenuation of > 55 HU (100% sensitivity and 79% specificity).[20] This study also found that hematomas were significantly larger than hyperplastic or malignant masses.[20] However, a more recent study using dual-phase contrast enhancement did not find any associations between imaging findings and etiology of splenic masses due to considerable overlap in findings.[18] Thus, we still have not found a definitive means of determining splenic mass etiology prior to biopsy.

Diffuse neoplasia

Round cell neoplasia often causes diffuse infiltration of spleen and liver, among other organs, and identification of this infiltration is important for determining prognosis and developing a therapeutic plan in these cases. As is often the case, ultrasound has been the recent historical imaging standard for assessing organs for evidence of infiltration. While ultrasonography is often adequate, it certainly has limitations. One study found 73% sensitivity and 81% specificity for identification of hepatic lymphoma, and 100% sensitivity and 23% specificity for identification of splenic lymphoma (few true negatives, lots of false positives).[21] When the spleen was described as "motheaten," positive predictive value for lymphoma jumped from 65% to 100%.[21] However, modern ultrasound machines have demonstrated a dramatically increased ability to detect benign nodules, so determination of "motheaten" may not be as clear-cut as it used to be. Ultrasound is also relatively poor in identifying mast cell neoplasia in the spleen and liver – in one case series, ultrasound had only 43% sensitivity

for identification of splenic mast cell neoplasia and 0% sensitivity for identification of hepatic mast cell neoplasia.[22]

Once again, CT has an opportunity to provide real value in oncologic diagnosis, but it failed to do so in one small study. In 12 dogs with multi-centric lymphoma involving both spleen and liver, the spleen appeared normal in 7, and the liver appeared normal in 10.[23] Thus, the CT sensitivity for identification of lymphoma in either of these organs seems poor, although more research is likely warranted.

Final thoughts

CT is quite beneficial in characterizing hepatic masses, and it may also be superior to ultrasound in evaluation of pancreas, parathyroid glands, and adrenal masses. However, the jury is still out regarding the utility of CT is assessing splenic masses, and at this point it is difficult to recommend CT for examination of suspected round cell neoplasia.

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PERSONAL INFECTION CONTROL PRACTICES OF COMPANION ANIMAL VETERINARIANS AND VETERINARY NURSES IN AUSTRALIA – A PRELIMINARY REPORT

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Background: Personal infection control practices are critical to prevent nosocomial and zoonotic infections in veterinary practice.
Aims: To ascertain rates of compliance and individual beliefs with recommended personal infection control practices.
Methods: A digital questionnaire was distributed to practicing companion animal veterinary staff over four months. The Australian Veterinary Association Guidelines for Veterinary Personal Biosecurity were used for recommended practices.
Results: Respondents numbered 182 (119 veterinarians, 63 veterinary nurses). Sharps injuries were sustained by 78.7% of respondents in the preceding six months with 23.1% completing an incident report. Needle recapping occurred frequently with 74% veterinarians and 81.3% of nurses recapping greater than 5 times a day/ every time. Uncapping needles with teeth was performed by veterinarians 47.1% and 37.5% nurses most/ every time. Gloves were worn less than half the time when handling bodily fluids (49%) and only 19.2% of the time for dirty linen. 30% of veterinary staff did not wear gloves for obstetric examinations. Unprotected neonatal mouth to mouth was performed by 5.6% of respondents in the previous 12 months. More than half (52.9%) of respondents believed their risk of acquiring a zoonotic disease was unlikely.
Vaccination rates were variable: Q fever at 70.3%, tetanus at 99.25%, influenza at 59.7% and rabies at 37%. Only 73.7% of respondents were current with tetanus vaccinations.
Conclusions: Veterinarians and veterinary nurses participate in numerous high-risk behaviours that can predispose them to infection with zoonotic pathogens and physical injury. Results confirm poor compliance with veterinary guidelines for safe infection control practices.

BRUCELLA SUIS SEROPREVALENCE IN AT-RISK HUNTING DOGS IN NEW SOUTH WALES AND QUEENSLAND AND THE CLINICAL APPROACH TO SEROPOSITIVE CASES

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Background: *Brucella suis* is an emerging zoonotic disease predominantly affecting pig hunting dogs, pig hunters and veterinary staff.
Aims: The aims of this study are to assess the prevalence and geographical extent of this infection in hunting dogs and monitor seropositive dogs over twelve months.
Methods: A seroprevalence survey is being conducted in at-risk dogs in six areas throughout NSW and southern QLD (target sample size = 612). Information regarding hunting, feeding practices and the health of the dogs, is being collected from their owners via a questionnaire. *Brucellosis* testing is conducted at the State Veterinary Diagnostic Laboratory (EMAI) using the Rose Bengal agglutination Test (RBT) and the Complement Fixation Test (CFT). All inconclusive and anti-complementary samples are retested with an ELISA. Positive dogs from the serosurvey and those detected by general sampling in the QLD area (Goondiwindi region) are invited to enrol for further investigation (clinical examination, imaging, sampling for serial serology, culture and PCR, possible therapy with rifampicin/doxycycline) and ongoing monitoring. Decisions on monitoring and/or treatment are made based on clinical health and test results in consultation with owners.
Results: 23 of 194 dogs in the serosurvey tested positive on RBT (12%) with 10 confirmed using CFT and/or ELISA. 16 dogs are being monitored.

Conclusion and future perspectives: Preliminary results confirm brucellosis is an important problem in pig-hunting dogs, with a finite percentage of subclinical infections requiring monitoring and possibly therapy. Monitoring results will help to develop evidence-based guidelines for the diagnosis and management of brucellosis in dogs.

LISTERIAL MESENTERIC LYMPHADENITIS IN THREE CATS

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Case series summary: This series describes the presentation, clinical findings, and outcome of three cats infected with *Listeria monocytogenes* (*Lm*). *Lm* is a gram-positive bacillus and facultative anaerobe with variable pathogenicity. Listeriosis is a rare disease in cats and only five cases have been reported to date in this species. The cats included in this case series were aged between four and six years of age, and presenting signs included combinations of weight loss, vomiting and anorexia. All three cases had profound abdominal lymphadenomegaly (2.8 cm, 4 cm and 5 cm on sonographic assessment respectively) identified by palpation and abdominal ultrasound, and later confirmed at exploratory laparotomy. *Lm* infection was confirmed by combinations of culture and immunohistochemistry. In all three cases, survival was prolonged, with one case surviving 64 months from diagnosis, and the remaining two cases are still alive, 16 and 21 months from diagnosis respectively. Two of the cats in this case study had been fed a raw meat-based diet prior to the development of clinical illness.
Relevance and novel information: Each of these cases were likely a consequence of gastrointestinal translocation of the bacterium, or haematogenous spread secondary to *Lm* septicaemia. The lymphadenitis phenotype of the disease undergoes a protracted time course and is a differential for abdominal lymphadenopathy in middle aged cats. Feeding of a raw meat-based diet may be a contributing factor for development of listeriosis in cats.

LINKING MACRONUTRIENT INTAKE AND BEHAVIOUR IN DOMESTIC CATS (*FELIS CATUS)*

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Background: Given the opportunity, domestic cats naturally self-select their diet based on the macronutrient components. The domestic environment, however, rarely allows for macronutrient selection. Other species of small carnivore are known to change their predation and play behaviour based on their diet, however the role of macronutrients and its interaction with predation and play behaviour is yet not identified in the domestic cat.
Aim: To determine whether behavioural links to macronutrient intake exist for domestic cats.
Methods: Pedometers were attached to seven cats kept in identical enclosures with Fitbark © pedometers attached to their collars. Their activity was measured over their usual diet, and then across a high-protein, high-carbohydrate and high-fat diet for three weeks per diet, with an adjustment period of one week. A restricted maximum likelihood (REML) analysis was conducted in GenSTAT 18 (VSNi), with the fixed effect of diet, and a random effect of cat ID. A separate model was run for each outcome variable.
Results: During the high-fat diet, cats showed significantly higher activity totals compared to all other diets ($P < 0.01$). During the high-protein diet, cats exhibited the lowest activity levels ($P < 0.01$); high-protein also generated the lowest 'high-energy' behaviours ($P < 0.04$). Diets were not iso-caloric, however BMI was not significantly different across diet ($P = 0.5$).
Conclusion: Cats on a high-fat diet showed a significant increase in activity compared to the other diets. Inversely, cats on a high-protein diet displayed significantly lower total activity, and lower high-level activity levels. The results are suggestive that there may be a behavioural response to macronutrient ingestion. Further replication would improve reliability of correlation.

