CITYU VETERINARY DIAGNOSTIC LABORATORY

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城大動物醫療檢驗中心

MESSAGE FROM THE DIRECTOR

Welcome to issue 3 of the 2019 CityU VDL newsletter. Thank you for all your interesting submissions and support of the laboratory.

In this newsletter we provide more information on new test offerings, special thyroid testing pricing, canine Leptospirosis cases in 2019, some testing tips and an interesting case of uveitis in a rabbit and heartworm in a cat.

Our Hong Kong based pathologists enjoy assisting practitioners with case investigations and welcome your contact by telephone or email to discuss cases.

- Dr. Fraser Hill, Anatomic Pathologist, Director of CityU VDL

IN THIS ISSUE

What's new at CityU VDL

Encephalitozoon cuniculi tests

CityU VDL has introduced an ELISA plus IgM and IgG IFA to measure antibodies produced against E cuniculi infection in rabbits. The ELISA demonstrates if the rabbit has been exposed to the pathogen or not while the IgG and IgM help establish how recent infection was.

Thyroid Testing Autumn Promotion

Don't miss the special pricing on T4, free T4 and TSH testing this autumn. Take the opportunity to thoroughly investigate those hypothyroid or hyperthyroid cases during this promotional period.

For details and pricing contact the laboratory by calling 3442 4849 or by email infovdl@cityu.edu.hk

Leptospira interrogans serogroup Hebdomadis infection in Hong Kong: 2019 cases

In 2018, ten cases of leptospirosis in Hong Kong dogs were diagnosed during the rainy season. So far in 2019, 30 confirmed cases of *Leptospira interrogans* infection have been identified by PCR on either urine or EDTA blood samples. In seven cases where follow-up serum samples were available for testing by microscopic agglutination titre (MAT), only antibodies to *Leptospira interrogans* serogroup Hebdomadis were found. MAT to 14 other serogroups were negative. Additional information from 19 dogs found nine survived, while 10 had died of the infection or complications. The clinical signs included anorexia, lethargy and vomiting and occasionally jaundice. If you suspect leptospirosis in any of your patients collect EDTA blood and urine for PCR testing (test both as results vary depending of the length of infection). Follow up a few weeks later with MAT serology to confirm the infective serogroup, as this influences vaccination strategies. Support from the Jockey Club College of Veterinary Medicine and Life Sciences has allowed sequencing of some of the isolates to be undertaken and assisted with MAT serology testing. Research projects are being planned to investigate the specific epidemiology of leptospirosis infection in Hong Kong.

For a recent report see https://www.scmp.com/lifestyle/health-wellness/article/3027072/hong-kong-dog-owners-warned-be-vigilant-around-water

TESTING TIPS

Collection tube type

It is important to use the correct type of tube for a designated test. Complete blood counts (CBCs) should be evaluated on samples collected into EDTA tubes for mammals (or alternatively heparin). A disadvantage of heparin is the occurrence of platelet clumping, falsely decreasing the platelet count. Additionally, after exposure to heparin, cells stain with a pinkish hue making cell morphology evaluation difficult. For birds and reptiles, the preferred anticoagulant is heparin, as blood from these species will hemolyse after exposure to EDTA.
Either serum or plasma from a sample collected into a plain (without anticoagulant) or heparin tube, respectively, are adequate specimens for a chemistry panel. Chemistry panels **should not be done** on plasma from blood collected into EDTA. Sodium citrate tubes are generally used for hemostasis testing.

Feel free to contact the CVDL at 3442-4849 if you have questions about what tube to use.

Post-prandial lipaemia

Lipaemia is characterized by the turbid to milky appearance of plasma or serum and is secondary to the presence of large lipoproteins, especially chylomicrons and very low-density lipoproteins (VLDLs). Lipaemia can be physiologic or secondary to metabolic disturbances (e.g. Diabetes mellitus, hyperadrenocorticism). Physiologic lipaemia occurs after a meal (post-prandial lipaemia) and consists of chylomicrons derived from food; therefore it can be minimized by implementing a 12 h fasting period prior to blood collection. The lipaemia caused by metabolic disturbances is unfortunately unavoidable, but the testing laboratory can employ techniques such as repeated high-speed microcentrifugation or clearing agents to minimize it as much as possible.

Lipaemia will cause a false increase in hemoglobin and consequently a false increase of the red cell indices calculated using hemoglobin: MCH and MCHC. Changes in red cell morphology may be seen, in which red cells acquire a fuzzy appearance and angular shapes. Lipaemia also predisposes red cells to in vitro hemolysis. Lipid-droplets may be mistakenly counted as platelets and total protein by refractometry is also inaccurate (false increase). Lipaemia will affect chemistry analytes in different ways due to interference with photometric measurements or water displacement for electrolytes.

