Vetnostics

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The integration of the pathology laboratories of Symbion Laverty Pathology and SDS Pathology has now been completed. We have installed new equipment and software systems and are now functioning extremely effectively in ensuring the rapid turnaround of veterinary specimens. A separate specimen reception area and biochemistry and haematology analysers are being used to process veterinary samples.. We thank you for your understanding and loyalty during this time of transition.

Two evening seminars at our North Ryde laboratory will allow veterinarians to see how the laboratory is now operating. There will also be a late afternoon and evening seminar in the Western Suburbs to allow easier access for those clients in this and outlying regions. These will be at no cost to Vetnotics users as the initial low cost can be recovered by practices submitting vouchers to be used against pathology tests. Details on these seminars are being circulated.

Now that the integration is complete, Vetnostics can offer fast turnaround and a large number of tests. Cytology is available the next day after submission six days a week. In addition, in most cases (dependent on size and nature of the specimen)Vetnostics offers a 24-48 hour turnaround of histopathology specimens.

Often we get questions from vets about the risk of pregnant women acquiring Toxoplasmosis from cats (this is often generated from the clients' doctor). Sue Foster has prepared an informative document that can be handed to owners. Angela Begg and Kristen Todhunter have prepared an excellent article on culturing urine samples. Both of these can be found in this newsletter and on our website.

Last year George Reppas and Brett Stone presented an excellent seminar on how to get the most from cytology, attended by almost 100 veterinarians. They have summarised the key points of this seminar and this can be viewed on our web site (under 'Test Reference Manual' – 'Information Sheets'). This talk will be one of the topics presented at our Western Sydney seminar.

KRISTEN TODHUNTER

BS, DVM, MACVSc (pathology)

Early this year Kristen joined us as a part-time clinical pathologist working with Angela Begg at our Newcastle laboratory.

Kristen is a 1994 graduate from the Auburn University School of Veterinary Medicine. She is married to the equine surgeon Paddy Todhunter and in 1996 they moved to Australia. They have four children. Kristen has worked in general practice for 15 years as a small animal – exotic pet practitioner. She obtained her pathology training working for Angela Begg at Scone Diagnostics Veterinary Laboratory where there was an emphasis on equine gross and clinical pathology. While in Scone, Kristen was part of a group of people investigating a newly described cause of equine abortion, termed Equine Amnionitis and Fetal Loss (EAFL), caused by exposure to the processionary caterpillar. Angela has continued to assist in Kristen's training and last year she obtained her MACVSc (pathology). Kristen is also enrolled in a masters programme at the University of Queensland where she is continuing research on the pathology of EAFL.

WEB SITE www.vetnostics.com.au

This site provides detailed information on many of our tests, access to our newsletters and the ability to order collection tubes, request forms and courier pick ups (Sydney area) by filling out the appropriate form and submitting it electronically. We would encourage veterinary practices in the Sydney area, during normal hours, to try ordering courier pick ups electronically. The username and password required for the restricted areas is the same as those that are used to obtain online results from our mainframe computer. Should you encounter any problems please contact Bruce Duff on (02) 9005 7474 and he will direct the enquiry or solve the issue. Many informative scientific articles are present, including some past issues of our newsletter.

TOXOPLASMOSIS - IS IT A CONCERN?

Toxoplasma gondii is a protozoan parasite that infects virtually all warm-blooded animals including humans. Domestic cats are the definitive host for the parasite but infection does not necessarily result from contact with cats or cat faeces.

When cats become infected with toxoplasmosis they pass unsporulated (non-infectious) oocysts (eggs) in faeces for 1 – 2 weeks. These non-infectious eggs mature in 1 – 5 days to become infectious (sporulated oocysts) and the sporulated oocysts survive for months to years. People can become infected by ingestion of these sporulated oocysts in contaminated soil or water.

However, most people become infected from ingesting the tissue cysts in meat. When animals (and people) become infected with toxoplasmosis, they may end up having tissue cysts in their body. Ingestion of poorly cooked meat (usually pork, goat or lamb) or failing to wash hands thoroughly after handling raw meat is probably the most common means of human infection with toxoplasmosis.

The majority of people never realize that they have been infected as the signs are self limiting fever, enlarged lymph glands and malaise (just not feeling well). However, toxoplasmosis can cause serious disease in the unborn foetus, a major concern for pregnant women, and can also cause severe disease in immunosuppressed people (eg on chemotherapy or with AIDS). In immunosuppressed people, infection is usually due to a reactivation of tissue cysts in the person's own body (eg from prior chronic infection not new infection). In pregnant women, infection of the foetus occurs after acute (new) infection. Stillbirths and serious foetal damage (especially eye and brain damage) can result.