STRONG ASSOCIATIONS OF BODY CONDITION WITH MORBIDITY, SURVIVAL AND LIFESPAN IN CATS

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Objectives: The objective was to investigate the associations of a 9-point body condition score (BCS) with (a) health conditions that have been related to overweight and/or obesity in cats, dogs or humans and (b) survival and lifespan in cats.
Methods: Patient records from a cat-dominant primary practice in metropolitan Sydney were obtained. Binomial logistic regression investigated the associations between 21 health conditions and regrouped BCS (3-4, 5, 6, 7 and 8-9) after adjusting for age, sex and breed. Two survival analyses evaluated the associations of BCS (excluding BCS 1 and 2) with cats' survival and lifespan.
Results: The median of the BCS was 6 in 2609 cats. Fourteen of the 21 health conditions examined showed significant associations with an increased BCS, particularly BCS of 7 and above. Atopic dermatitis, hypertension, asthma, diarrhoea, ophthalmic conditions and allergic conditions were reported to be positively associated with an increased BCS for the first time. Relative to cats with BCS of 6, cats with BCS of 3 (hazard ratio [HR]: 4.67), confidence interval [CI]: 3.00–7.27), 4 (HR: 2.61, 95% CI: 1.95–3.49), 5 (HR: 1.43, 95% CI: 11.5–1.76) and 9 (HR: 1.80, 95% CI: 1.11–2.93) had significantly increased hazards of death. Compared to cats reaching BCS of 6 in the same age group, cats reaching BCS of 4 (HR: 4.15, 95% CI: 1.26–13.67) or 5 (HR: 1.75, 95% CI: 1.07–2.85) between age 1 and 3 years and BCS of 3 (HR: 6.09, 95% CI: 1.47–25.25) and 9 (HR: 2.27, 95% CI: 1.27–4.04) between the age of 3 and 11 years had significantly shortened lifespans.
Conclusions: A large number of health conditions are associated with increased BCS in cats. BCSs ≤ 5 and of 9 are negatively associated with survival and lifespan in cats.

DGGR LIPASE CONCENTRATIONS IN DOGS WITH NATURALLY OCCURRING HYPERADRENOCORTICISM

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Background: The association between 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) lipase assay and dogs with naturally occurring hyperadrenocorticism (HAC) is unknown.
Aims: To determine if there is a relationship between DGGR lipase assay and newly diagnosed naturally occurring HAC.
Methods: Review of medical records from 2016-2018 was conducted to identify newly diagnosed dogs with HAC with concurrent DGGR lipase assays performed. All dogs were diagnosed with naturally occurring HAC using both clinical signs and adrenocorticotropic hormone (ACTH) stimulation/low-dose dexamethasone suppression test +/- ultrasonography. Results were compared to healthy age-matched controls. Unpaired t-test results were used and a P value < 0.05 was considered significant.
Results: Criteria identified twenty dogs with HAC. Seven dogs had DGGR lipase activity within the normal laboratory reference range (1-70 U/L). Nine dogs had DGGR lipase activity in the range 70-216 U/L. Four dogs had DGGR lipase activity > 216 U/L and one of these dogs (DGGR lipase activity 830 U/L) had ultrasonographic signs of pancreatitis. The DGGR lipase assays were compared to twenty control dogs. All healthy patients had DGGR lipase activity within the reference range (1-70 U/L) except for two dogs (87 U/L and 79 U/L). DGGR lipase was higher in dogs with HAC (243 +/- 348 U/L; mean +/- SD) than in healthy controls (46 +/- 20 U/L; P = 0.016).
Conclusions: Dogs with naturally occurring HAC on average had a higher DGGR lipase activity than clinically healthy dogs at the time of diagnosis. DGGR lipase activity should be interpreted with caution in dogs newly diagnosed or untreated for HAC. Further research is necessary to ascertain the reason for this potential association.

A MULTI-CENTRE EPIDEMIOLOGICAL STUDY OF CANINE CUTANEOUS AND SUBCUTANEOUS MAST CELL TUMOURS ACCORDING TO HISTOLOGICAL GRADE AND MITOTIC INDEX

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Background: Despite previously well described breed predispositions to canine mast cell tumours (MCTs), limited studies have been performed to assess patient and tumour factors that may influence MCT histological grades. This information would allow for better prediction of MCT biological behaviour within patients.
Aims: To identify patient and tumour factors most frequently associated with high histological grades of canine MCTs.
Methods: Search criteria in a shared database of referral veterinary hospitals generated 410 canine MCTs within 295 patients. Patient and tumour data were extrapolated with frequencies calculated and statistical methods performed. The probability of the tumour being histologically high grade was calculated compared to the patient and tumour factors.
Results: The study consisted of 90 (21.9%) tumours meeting histological high grade criteria. Staffordshire Terriers (American and English) were the most common breed represented with MCTs, comprising 16.1% (n=66) of all tumours. Shar Peis were the most likely breed to have high grade MCTs, with an OR of 3.74 (p=0.03), whereas the Pug (OR = 0.05, p = 0.04) and the Golden Retriever (OR = 0.13, p =0.04) were the least likely breeds to develop high grade MCTs. No significant difference in risks could be established between the genders or neuter statuses of patients. MCTs of the inguinal region were the most likely to be high grade (OR = 3.71, p = 0.04). Tumour size did not influence the likelihood of a tumour being high grade or low grade.
Conclusion: Patient and tumour factors may play a role in the histological outcomes of canine mast cell tumours.

EFFECT OF MELATONIN ON CYCLICITY AND LACTATION OF 7 POST-PUBERTAL QUEENS - A CASE SERIES

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Background: Subcutaneous melatonin implants represent an effective method of suppressing oestrus in queens without causing uterine pathology such as cystic endometrial hyperplasia (CEH) or pyometra, commonly seen following the use of progestins. Previous studies have demonstrated that 18 mg subcutaneous melatonin implants reliably inhibit oestrus. However, there is limited data describing the duration of suppression, effect on fertility, return to call, and effect on lactation.
Methods: A retrospective analysis of the medical history of seven post-pubertal queens treated off-label with 18 mg subcutaneous melatonin implants to temporarily suppress oestrus was made.
Results: Mean age of all patients was 13 months (6 to 48 months). At the time of suppression, 29% were multiparous, 14% were uniparous, and 57% were nulliparous. All patients displayed suppression of oestrus following implantation, with one demonstrating a delay in suppression. Duration of suppression varied between 9 to 17 weeks with an average of 12.1 weeks. At the time of analysis, 4 of 7 cats had returned to call following suppression and were mated successfully. Ultra-sonographic assessment of the uterus and ovaries revealed no abnormalities or evidence of pathology. One patient was treated post-partum; following this the mammary glands appeared more distended with milk and the patient continued to produce milk for 2 weeks longer than normal.
Conclusions: The administration of 18 mg subcutaneous melatonin implants represents an effective, safe, and reversible method for inducing short-term suppression of oestrus in post-pubertal queens with an added effect on lactation in postpartum queens. Administration post-partum appears safe, effective, and induces a positive effect on lactation.

DISEASE DISTRIBUTION IN 955 DOGS PRESENTING FOR LOWER MOTOR NEURON PARESIS AND ASSOCIATION WITH RAW CHICKEN: A RETROSPECTIVE STUDY

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Background: Diffuse lower motor neuron paresis is a common presentation to both general practitioners and referral hospitals, but both the numbers of patients with a particular differential diagnosis and the overall prevalence of this syndrome remain generally anecdotal due to many patients going without a definitive diagnosis.
Aim: Aims were to determine the distribution of differential diagnoses in dogs presenting with lower motor neuron paresis, and to characterise the diagnostic process used by referral clinicians when establishing a presumptive diagnosis of polyradiculoneuritis. A secondary aim was to determine if a significant association was present between dogs presenting with non-toxigenic lower motor neuron paresis and feeding raw chicken.
Results: Inclusion criteria identified 955 cases. Overall prevalence was 1.4%, and the most prevalent conditions observed were tick paralysis (66.5%) and open diagnosis (20.3%). No dog with presumed polyradiculoneuritis underwent the full set of diagnostics required to exclude all other differentials, but all had some sort of diagnostic testing. After exclusion criteria for diet analysis were applied, 61 cases remained. Across the various diagnosis groups, no significant difference was found in the proportion of dogs fed a raw diet (non-parametric Chi-squared test, $X^2 = 1.067$, $P = 0.302$).
Conclusions: Most cases of lower motor neuron paresis in the source population were caused by organic toxins, namely tick paralysis. Although most dogs with polyradiculoneuritis undergo some diagnostic testing, it remains a presumptive diagnosis. Finally, there was no significant difference in raw chicken consumption for animals with varying causes of lower motor neuron paresis in this population.