Sample volume

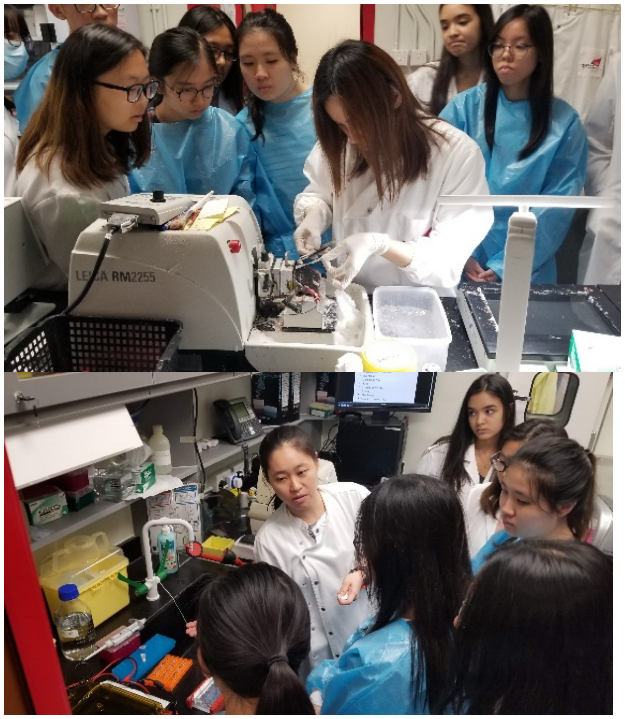
A frequent but preventable pre-analytical error is the under-filling and less frequently overfilling of EDTA or citrate tubes with blood. For example, excess EDTA (due to under-filling with blood) will alter the red blood cell indices, typically resulting in low MCV and/or high MCHC. Under-filling of citrate tubes prolongs the times of clotting assays. Overfilling the tubes, will result in clotting of the specimen. Therefore, the person collecting the sample should always attempt to fill the tubes at the appropriate ratio to avoid inaccurate results. This is typically accomplished by filling the tubes to the indicated mark on the collection tube. Additionally, there are different sizes of tubes available, including EDTA and citrate tubes that only require about 0.5 mL (500 uL) of blood. Maintaining the appropriate ratio of blood to anticoagulant is especially important for hemostasis testing.

Container size for biopsy submission

For ideal biopsy sample fixation and preservation, the tissue biopsy sample should be placed in 10% formalin at a ratio of 10:1 (ie for every 1 volume of tissue put 10 times the volume of formalin). For very large samples, this can seem daunting. CityU VDL can provide 5L containers of formalin, 1.5, 3, 5.6 and 10 L plastic pails to store and submit the samples. Please call the laboratory to request order placement and pricing. Use containers with wide necks so samples can be removed easily once fixed. Only seal containers with parafilm and avoid using sticky tape. For transportation, handling and staff safety, please do not submit samples in glass containers.

VETERINARY EDUCATION: A KEY ROLE FOR THE LABORATORY

Teaching current and future veterinary students is an important component of work undertaken at CityU VDL. In the pictures on the right, technologists Miss Candy So (top) instructs final year secondary school students in the procedure for cutting histopathology slides and Miss Chu Lai On (bottom) demonstrates the preparation of a gel for PCR testing.



INTERESTING CASES

Encephalitozoon cuniculi induced phacoclastic uveitis in a rabbit (Dr Fraser Hill)

Clinical History: A two-year-old, female spayed rabbit was present for veterinary attention and had a six month history of phacoclastic uveitis. Treatment with anthelimintics and antibiotics had been ineffective so eye enucleation was performed and the eye submitted for histopathology.

Histopathology found inflammation centred on the iris and cilary body (figure 1) associated with a dense infiltrate of heterophils mixed with fibrin, necrotic debris and multinucleated giant cells containing lens remnants. Inflammatory cells and debris spilled into the aqueous humour and posterior chamber and were adhered to the lens and also Descemet's membrane. Gram stains revealed gram positive spores within the inflammatory infiltrate and lens.