Touching cats is an extremely unlikely way to acquire toxoplasmosis and because of this, there is no correlation with toxoplasmosis and cat ownership. Similarly, veterinary health care providers are no more likely than the general population to be infected with toxoplasmosis, and people with HIV infection who own cats are no more likely to acquire toxoplasmosis than those who do not. There is one obvious conclusion from this:

THERE IS NO REASON TO REMOVE CATS FROM THE HOUSES AND LIVES OF PREGNANT WOMEN.

There is every reason to take sensible precautions:

1. wear gloves when gardening and wash hands thoroughly after any gardening
2. wash any produce from the garden very carefully
3. gloves should be worn whenever raw meat is handled and hands washed thoroughly afterwards
4. litter boxes should be cleaned daily to prevent any sporulated oocysts (it takes at least 1 day for eggs to become infective). Removing sporulated (infective) oocysts is very difficult but if oocysts do not have time to sporulate, it is not a problem
5. ideally, immunosuppressed people or pregnant women should not be cleaning the litter box but where this is impractical, ensure any faeces are removed promptly, ensure the litter box is not left longer than 24 hours before cleaning, use gloves when cleaning the litter box and wash hands thoroughly after removing the gloves
6. washing hands thoroughly after any cat handling

NO CAT SHOULD EVER HAVE TO BE EUTHANASED OR REHOMED DUE TO HUMAN PREGNANCY.

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Registered feline specialist (WA)

References:

1. Feline ZoonosesGuidelines from American Association of Feline Practitioners.
   Journal of Feline Medicine and Surgery 2005: 7, 243 – 274
2. Toxoplasmosis. By Dubey JP and Lappin MR In: Green CE (ed) Infectious Diseases of the Dog and Cat, Saunders, Philadelphia, 1998: 493-503

URINE CULTURES

Urine culture can be an invaluable tool in the diagnosis and treatment of acute and recurrent urinary tract infections in dogs and cats. Bacteria most commonly associated with urinary tract infections in the dog are *Escherichia coli, Klebsiella, Staphylococcus, Enterococcus, Proteus, Pseudomonas, Enterobacter,* and *Streptococcus*[1]. *E. coli, Enterococcus faecalis,* and *Staphylococcus felis* (coagulase-negative) are the most common isolates from feline urinary tract infections[2,3]. Proper sample collection and transport is essential for getting a diagnostic culture result. Voided samples are generally considered contaminated due the presence of bacteria from mid to distal urethra, vaginal vault, and prepuce. Cystocentesis is the gold standard method to collect samples for culture and/or cytology. Catheterised samples are second best. Samples should be collected in an aseptic manner and transported in a sterile container (not syringes) for culture as soon as possible. Refrigeration before submission to the laboratory is essential for preservation of cell integrity and to prevent bacterial multiplication. Delays of greater than 12 to 24 hrs in processing compromises results. It is not advisable to place urine in a media bottle for culture even when the animal has been on antibiotics as any normal flora or contaminants can easily overgrow significant bacteria or a single bacterium can grow to large numbers and made to look significant even when it is not.

Contamination of cystocentesis samples can occur from improper technique (skin flora), or inadvertent sampling of gut (mixed bacteria). Results should always be correlated with clinical history and presence of leucocytes within the urine (sign of urinary tract inflammation in response to bacteria). Greater than 10 white blood cells per high-powered field (wbc/hpf) is considered significant. Growth of bacteria without indications of inflammation in the urine (<10 wbc/hpf) should be interpreted in relation to history and clinical findings in the individual patient since other disease conditions like hyperadrenocorticism and FIV infection can suppress the inflammatory response in affected animals.

We perform quantitative culture using calibrated loops and a variety of agar plates, some quite specialized. Each plate is incubated overnight before examination and reporting. Negative cultures are incubated another 24hrs. Urines with greater than 10 WBC/ul will have a direct sensitivity test performed. Initial antibiotics tested for dogs and cats are enrofloxacin, amoxycillin/clavulanic acid, sulpha/trimethoprim, cephalexin, ampicillin (extrapolate for amoxycillin as well) but others will be tested for specific isolates. Samples are also cultured for purity and if the culture grows mixed bacteria, the sensitivity is repeated for the individual bacteria in pure cultures.

Urine culture results are correlated with microscopy findings and significance is assessed according to method outlined by Love[4] and Barteges[5]. In brief, most isolates, equal or greater than 10^6/L in cystocentesis samples are considered significant unless mixed with greater than 2 bacteria in samples without inflammation. If samples are very mixed, then samples may have been contaminated at collection but this needs to be correlated with the clinical history for significance. For example if the animal has had an indwelling urinary catheter, this may allow ascent of bacteria into the bladder and a significant mixed infection in a cystocentesis sample. In voided samples, mixed cultures less than 10^8/L in dogs and less than 10^7/L in cats are more often contaminants although there is evidence that significant urinary infections in cats can involve lower bacterial counts[5,7]. Please tell us what the collection method is so we can appropriately assess significance.