Management of multi-drug resistant urinary tract infections and cholangitis

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Introduction

Multi-drug resistant (MDR) bacteria are defined as bacteria that are resistant to three or more classes of antimicrobials.[1] In dogs and cats they are primarily cultured from animals that have comorbid conditions which reduce host defences and/or predispose to entry and persistence of bacteria. Prior antimicrobial use and hospitalisation (≥3 days) also predisposes to the presence of MDR bacteria.[2, 3]
The disease processes and important diagnostic criteria are reviewed in the following sections, followed by a suggested practical approach to management of cases with MDR bacteria.

Cholangitis in dogs and cats

Percutaneous cholecystocentesis should be considered when cholangitis is suspected, to minimise unnecessary empirical antimicrobial use and to obtain information that directs treatment. When performed correctly, the potential risk for major complications (such as bile peritonitis) is low (<4%).[4, 5] In cats, bile cultures often return negative when the biliary tract is ultrasonographically unremarkable, hence bacterial cholangitis or cholecystitis may be less likely in these cases and cholecystocentesis for culture may not be warranted.[5] In dogs, the decision to perform cholecystocentesis cannot be based on ultrasonographic appearance of the biliary tract, as positive bile cultures may be obtained in dogs with an ultrasonographically normal biliary tract.[4, 5] Bile culture results should be evaluated in light of the clinical picture and bile cytology. In some cases, bile culture and cytology results do not correlate, i.e. bile cytology detects bactibilia but bile culture returns no growth or *vice versa*. This may be due to previous antimicrobial use, bacteriostatic properties of bile or because contaminants were cultured. Approximately 75% of positive cultures in dogs are not associated with an inflammatory picture on cytology. In some of these dogs, inflammation may be associated with the biliary tract wall and hence not detectable in bile.[6] However, in some dogs, bacteria may be non-pathogenic rather than associated with disease.

Culture may detect bacteria in cholecystocentesis-derived bile of up to 41% of healthy dogs sampled over time.[7] The most common organisms identified are of enteric origin: *Escherichia coli*, *Enterococcus* spp. and *Bacillus* spp. Positive bile culture in healthy dogs is usually associated with bile cytology that is unremarkable (apart from potentially detecting bactibilia). Bile is thought to be sterile in healthy cats but this information is based on bile culture results of only six cats.[8]

Culture may be positive in 18-36% of cholecystocentesis-derived samples from dogs and cats with hepatobiliary disease.[4-6, 9] Many dogs have comorbid conditions that may predispose to the presence of bacteria; this complicates interpretation of positive culture results.[6] In cats, all bacteria are thought to be pathogenic, particularly when ultrasonographic changes of the biliary tract are present.[5, 8] Predominant bacteria that are isolated from dogs and cats include enteric aerobes (e.g. *E. coli*, *Enterococcus* spp.) and enteric anaerobes (*Bacillus* spp., *Clostridium* spp., *Bacteroides* spp.). *Staphylococci* or *Streptococci* have also been identified; although these species can be pathogenic, contamination from skin must be considered in these cases, particularly as cutaneous bacteria may be resistant to multiple antimicrobials.

There is limited information regarding antimicrobial susceptibility results of bacteria isolated from bile, particularly for anaerobes. MDR bacteria have been reported uncommonly,[10, 11] yet resistance to at least one of the commonly used antimicrobials, such as beta-lactams, potentiated beta-lactams or fluoroquinolones is frequently detected.[9]

Subclinical bacteriuria and urinary tract infections in dogs and cats

To properly address management of MDR bacteria in urine, subclinical bacteriuria must be discussed in addition to classical urinary tract infection. Subclinical bacteriuria is the presence of bacteria in the urine in the absence of clinical signs.

The diagnosis of UTI or subclinical bacteriuria should be based on culture, as urine sediment findings may not correlate with culture results.[12] Cystocentesis is the aseptic sample-acquisition method of choice. If cystocentesis is contraindicated (e.g. due to a coagulation disorder or risk of neoplastic spread along the needle tract), samples must be obtained by catheterisation. Isolation of the same bacterial species from two consecutive catheter samples, taken one week apart, is necessary to confirm subclinical bacteriuria.[13] Free-catch urine should not be used to diagnose subclinical bacteriuria or urinary tract infection. Cultures from catheter tips or urine collection bags are not representative of *in vivo* infection and treatment should never be based on results of cultures taken from these sites.

Recent studies suggest that the urinary tract of healthy dogs and cats is not sterile.[14, 15] Microorganisms have been cultured in up to 9% of healthy dogs[16, 17] and up to 13% of older, non-azotaemic cats,[15] with *E. coli* being the most common bacteria identified in both species. All these animals had subclinical bacteriuria that was not treated and this was not associated with a detrimental outcome.

Subclinical bacteriuria is common in dogs and cats with comorbidities (such as obesity, diabetes mellitus, canine hyperadrenocorticism, feline hyperthyroidism, chronic kidney disease or bladder atony) or animals treated long-term with glucocorticoids or other immunosuppressants.[18] Many of these animals have persistent or recurrent bacteriuria and, as mentioned, prolonged antimicrobial treatment predisposes to presence of MDR microorganisms. As a result, MDR microorganisms are particularly common in these dogs and cats, yet whether they benefit from antimicrobial treatment remains unclear (see below).[19, 20]

E. coli, *Enterococcus* spp. or *Staphylococcus* spp. are the most commonly isolated urinary bacterial species associated with MDR.[21] Saputra et al. (2017) recently surveyed antimicrobial resistance patterns in Australian *E. coli* (many of them of urinary origin) and reported an MDR prevalence of 18.1% and 11.7% in canine and feline isolates, respectively. Whilst some of these MDR *E. coli* may have been isolated from the same animal, it is important to note that many MDR bacteria were resistant to fluoroquinolones and most beta-lactams. MDR prevalence was not discussed in the recent New Zealand survey of canine and feline urinary bacteria.[22] However, a rise in antimicrobial resistance to amoxicillin-clavulanic acid, cephalothin and enrofloxacin over the survey period (2005-2012) was seen. Notably, Karkaba et al. reported that many of the amoxicillin-clavulanate-resistant *Enterobacteriaceae* may produce extended-spectrum- or AmpC beta lactamases[23] and concurrent resistance to fluoroquinolones is also a concern.[24]

A suggested practical approach to management of cases from which MDR bacteria have been recovered

As outlined above, the MDR bacteria recovered from dogs or cats with cholangitis or urinary tract disease may be normal microflora, contaminants or facultative/obligate pathogens. Ideally, only pathogens should be treated; yet differentiation between aforementioned groups remains difficult with the veterinary information currently available. An approach is outlined in Figure 1.