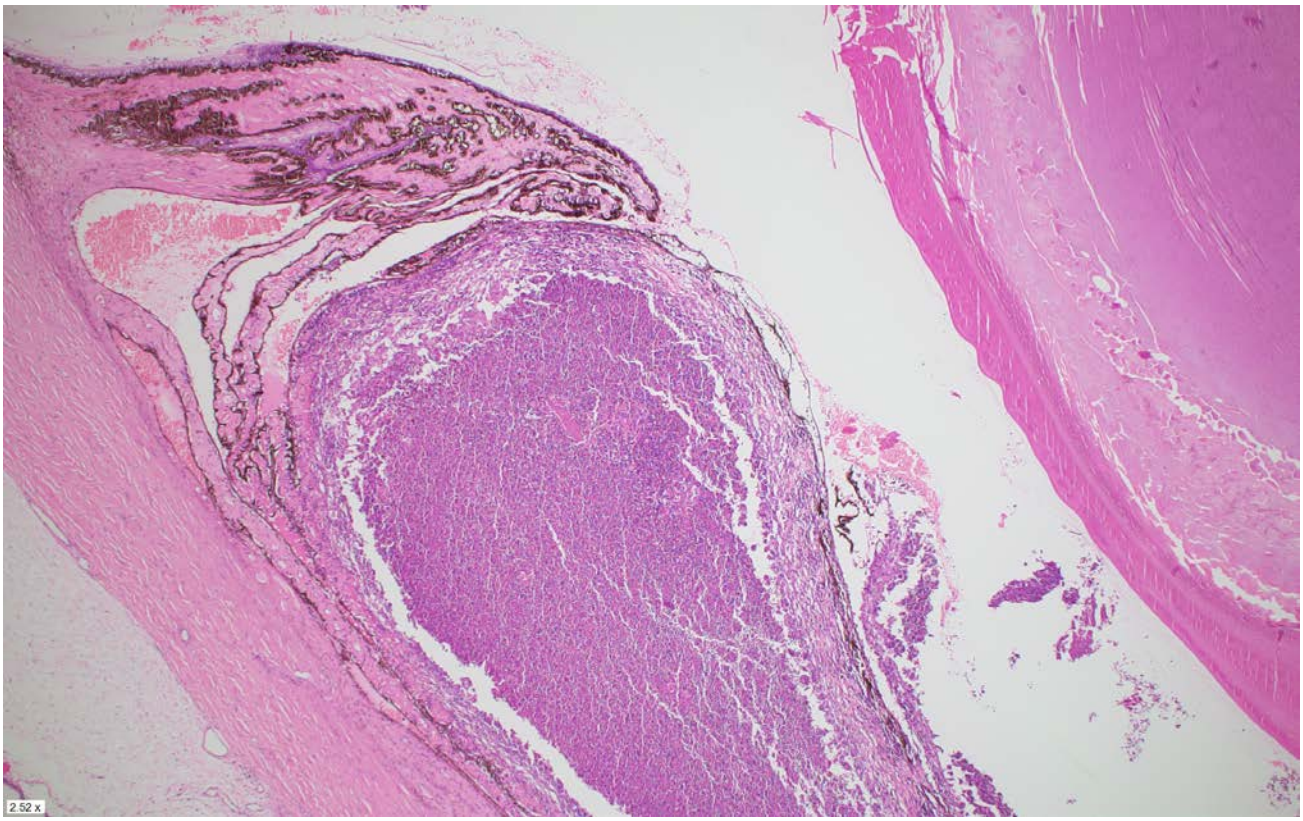


Figure 1. Cilary body on the left filled with inflammatory cells and the lens on the right (HE stain 40 x)