Sensitivity testing is performed using disc diffusion susceptibility (CLSI – an international guideline for testing veterinary antimicrobial agents based on the Kirby-Bauer method). After 16-18 hours of incubation on specialised agar, the diameters of the zones of complete inhibition are measured to the nearest millimeter. Zone size limits are outlined in the CLLS method and the organism is reported to be either susceptible or resistant. Intermediate susceptibility is available for some antibiotics. These are normally reported as resistant. However, with the increased prevalence of multi-resistant bacteria in recurrent UTI's, certain antibiotics that fall into the intermediate category may be effective in recurrent UTI's due to increased concentrations of the antibiotic in the urine. We are currently looking at changing reporting on these antibiotics for multi-drug resistance. If your urine culture report does not identify any intermediate sensitivity results for multi-drug resistant organisms please enquire about this with one of our pathologists. Detection of multi-drug resistance is complicated and if we suspect this, further testing may be required to verify some initial reported sensitivity results.

We can perform Minimum Inhibitory Concentrations (MIC), the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism for many organisms and antibiotics. This may be of use in urinary infections where some antibiotics excreted by the renal

system can achieve greater than 10x MIC in urine. There is an additional charge for MIC determination of specified antibiotics in addition to the standard culture and sensitivity. The benefit is determining whether certain antibiotics may be appropriate to use because of higher achievable urinary concentrations on cultured organisms that appear resistant. Increasing we are seeing urinary bacterial isolates with multi-drug resistance. These include *Enterobacteriaceae (Klebsiella spp, E coli* and others) with extended-spectrum β-lactamases (ESBLs), staphylococci with methicillin-resistance, highly resistant *Enterococcus* spp and *Pseudomonas* spp. Affected animals typically have a history of concurrent disease (hyperadrenocorticism, diabetes mellitus, recurrent urinary infections, multiple antibiotic use or debilitating disease). ESBLs are enzymes that mediate resistance to extended-spectrum (3rd generation) cephalosporins like Convenia®, ceftazidime and cefotaxime and this infers resistance to most beta-lactam antibiotics. The development of multi-drug resistance (MDR) by bacteria is a complex discussion beyond the scope of this article; however, a predisposing factor is empirical antimicrobial use (more prevalent with certain antimicrobials eg fluroquinolones) as well as exposure to multiple antibiotics. Prevention of the development of MDR bacteria is suggested by ensuring the necessity of antimicrobials for treatment of a urinary problem (ie correct clinical signs and positive urine culture from cystocentesis sample not voided samples), choosing the correct antimicrobial for the cultured pathogen, and ensuring that adequate drug concentrations are reached within the urinary tract for the treatment period required[6]. Therapeutic culturing of urine (3-7 days after instigating treatment and while on therapy) can be of use in certain situations to ensure the selected antibiotic is achieving the goal of killing the pathogenic bacteria involved in the urinary tract infection[6].

Convenia® (cefovecin sodium) is a new extended-spectrum (3rd generation) bactericidal cephalosporin antibiotic developed for treatment of skin and urinary tract infections in dogs and cats. In Australia, it is labeled for use in urinary tract infections in dogs associated with *E. coli, Proteus mirabilis,* and *Staphylococcus intermedius* and urinary tract infections in cats associated with susceptible strains of *E.coli*. It is not effective for urinary tract infections associated with *Pseudomonas* spp, *Enterococcus* spp., or *Enterobacter* spp. Vetnostics is currently developing guidelines for adding this antibiotic to the sensitivity panel of urine cultures for dogs and cats when indicated by culture results. In the mean time, a general rule is that if the bacteria are reported as sensitive to cephalexin it will be sensitive to Convenia. However amoxycillin is still one of the most useful drugs in treating UTI and is less likely to induce MDR. Convenia, amoxicillin-clavulanate and fluoroquinolones should really only be used when first-line antimicorbials such as amoxycillin are not appropriate and on the basis of culture and sensitivity due to the concern re emerging MDR.

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3. Lister A, et al (2009) Occult bacterial lower urinary tract infections in cats-urinalysis and culture findings Vet Micro in press
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7. Eggertsdottir AV et al (2007) Bacteriuria in cats with feline lower urinary tract disease: a clinical study of 134 cases in Norway, J Fel Med Surg 9, 458-465

Article by Kristen Todhunter and Angela Begg

Most of the above articles are available in pdf format from Angela.Begg@symbionhealth.com