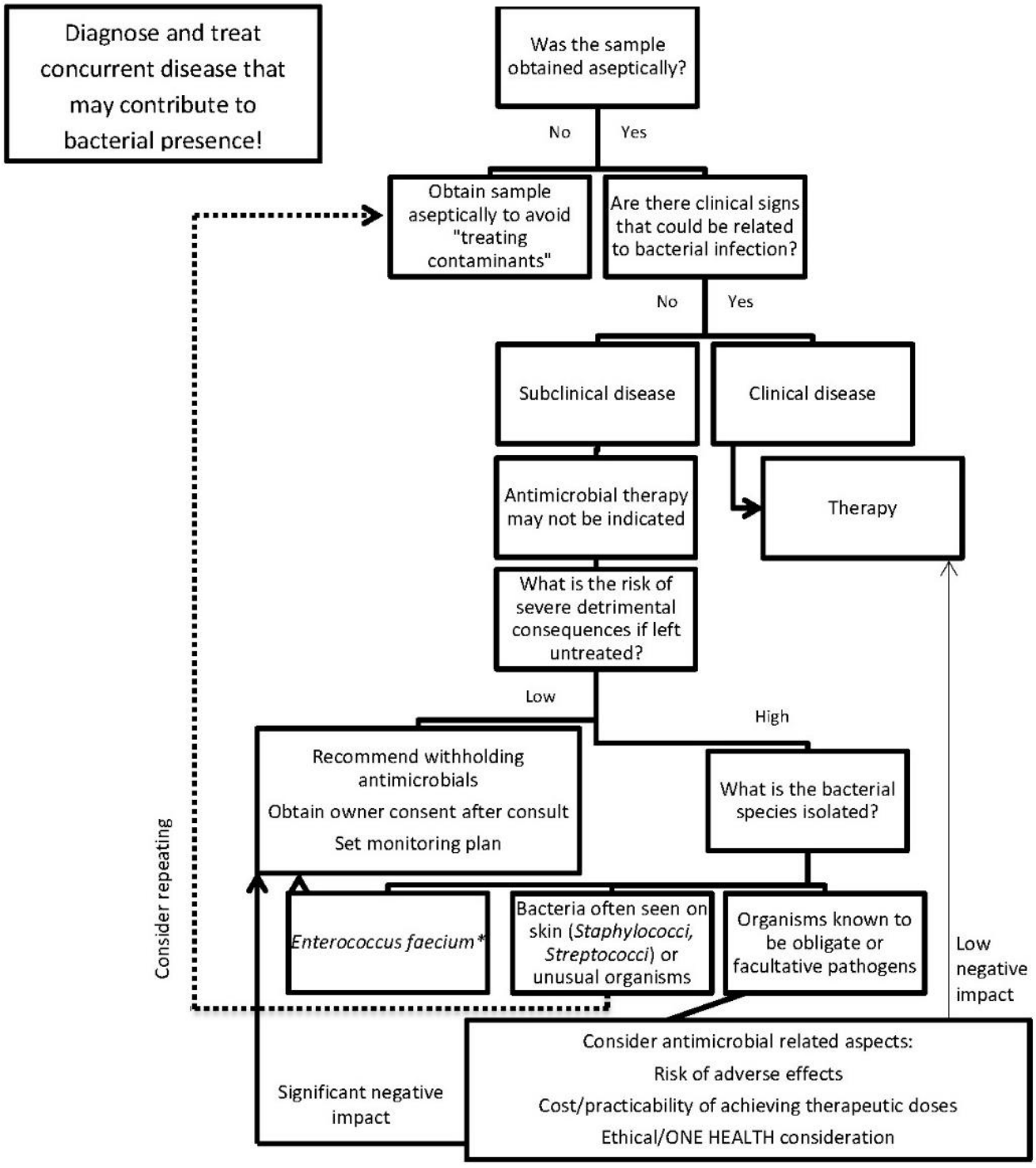

Figure 1 Approach to management of cases from which MDR bacteria have been recovered.

Evidence for withholding antimicrobial treatment

There is no peer-reviewed published veterinary information that I could find regarding the outcome of dogs and cats with comorbid conditions and subclinical bacteriuria or bactibilia in which antimicrobial treatment was withheld. In people, asymptomatic bacteriuria is common when aforementioned comorbidities are present, and isolated microorganisms are similar. Current human guidelines recommend avoiding antimicrobial treatment in most instances of asymptomatic bacteriuria.[25] Veterinary experts of the International Society for Companion Animal Infectious disease (ISCAID) support this approach.[13]

It has been suggested that the presence of *Enterococcus* spp. in clinical samples may not always require treatment, particularly if *Enterococcus* spp. is present in addition to another, potentially more pathogenic microorganism that could explain clinical signs.[26] *Enterococci* are intrinsically resistant to several antimicrobials and acquire antimicrobial resistance quickly. However, particularly *Enterococcus faecium* is thought to be largely devoid of virulence factors.[27] Veterinary studies assessing the outcome of dogs or cats after enterococcal isolation are, to my knowledge, not available. Hence, current advice is based on expert opinion.

Treatment options

Bacterial susceptibility results should direct treatment. In selected cases, *in vivo* response to first- or second-line antimicrobials is seen despite apparent *in vitro* resistance, possibly because *in vivo* antimicrobial concentrations were higher than those necessary to inhibit bacterial growth.[2] Hence, it may be useful to request results of minimal inhibitory concentrations for antimicrobials that concentrate in bile or urine, particularly if the disease is not thought to be tissue-associated.

Aminoglycosides could be considered for infections with bacteria that are susceptible to this antimicrobial group, though nephrotoxicity and the necessity to give these drugs parenterally may limit their use. Chloramphenicol or nitrofurantoin may be considered if resistance to all commonly used antimicrobials is observed. Fosfomycin can also be considered in the treatment of canine urinary tract infection (potential for nephrotoxicity prevents its use in cats). Although these drugs may be active *in vitro*, they have a less favourable therapeutic profile than commonly used antimicrobials and their contraindications, potential to cause adverse effects and drug interactions should be studied before they are used.[28]

The use of vancomycin, carbapenems (e.g. imipenem) or linezolid is strongly discouraged. According to ISCAID guidelines, their use is not justified in subclinical disease.[12] Use in clinical infections is only justified when the infection is documented on the basis of clinical or cytological abnormalities and culture, there is resistance to all other reasonable options and susceptibility to the chosen antimicrobial is documented, the infection is potentially treatable (if the underlying cause cannot be managed or removed these drugs should not be used) and finally, consultation with an expert in infectious diseases and antimicrobial treatment has been undertaken to determine whether there are any other viable options and to confirm that treatment is reasonable.

There is currently insufficient evidence to support the use of adjunctive therapy (e.g. cranberry juice) or antiseptics (methenamine hippurate) in urinary tract infections. There is also no evidence that supports the use of additional antimicrobials (e.g. clarithromycin) to break biofilm formation or for direct instillation of antimicrobials, antiseptics or DMSO into the urinary bladder.[12] Treatment by instillation of harmless bacteria (live biotherapeutic products) is currently being investigated.[29]

"*A clever person solves a problem. A wise person avoids it.*" (*Albert Einstein*)

Infections caused by MDR bacteria are extremely difficult to treat, may cause significant morbidity in animals and cost to clients. MDR bacteria are a great public health concern. Antimicrobial stewardship programs are important to reduce the presence and spread of MDR bacteria.

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Chronic enteropathy: Faecal microbiota transplant or antibiotic trial?

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Introduction

Antibiotics are often used as a second line treatment if a diet trial has failed in dogs with chronic enteropathy (CE). However, there are concerns about how effective antibiotics are long term and the potential risk of bacterial resistance.

Faecal microbiota transplant has been used in human medicine to treat *Clostridium difficile* and has the potential to be useful in patients suffering from inflammatory bowel disease. Can it be used in veterinary patients?

Antibiotic-responsive enteropathy – CE

Antibiotics are often used as a second line treatment in CE if a diet trial has not been successful. Usual antibiotics used are listed in table 1 and dogs responding are classified as having antibiotic-responsive enteropathy (ARE).

The exact role of the antibiotic remains unclear, but possible effects include modification of the gut flora through anti-microbial effect and modulation of the immune-system.[1]

Granulomatous colitis (GC) of boxers is the only CE where bacterial invasion of the intestinal wall has been documented, with resolution of clinical signs apparent after clearance of bacteria. [2,3] Recent guidelines have been published for the use of enrofloxacin for canine colitis.[4] Treatment with 10-15mg/kg q24h PO has been suggested with reassessment after 2 weeks and total treatment duration of 8 weeks in responders.

Dogs responding to antibiotics (excluding GC) are usually young and large breed. German Shepherd dogs are over-represented in the UK and tylosin-responsive middle-aged, large dogs have also been described.[5–7] Several publications from Finland have been unsuccessful in identifying an underlying infectious aetiology and relapses are reported frequently after cessation of antibiotics. Remission is typically achieved by reintroducing the antibiotic, whereas relapse is not prevented with a probiotic or prednisolone. The effect of tylosin was confirmed in a double-blinded prospective clinical trial and a dosage as low as 5mg/kg q24h was found to be effective with resolution of the diarrhoea within days.[8,9]

Antibiotic treatment is typically recommended for 4 to 6 weeks, but there is no published information to determine what the optimal duration is. A trial with a one-week course of tylosin had similar success rates to a 6 week course, and there were similar relapse rates of 88% of dogs within two months and 86% within 30 days, respectively.[7,9] Another study raised concerns about long term success in dogs treated with antibiotics, where out of 33 dogs, all had relapsed after 6 to 12 months.[6]

Another concern when using antibiotics is the effect on the microbiome. Metronidazole treatment in healthy dogs has resulted in increases in *E. coli* and decreases in Firmicutes as well as alterations in about 20% of faecal metabolites.[10] Some of these changes were still present four weeks after stopping antibiotics at the end of the study.

As antibiotic response seems short lived, this raises the question of how useful antibiotics truly are to achieve long term remission with the additional risk for development of antibiotic resistance.[11]

Faecal microbiota transplant

The microbiome is suspected to play a central role in inflammatory bowel disease and alterations in microbiota have been reported in dogs and cats with acute or chronic enteropathies.[12] In human medicine, this finding has led to attempts of modifying the intestinal flora using faecal micriobiota transplant (FMT) from healthy donors.[13] The technique consists of administering faecal material from a “healthy” donor to a patient. It’s utility

has been accepted in human patients with recurrent *Clostridium difficile* infection, but it might also successful for other types of GI diseases. Central to the technique is to identify the right diseases and the right donors.

Currently, very little information has been published in veterinary medicine, but key aspects of the technique for use in small animal practice are available.[14] However, studies are still needed to determine the real benefit of this technique, as well as the best way to administer FMT. A recent study assessed the use of FMT in puppies with parvovirus infection.[15] Although mortality rate was similar between dogs treated with standard treatment or standard treatment and FMT, puppies treated with FMT had faster resolution of diarrhoea and a shorter duration of hospitalisation. There are currently no large studies assessing the use of FMT in dogs or cats with CE.

Conclusion

The microbiome is central in the development of CE and FMT has the potential to be used to modify it. There is currently very little information on its use in dogs and cats, but there is active research on this subject.
Although antibiotics have been used to treated animals with CE not responding to food trials, the need for long term antibiotic treatment or short-lived response raises the question of how useful antibiotics truly are.

FMT might be a new tool to treated dogs with CE although more studies are needed to determine its utility.

Table 1. Common antibiotics used in CE.

Drug	Type	Dosage
Oxytetracycline	Antibiotic	10mg/kg, every 8 hours, orally
Metronidazole	Antibiotic	10mg/kg, every 12 hours, orally
Tylosin[1]	Antibiotic	20mg/kg, every 8 to 12 hours, orally
Enrofloxacin[2]	Antibiotic	10-15mg/kg, every 24 hours, orally

(1) Alternate dosage of 5mg/kg q24h PO (Kilpinen, 2011). (2) Recommended for granulomatous colitis in Boxer and French bulldogs.

Table 2. Response to antibiotic in dogs with CE.

Antibiotic	Number of dogs[a]	Treatment success[b]	Study duration	Evidence	Reference
Oxytetracycline[1]	12 (27)	44%	10 weeks	IV	German, 2001
Tylosin[2]	9 (9)	100%	N/A	II (no blinding)	Westermarck, 2005
Tylosin[3]	20 (27)	74%	N/A	I	Kilpinen, 2011
Metronidazole[4]	33 (203)	0%	6 to 12 months	III	Allenspach, 2016

[a] Number of dogs on antibiotic trial, with total dogs in the study in brackets. [b]Dogs responding to antibiotic. [1]Primary treatment. 4 week course of oxytetracycline at 10mg/kg, every 8 hours, orally or 4 week course of tylosin (10mg/kg, every 8 hours, orally) if oxytetracycline had been used prior. [2] Dosage of 12mg/kg, every 24 hours, orally (mean) for a 2-week course. Diarrhoea relapsed after stopping treatment despite treatment with a probiotic. No clinical improvement was seen with steroids. [3]Dosage of 25mg/kg, every 24 hours, orally for 7 days. Seventeen dogs out of 20 treated with tylosin responded versus 2 out of 7 with placebo. Out of 5 non-

responders on placebo, one was lost and 3 responded to tylosin. [4]Metronidazole was used as second line treatment after failure of diet trial.

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Optimization of immunotherapy

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Introduction

Although immunosuppressive drugs are widely used in companion animals, treatment monitoring remains crude. Typically, treatment is tailored by assessing clinical response and monitoring for adverse effects. For example, adverse effects that can be seen with azathioprine treatment include gastro-intestinal signs, bone marrow suppression and rarely liver necrosis (both dosage related) as well as idiosyncratic pancreatitis.[1]

Usually, animals are started at a standard dose of an immunosuppressant, but there is evidence that individuals will respond differently. For example, when seven healthy dogs were given a dose of 5mg/kg q24h of cyclosporine, two of them did not show suppression of IL-2 production.[2] This implies that when using a given dose, some animals will be under-dosed with a risk of treatment failure, while others will be over-dosed with a higher risk of dose-dependent adverse effects and secondary infections that can lead to morbidity or mortality.[3,4]

Newer tests are being developed to assess the immunosuppressive effects of cyclosporine and other drugs. Such strategies have the potential to be used to finely tune the amount of drug that is needed to achieve individualised immunosuppression.

The following assays will be discussed: Therapeutic drug monitoring and pharmacodynamic assays.

Therapeutic drug monitoring (TDM)

TDM consists of measuring a drug's concentration in plasma, serum or blood. TDM is considered in the following instances:

- The drug has a narrow target range
- The drug's pharmacokinetics are highly variable between animals
- A relationship exists between the drug concentration and clinical effects
- To establish target concentration ranges
- A cost-effective drug assay is available

TDM can be very useful if the intra-vascular concentration is correlated to the concentration at the site of action. For example, cyclosporine accumulates in the skin and the intestines. For this reason, the blood concentration will be less useful in ensuring an adequate concentration for dermatologic indications (for example atopic dermatitis treatment), but can be considered to avoid overdosing.[5] However, TDM can be more relevant if used for an animal with immune-mediated haemolytic anaemia to optimise treatment.

TDM not only involves measurement of drug concentration, but also interpretation of the result clinically. To achieve this, information regarding the clinical condition of the animal as well as drug history and sampling time are required. For this reason, discussion with the laboratory staff can be necessary to obtain a comprehensive interpretation.

TDM can be considered in the following scenarios:

1. At the **start of treatment** in an animal responding clinically. This can be used as a baseline (steady-state). This can be very helpful for comparison at a later stage if an animal relapses.

2. As a **follow-up** after a change in dose or medication brand to re-assess the steady-state concentration. For example, to monitor a dog after changing the cyclosporine brand used (e.g. Atopica to a generic).

3. If an animal shows clinical signs that could indicate drug toxicity.

4. In **treatment failure**: In an animal not responding to initial treatment or where control of the disease is lost over time.

Cyclosporine TDM
Monitoring cyclosporine concentration is technically challenging and results are very dependent on the methodology used (high-performance liquid chromatography versus use of antibodies). Another complicating factor is that there are very few studies correlating cyclosporine concentration and immunosuppression. Most of the information available comes from experimental data using cyclosporine in dogs to prevent renal rejection after transplantation.[5]
Because cyclosporine concentrates in red blood cells; whole blood should be submitted for cyclosporine TDM. As mentioned, laboratory recommendations are dependent on the assay used and may not be appropriate for other laboratories. As an example, the current recommendations from the clinical pharmacology laboratory of the Auburn University (http://www.vetmed.auburn.edu/veterinarians/clinical-labs/) are outlined below:

- For **immune-mediated disease**, dosing is recommended at q12 hour intervals to achieve adequate suppression. A trough concentration (prior to the next dose or 12 hours after dosing) of 400 to 600ng/ml or a peak concentration (2 hours after dosing) of 800 to 1,400ng/ml is recommended.

- For **chronic inflammatory disorders**, a trough concentration of 250ng/ml is recommended or 100 to 600ng/ml (the high end of the range is preferred during induction and the lower end during maintenance).

Cyclosporine concentration can be measured within a week of new dosing regimen, but can also be measured if another drug is added that affects cyclosporine pharmacokinetics (e.g. concurrent treatment with ketoconazole). Aggressive monitoring at peak and trough can be helpful in animals with severe disease (e.g. pure red cell aplasia, transfusion dependent immune-mediated haemolytic anaemia) to optimise treatment in a timely manner. Monitoring can be considered weekly while adjusting cyclosporine dosage and then monthly for the first few months once steady state has been reached.

Leflunomide TDM
Leflunomide is a prodrug that is converted to teriflunomide (active metabolite). There is a lack of studies in vivo in dogs and cats, but in vitro data and Auburn laboratory data suggests that responders have trough concentration between 5-45mcg/ml. A trough sample is recommended within 5 to 7 days of a dose change if TDM is deemed necessary.

Pharmacodynamic monitoring (PD)

TDM is especially useful if a drug has a narrow target range to avoid toxicity or if there is a clear relationship between drug concentration and clinical effect. PD monitoring on the other hand is assessing the effect of the drug rather than its concentration.

Cyclosporine mechanism of action is by inhibition of IL-2 production by lymphocytes. The pharmacodynamic laboratory from Mississippi State University (http://www.cvm.msstate.edu/animal-health-center/pharmacodynamic-laboratory) has developed an assay to measure IL-2 production in vivo using a PCR-based assay.[6]

In depth studies of the pharmacodynamics of cyclosporine in healthy dogs have been performed during validation of this assay and have shown that:

- The effect of a given dosage of cyclosporine can be **very different from one dog to the other**

- **High cyclosporine dosage** (5-10mg/kg q12h) is often necessary to reliably achieve immunosuppression.

The test can be performed within a week of starting a high dose of cyclosporine, especially in critical animals. This enables the clinician to rapidly determine if the dosage is adequate for a given dog. A peak sample (2 hours post-pill) is used in dogs receiving cyclosporine twice a day.

Testing can also be considered in an animal treated with cyclosporine that is not responding adequately clinically to rule out under-dosage as a cause for the poor response.

In collaboration with Mississippi State University, we have shown that measurement of cytokine concentrations can be used not only to assess the effect of cyclosporine, but also of prednisolone in healthy dogs.[7]

Conclusion

New methods are being developed to monitor dogs on immunosuppressive drugs. Although few veterinary laboratories offer this service (currently none in Australia), these tests are likely to become more widely available in the future. This is a first step towards personalised veterinary medicine.

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A novel treatment option for canine myxomatous mitral valve disease in Australia - Surgical mitral valve repair

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Introduction

Mitral regurgitation (MR) due to myxomatous mitral valve disease (MMVD) represents by far the most common cause of cardiac disease in dogs (75 to 80%). This disease is more prevalent in middle- to old-aged small breed dogs, with a heritable, genetically determined component in certain breeds, such as Cavalier King Charles Spaniels and Dachshunds. [1-3] The aetiology of the myxomatous degeneration is still unknown. However some experimental evidence seems to support a dysregulation in serotonin receptor signaling leading to pathological remodeling of the mitral valve. [4] Valves affected by myxomatous degeneration appear thick and nodular and the degree of severity and distribution of the lesions is age related. Lesions start as isolated nodules on the free edges of the leaflets and will progress to involve larger areas of the valve and chordae tendineae, therefore compromising leaflet coaptation and generating MR. [5, 6]

MMVD is characterised by a protracted course of asymptomatic disease (stage B), often spanning over several years. During this time, gradual cardiac remodeling secondary to elevated left atrial pressure and left ventricular preload occurs. In the majority of dogs, the disease never progresses to the symptomatic phase of congested heart failure (CHF) or stage C. [7] When escalating drugs and dosage does not result anymore in the expected improvement, dogs with severe MMVD and CHF are considered in the refractory stage (stage D).

Current therapies

Dogs affected with MMVD are mostly treated medically (furosemide, pimobendan, ACE-inhibitors, spironolactone) with the purpose to suppress their symptoms and prolong their survival time in stage C, or to slow down the progression of the disease in dogs with stage B2.[7] The medical therapy has no effect on the main pathology, i.e the progressively worsening valvular lesions.

Mitral valve repair is the procedure of choice for correcting significant MMVR in humans, providing long-term results that are superior to mitral valve replacement. More specifically, while maintaining both valvular tissue and the subvalvular apparatus, this procedure has lower operative mortality, with better preservation of left ventricular function and superior survival rates in the long term, than does prosthetic valve replacement. [8].

Mitral valve replacement has been attempted in veterinary medicine, using either mechanical or bioprosthetic valves. Reports with the mechanical valve describe short-term good results but very poor post-operative survival due to the thrombogenicity of the artificial valve. [9] The bioprosthetic valves use animal tissue material in order to improve the biocompatibility of the implant. The few papers reporting the use of these implants describe encouraging short- and long-term results. [10]

Unfortunately, mitral valve replacement as well as conventional mitral valve repair techniques involve an open-heart surgery. These procedures requite a very well-trained team, including cardiologists, anaesthetists, perfusionists, cardiovascular surgeons and ECC specialists, as well as some special equipment including a heart/lung machine and transesophageal echocardiography, which complicate the widespread of these techniques in veterinary medicine. To date in fact, routine and successful mitral valve repair in dogs is performed only by two team worldwide. The University Veterinary Teaching Hospital in Sydney (UVTHS) has been fortunate to collaborate with the team led by Dr M. Uechi, who performed the first successful mitral valve repair in Australia on Prince, a 10 year-old male Cavalier King Charles Spaniel, in November 2017. Since then, two additional cases, Charly and Keisha, had surgery in April 2018.

Mitral valve repair

Indications and patient selection

Dogs with severe MMVD (stages C and D) and without any co-morbidities, are good candidates for mitral valve repair, since the median survival time in these groups of dogs is generally considered 12 months at best. However, relatively young dogs with severe or rapidly progressing stage B2 MMVD are also suitable candidates for the surgery.

Description of the technique

The mitral valve repair technique consists in a mitral annuloplasty and artificial chordal repair via a left atriotomy.[11-13] The final goal of the technique is to restore leaflet coaptation and abolish/diminish MR. The left atrium is approached through a fourth intercostal thoracotomy and the left atrial appendage incised to visualize the mitral valve and the ruptured chordae tendineae. A double semicircular suture annuloplasty is placed in the mitral annulus around the mural leaflet to reduce and stabilize the annulus. Artificial chordae made of expanded polytetrafluorethylene (ePTFE) are sutured between the mitral leaflets and the papillary muscles. The lengths of the artificial chordae are adjusted to ensure appropriate leaflet coaptation.

In human medicine, a prosthetic ring is favored over a suture annuloplasty due to its stability and durability. [14] However prosthetic rings designed for humans are not a suitable size for small-breed dogs. The best alternative is a soft prosthetic ring made of ePTFE that can be trimmed to an appropriate size. Moreover the ring has drawbacks and potential hazards [14] and is a potential site of haemolysis or thrombosis while suture annuloplasty is free of these potential sequelae. The selection of the annuloplasty technique remains controversial and the most effective option in dogs still needs to be further investigated. [11, 12]

The main challenge in chordae replacement is the difficulty of adjusting the artificial chordae to the appropriate length. The basic principle is that during systole, with the chordae in straight lines, the apposing free edges of the mitral valve are normally at the same level (annulus level). Several methods, including application of a caliper and transesophageal echocardiography have been reported.[12, 15] Recently in human medicine, the introduction of premeasured e-PTFE chordal loops with integrated sutures for attachment to the papillary muscle and leaflet edges, facilitates mitral valve repair by eliminating the need for surgeon to create chordal loops, potentially reducing operative time. [8, 16-18] This has to be further investigated in veterinary medicine. Over the first three surgeries performed at the UVTHS, no per-operative complication occurred.

Postoperative care and follow-up

Cardiac surgery using cardiopulmonary bypass causes a systemic inflammatory response syndrome (SIRS).[19, 20], which includes IL-6 release and an elevation in WBC and C-reactive protein. Such an inflammatory response may cause acute lung injury, myocardial dysfunction, renal failure, thromboembolism and multiple organ dysfunction syndrome, which ultimately could result in postoperative mortality and morbidity. Moreover, transfusion and hemodilution enhance the inflammatory response during CBP and it is therefore important to reduce the amount of transfusion and minimize the priming volume, hemodilution and blood loss. Other postoperative complications include congestive heart failure and temporary tricuspid regurgitation, arrhythmias, thromboembolism (neurologic, peripheral, visceral, cardiac or pulmonary), diaphragmatic paralysis, intracardiac hematoma, hemorrhage, anemia, thrombocytopenia and infection. Postoperative complications are most common in the first 4 days.

Patients following cardiac surgery under CPB require thorough postoperative monitoring in appropriate intensive care units, which is another limitation to the widespread of the procedure in veterinary medicine. Patients are monitored in intensive care during the first week; they receive an initial blood transfusion (autotransfusion) and are on oxygen supplementation for the first 3 days. Intensive diagnostic tests (CBC, biochemistry, blood gases, chest X-Rays, echocardiogram, blood pressures, ECG, TEG/PT/aPTT etc…) are performed daily until discharge. Anti-clotting medications are required for 3 months postoperatively.

Regarding our first three patients, both Prince and Keisha were discharged after 1 week. Prince had an uneventful recovery while Keisha had a self-limiting peripheral thromboembolism 4 days post surgery. Charly

passed away 3 days postoperatively from thromboembolism. At 1-month and 3-months post mitral valve repair, Prince's owner reported that he was going very well at home, with normalization of his respiratory rate and effort, reduced cough, increased appetite and exercise tolerance. On cardiac auscultation he had a persistent grade 2/6 systolic murmur, which was much reduced than preoperatively (grade 5/6). A recheck echocardiography showed a reduction in the heart size and an improvement in systolic function, despite a residual minimal MR. At the 3-months recheck Prince's medication only consisted of pimobendan. Indeed reverse remodeling is quite slow postoperatively in Cavalier King Charles Spaniels compared to other breeds.

Outcome

Dogs with severe MMVD associated with CHF have a poor prognosis (<1 year) even with medical treatment. [21] Mitral valve repair can treat those patients, especially if these dogs are relative young, and have no co-morbidities. Uechi et al reported 93% survival at 38 months post mitral valve repair and long-term survival (>5 years) have also been reported. [11, 12, 22] A retrospective study compared 105 dogs treated medically to 93 dogs treated surgically between July 2016 and October 2017. In the internal medicine group, median survival times were 649 days (stage B2), 220 days (stage C), and 52 days (stage D). In all cases, the dogs died due to heart failure. In the surgical group, the discharge ratios were 100% (19/19), 93.1% (54/58), and 80% (12/15) for stages B2, C, and D, respectively. Median survival times were undefined because almost all patients survived. Survival ratios were 94.7% (18/19), 90.0% (52/58), and 73.3% (11/15) for stages B2, C, and D, respectively. These results suggest that mitral valve repair is more effective than medical therapy for the treatment of MMVD, and earlier surgery is recommended. [23]

Future perspectives

The next mitral valve repair surgeries are scheduled in October 2018 at the UVTHS and it is expected that the collaboration with the team led by Dr M. Uechi will bring more successful surgical cases in the following years. It is also hoped that the UVTHS will be able to run autonomously a mitral valve repair program in the long term.

In the last years, minimally invasive approaches have been developed for performing mitral repair without CPB in human medicine. The MitraClip is a transcather mitral valve repair system requiring a transeptal puncture and real-time guidance under angiography and 3-D transesophageal echography. The device clips together two facing portions of the septal and mural leaflets, therefore performing an edge-to-edge mitral valve repair. Transapical artificial chordeae implantation has also been introduced with a device named NeoChord DS-1000. Borgarelli et al have recently tested this device in 6 healthy Beagle and demonstrated the feasibility of this technique in dogs. [24] Techniques performed off-pump would constitute a great asset for the treatment of MMVD in Veterinary Medicine. However those previous techniques are currently only recommended in human patients who are considered too high risk for conventional surgical intervention and reported mortality rates are as high as 50% at 1 year post MitraClip mitral valve repair in patients with severe MMVD [25, 26] while only early results of transapical implantation of neochord have been published yet. [27, 28]

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Recent advances in veterinary virology

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Introduction

The purpose of this talk is to present recent advances in virology published in the mainstream literature. It is not an exhaustive coverage of all viruses and I have designed it to not overlap with presentations by other members of the College who may present their own research findings.

Feline coronavirus and feline infectious peritonitis

Feline infectious peritonitis (FIP) is a fatal disease of cats caused by a feline coronavirus (FCoV). What we have managed to discover about this virus in the last 50 years has been a slow process of scientific attrition aided by new techniques in molecular biology and immunology. Our understanding is greatly enhanced but still incomplete. In my last presentation at Science Week, I discussed the emergence of new treatment options and advances in potentially treating cats with FIP [1]. Since then progress has been made and drugs to combat the disease show promise *in vivo.* [2, 3] So what do we know about this virus and what do we still have to do? Where is the research headed?

The *Coronaviridae* are positive sense, enveloped RNA viruses. Like many RNA viruses these viruses are prone to high rates of mutation meaning there is often not one serotype or form of the virus but a large number of mutants. What we know is that carriage of feline coronavirus (FCoV) as an enteric virus (FECV) is quite high in the feline population and is higher in cats that live in high-density situations (catteries or multiple cat households). FIP is more common in cats in this situation also. The virus causes a prolonged infection and it is not uncommon for cats with the enteric form of the disease to be positive on PCR testing for long periods of time while showing no clinical signs of disease. The disease FIP appears to be associated with viral mutants arising from the FECV that are able to replicate outside of the intestine in macrophages but importantly they are also more pathogenic. It is worth noting that FECV has been found in macrophages in the blood. In one study viraemia accompanied intestinal infection and FECV was identified in blood monocyte/macrophages in 40% of experimentally FECV infected cats by day 14 and 14% remained viraemic at day 48 post-infection. [4] Additionally, FECV was detected in several internal organs after faecal shedding ceased. The production of more pathogenic FIPV is therefore more than just acquiring the capacity to replicate in macrophages. The FIPV unlike the FECV clearly has the capacity to spread systemically and cause the disease FIP. The generation of FIP causing viruses (FIPV) appears to be associated with a loss of the FIPV ability to spread to other cats by the faecal-oral route. Hence, there is usually no transmission naturally of the virus to in contact cats. However, injection of large amounts of the virus (obtained in ascites fluid) via the intra peritoneal route will cause FIP in most cats. So what is to be concluded from this? Firstly, sequencing of the FIPV genome and correlation with sequences from local FECV shows that the FIPV is likely derived from the "local" FECV. That is, local FECV and FIPV are more closely related than FIPV from different geographic locations. Put simply the FIPV arises from de novo mutation of FECV. Secondly, specific mutations that cause FIPV to be more virulent than FECV have as yet not been identified although several mutations in the spike protein (S) sequence (M1058L and S1060A) are considered indicative of systemic spread of FECV from the intestine, rather than a virus with the potential to cause FIP. [5] Conversely most FIP affected cats will have this mutation present if the S protein is sequenced.

So what is missing in our understanding of FIP?

1. What is the cellular basis of the increased pathogenicity of FIPV? This has largely been attributed to changes (thought not proven) in the S protein that enable increased replication in macrophages. Hence there is improved ability to move systemically and a loss of the ability to replicate intestinally and spread via the faecal oral route. The finding of FCoV in blood in cats without FIP and with the mutation suggests there is more to developing FIP than purely receptor switching. The search for the cellular receptor for feline coronavirus is also

complicated by there being two different types of FIPV (I and II). The less common Type II FIPV S proteins are thought to have been caused by recombination with canine coronavirus S protein. The receptor for type II viruses is aminopeptidase N (APN). The receptor for type I viruses is not currently certain but both type I and II viruses may require the molecule DC SIGN as a co receptor. [6] Just how any change in receptor tropism is related to S protein sequences is also not completely understood. In addition to receptor binding, two biochemical events are thought to be necessary to successfully induce membrane fusion at the endocytic vesicle: a protease activation or cleavage of FCoV S, and a drop of the endosomal pH. Protease activation of S is a complex process and it is suggested that each CoV has its own proteolytic requirements. Furin-like proteases are a group of enzymes that have been reported to activate several CoVs proteins, including: SARS-CoV, Middle East respiratory syndrome-CoV (MERS-CoV). Specific sequences at the S2' protease cleavage site of the S protein that allow for cellular entry, and mutations on it can lead to changes in protease activation requirements, and possibly tropism of FECV to FIPV (reviewed in [7]). Mutations in the S protein gene encoding amino acid differences in the furin cleavage motif [8] and the heptad HR1 region of the S2 subunit [9] have been correlated with disease in. Truncations of the accessory protein 3c gene have also been associated with loss of ability to replicate within enterocytes and, as such, have only been reported in FIP-associated FCoVs. [10, 11] It is clear our understanding of FIPV would be greatly enhanced by determining the receptor usage of type I FCoV and how mutations affect the cellular entry of the virus.

2. The passage from intestine to macrophages and spread systemically likely involves the infection of local dendritic cells and transport of FIPV virus to lymph nodes where macrophages become infected and spread the virus systemically. This process is poorly investigated.

3. The key element of FIP is the loss of cell-mediated immunity to eliminate the virus with ongoing antibody production. A type III hypersensitivity is produced and largely is responsible for the vasculitis and pathology produced. A consistent cytokine pattern is not determined but large amounts of the inflammatory cytokine tumour necrosis factor alpha (TNFα) are produced which is known to be pro apoptotic. Why is there a failure of the immune system to eliminate the virus? There is some evidence for genetic susceptibility of cats with suggestions of polymorphisms IFN-g and TNFα reported as possible associations with development of fatal FIP. In my opinion, the failure of the immune response to eliminate the virus is the key element of the disease. Is there a mutation in an accessory gene in the virus responsible for this also or is it a just a feature of highly efficient entry and replication of coronavirus infection in macrophages? Lymphopenia in FIP is very common and intense lymphocyte apoptosis is a feature of the disease. [12-16] Studies have shown in FIPV infected cats that NK cells and Tregs are drastically depleted from the peripheral blood, mesenteric lymph node (LN) and spleen. Furthermore, lymphopenia has recently been shown (using antiviral 3CL protease inhibitors) to be reversible. In other words, if the FIPV can be stopped, the immune system function can be restored. What causes this intense lymphocyte apoptosis? If the process could be reversed or inhibited potentially FIP might be treated. In recent studies of the Middle East respiratory coronavirus (MERS- coronavirus), similar lymphocyte apoptosis is noted. It is possible the one of more of the proteins in the FIPV are responsible for the intense lymphocyte apoptosis directly or do they just stimulate production of a product (S) responsible for the apoptosis. In a study of MERS-CoV, viral replication was not necessary to induce apoptosis in infected T lymphocytes suggesting that a process for production of apoptosis in lymphocytes may not be always associated with the virus replicating within the cells. A candidate might be the TNF-receptor-related apoptosis-inducing ligands (TRAIL) protein; this has not been tested for so far. This protein is noted to bind to lymphocytes and cause death by apoptosis. As yet studies of TRAIL are lacking in cats with FIPV. However, the apoptotic effect was induced by heat-treated effusion fluid (presumably containing some cytokine or other humoral factor) but not tissue culture fluid.[13] This suggests a factor is produced by the macrophages that are responding to the FIPV infection rather than the virus or one of its proteins. Up regulation of multiple apoptotic genes is seen in FIPV infected CRFK cells [16] but FIPV infected macrophages has not been looked at yet and would probably be more effective. The reversal of lymphocyte apoptosis would be a key element for future therapeutic strategies. Although this might be achievable by antiviral therapy, direct inhibition of the viral effects on the immune system in FIP might be possible also and may enhance the effects of antiviral therapy.

Treatment options for feline infectious peritonitis

Antiviral drugs

In general, the most successful antiviral therapies have involved drugs that target specific regions of the viral genome that regulate key processes in infection or replication. Feline coronavirus protease inhibitors have been developed against the main protease (3CL) encoded by both coronaviruses. The one showing early promise for treatment of FIP is GC376. [3] Some of the structural proteins of coronavirus are first transcribed from mRNA as polyproteins and are cleaved by cellular proteases into their constituent parts. Others required the virally encoded 3CL protease to be cleaved. These inhibitors are effective in inhibiting FIPV replication at levels that are not toxic to cells and have been used in cats with clinical FIP.

In 2018, Pedersen reported on treatment of twenty cats from 3.3–82 months of age (mean 10.4 months) with various forms of FIP in a field trial. [3] Fourteen cats presented with wet or dry-to-wet FIP and six cats presented with dry FIP. GC376 was administered subcutaneously every 12 h at a dose of 15 mg/kg. Cats with neurologic signs were excluded from the study. The drug improved most cats clinically within 2 weeks and side effects were local and transient largely. However, only 7 cats achieved remission at the time of writing and several required on going therapy after relapse. It was determined that a minimum treatment period should be around 12 weeks. Most failures of treatment were associated with neurological signs and conformation of FIP lesions in the CNS of affected cats. For cats developing neurological signs a higher dose of 15 mg/kg every 12 h was considered to be possibly more effective. In cats treated before 16–18 weeks of age retarded development and abnormal eruption of permanent teeth was noted. The anti-malarial drug chloroquine has also been shown to inhibit FIPV replication in vitro. [17, 18] In vivo, chloroquine has even shown to improve clinical scores in experimentaly infected cats but ALT levels increased suggesting hepatoxicity.[18]

Cyclosporine A has also been shown to possess anti-coronavirus activity in vitro but clinical trials are lacking. A number of immunophilins interact with coronavirus nonstructural protein 1 (Nsp1) and that cyclophilin inhibitors such as Cyclosporine are effective in vitro. [19-21] Whether combinations of drugs at lower doses are effective is hard to establish at present.

Virus inhibitory peptides

Liu et al. (2013) designed five overlapping peptides and tested these in vivo against a type II FIPV. The peptides inhibited virus replication by blocking the intercalation of the HR1 and HR2 regions, required in the activation of S protein-mediated fusion. [22] They were able to inhibit FIPV replication by 97% using one of the five peptides at a concentration <20 μM. A synergistic effect was found with human interferon-α. These peptides have not been trialed in vitro.

Anti- TNF-α treatment

Tumor necrosis factor (TNF-α) is thought to be critical in the development of FIP. Recent treatment with monoclonal antibodies against TNF-α have shown improvement in 3 experimentally infected cats. [23] The efficacy of murine monoclonal antibodies will be limited by anti mouse antibodies but the future production of "felinised" monoclonal antibodies might make this a successful approach. I note with interest the production of recombinant felinised monoclonal against FPV [24] suggesting the methodology may soon be at hand. Polyprenyl immunostimulant (PI). PI has been used in cats with suspected dry FIP. The study has shown improvement in survival times. [25]

Feline morbillivirus

The discovery and isolation in 2012 by Woo et al, of a feline morbillivirus (FMV) was a novel finding. [26] FMV RNA was detected in 56 (12.3%) of 457 stray cats by RT-PCR in that study. Complete genome sequencing of three separate FMV strains showed phylogenetically the virus clustered with other morbilliviruses. Histological examination of necropsy tissues in two FMV-positive cats revealed interstitial inflammatory infiltrate and immunohistochemical staining revealed FMV N protein-positive renal tubular cells and mononuclear cells in lymph nodes. A case-control study showed the presence of tubule interstitial nephritis in 7/12 cats FMV positive cats but only 2/15 cats without FMV infection. These findings, suggested an association between FMV and

feline kidney disease, particularly tubulointerstitial nephritis. Since this time further research has been conducted to try and elucidate the pathology if any of this virus for cats.

Since this time several studies have been conducted that suggest a global distribution of FMV. [27-31] Furthermore an association with renal disease is both supported and refuted in separate studies. For example, in a Japanese study no statistically significant correlations were found between the grades of kidney lesions and infection with FMV. However, a statistically significant relationship with the presence of inflammatory lesions and FMV infection was found but independent of the presence of tubule interstitial nephritis (TIN). Consequently, a direct relationship between infection by FMV and chronic TIN was not confirmed in this study. It was concluded that it might be possible that FMV antigens in the lesions had been previously eliminated by host immune responses in the case of severe chronic TIN.

A recent study from the UK showed that although feline morbillivirus was detected in cats there was no association between virus prevalence or seropositivity and azotaemic CKD. [32] The study used RT-PCR of urine to examine 16 azotaemic cats and 24 non-azotaemic cats serology was performed on 72 cats. The results of this study suggest that exposure to, or urinary excretion of, FMV is not associated with the azotaemic feline CKD. Additionally, other paramyxovirus sequences were obtained as in previous studies suggesting genetic diversity of FMV. However, the authors could not exclude the possibility of a causal association between FMV infection and CKD. Intermittent shedding of FMV or generation of a chronic immunopathological process leading to the development of TIN and CKD after the viral infection had been removed are possible causes. At present research into the role of FMV involving in feline renal disease is hampered by problems of case selection, power of the studies and temporal relationship of chronic renal disease to seropositive cats.

Parvoviruses

A recent study by Balboni et al. [33] has shed some light on our previous findings related to the presence or chronic carriage of parvovirus in cats. [34] Cats are known to be infected with feline panleukopenia virus (FPV) and canine parvovirus (CPV). Detection of FPV and CPV variants in apparently healthy cats and their persistence in white blood cells (WBC) and other tissues when neutralising antibodies are simultaneously present, suggest that parvovirus may persist long-term in the tissues of cats post-infection without causing clinical signs. In the study of Balboni, 54 cats from Sardinia were analysed for the presence of both FPV and CPV DNA within buffy coat samples using PCR. The DNA viral load, genetic diversity, phylogeny and antibody titres against parvoviruses were investigated in the positive cats. Carnivore protoparvovirus 1 DNA was detected in nine cats (16.7%). Viral DNA was sequence and allocated to FPV in four cats and to CPV (CPV-2b and 2c) in another four cats; one cat showed genetic complexity with mixed infection involving FPV and CPV-2c. Anti parvovirus antibodies were detected in all subjects that tested positive by PCR for parvoviruses. The identification of FPV and CPV DNA in the WBC of seropositive cats, despite the presence of specific antibodies against parvoviruses, suggests an ongoing reservoir in the bone marrow or other continuous replicating cells of the cat.

In study by Meurs et al in 2000, the question of involvement of FPV in chronic myocardial disease in cats was examined. FPV was identified by PCR in 10 of 31 cats with cardiomyopathy but in none of the controls. [35] This paper suggested a possible role in the pathogenesis of cats with inflammatory cardiomyopathies such as endomyocarditis. In a recent study by McEndaffer, 2018 [36], PCR and sequencing for the parvovirus VP1/2 gene was performed on archived heart tissue from cats with endomyocardial disease and controls. Similar methods were used prospectively on myocardial tissues from shelter-source kittens. Although 8 of 36 (22%) shelter kittens had parvoviral DNA in myocardial tissue, VP1/2 DNA was not detected in 33 adult cases or 34 controls. These findings were confirmed by in situ hybridization: adult cats did not have detectable parvovirus DNA, although rare intra nuclear signal was confirmed in 7 of 8 shelter-source kittens. These findings conflict with previous results and the role of parvovirus in cats with endomyocardial disease is still debatable.

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