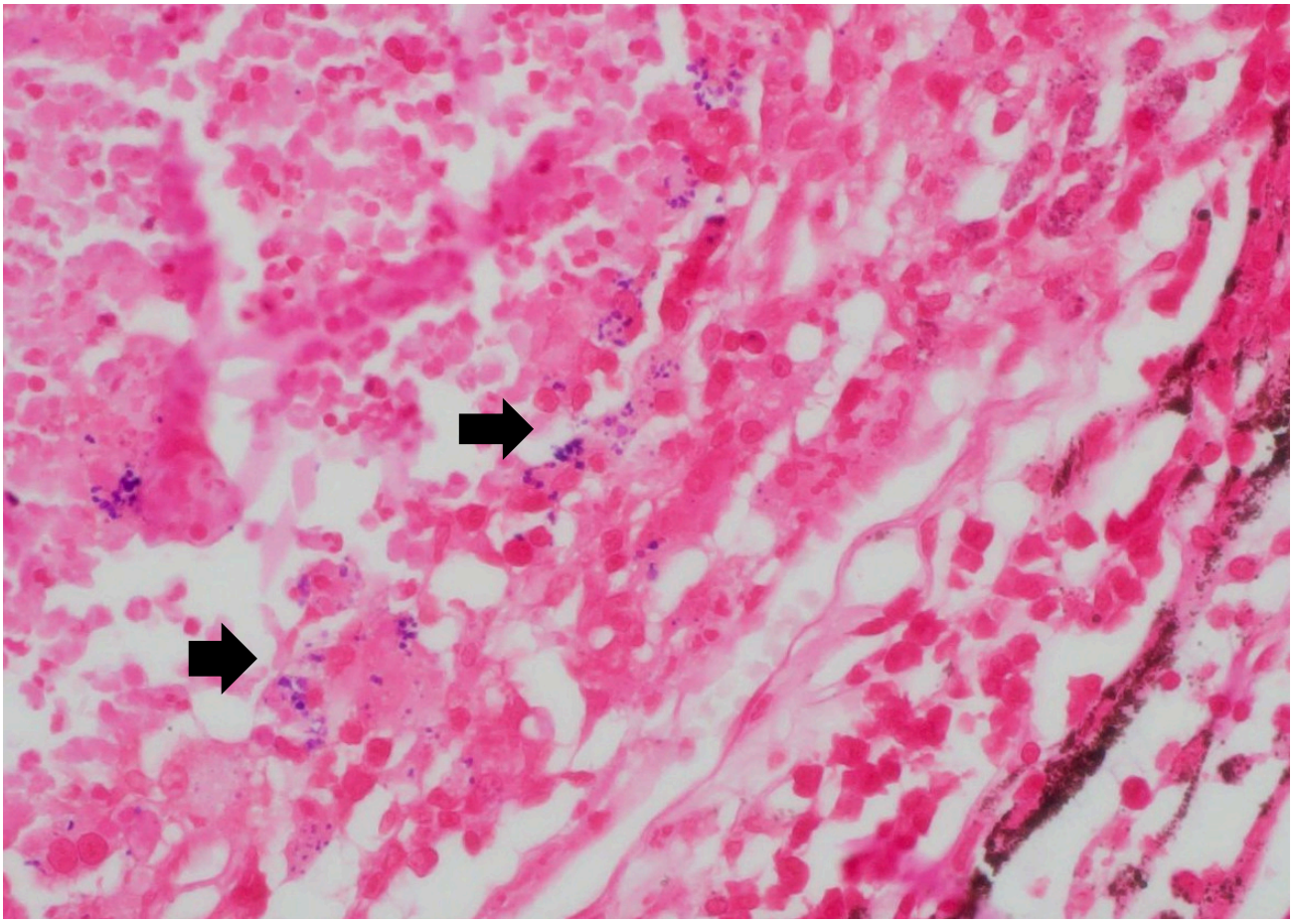


Figure 2. Gram stains revealed gram positive blue staining *E cuniculi* organisms (arrows) in the affected area (400 x).

Encephalitozoon cuniculi is an opportunistic, emergent, zoonotic, microsporidian parasite infecting a number of different species of mammals and humans. It can infect both domestic and laboratory rabbits, resulting in chronic interstitial nephritis, granulomatous encephalitis and phacoclastic uveitis (1). *E cuniculi* spores can be identified in paraffin-embedded, HE stained sections although they can be obscured by inflammatory debris. Gram and modified trichrome stains were concluded to be the best stains for enhancing spore detection (2).

In one study of 144 seropositive rabbits with clinical signs, 75% showed neurological symptoms, 14.6% demonstrated phacoclastic uveitis and 3.5% suffered from renal failure. (3)

Ocular *E cuniculi* infection can be responsible for cataract and lens-induced uveitis. Inflammation occurs in response to the release of lens protein after lens capsule rupture. Histopathological investigation of four rabbits with eye lesions found pyogranulomatous inflammation encasing an anterior or polar lens capsule break. Heterophils, macrophages and multinucleated giant cells containing lens fiber remnants were all visible. Occasional spores were detected by immunohistochemistry within lens epithelial cells (4).

The most frequent route of infection for *E. cuniculi* is probably through ingestion of spores shed in urine of infected animals and haematogenous spread via infected monocytes, but little is known about the pathogenic mechanisms of the disease. How *E. cuniculi* gains access to the lens is poorly understood. The lens is an avascular segregated compartment in the adult and it is thought there may be early infection in rabbit kits, when the lens has a thin capsule and rich vascularity (4).

Treatment with various therapies often proves ineffective although phacoemulsification of infected lenses has been successfully undertaken in rabbits with slowly progressive unilateral phacoclastic uveitis and cataract formation (5).

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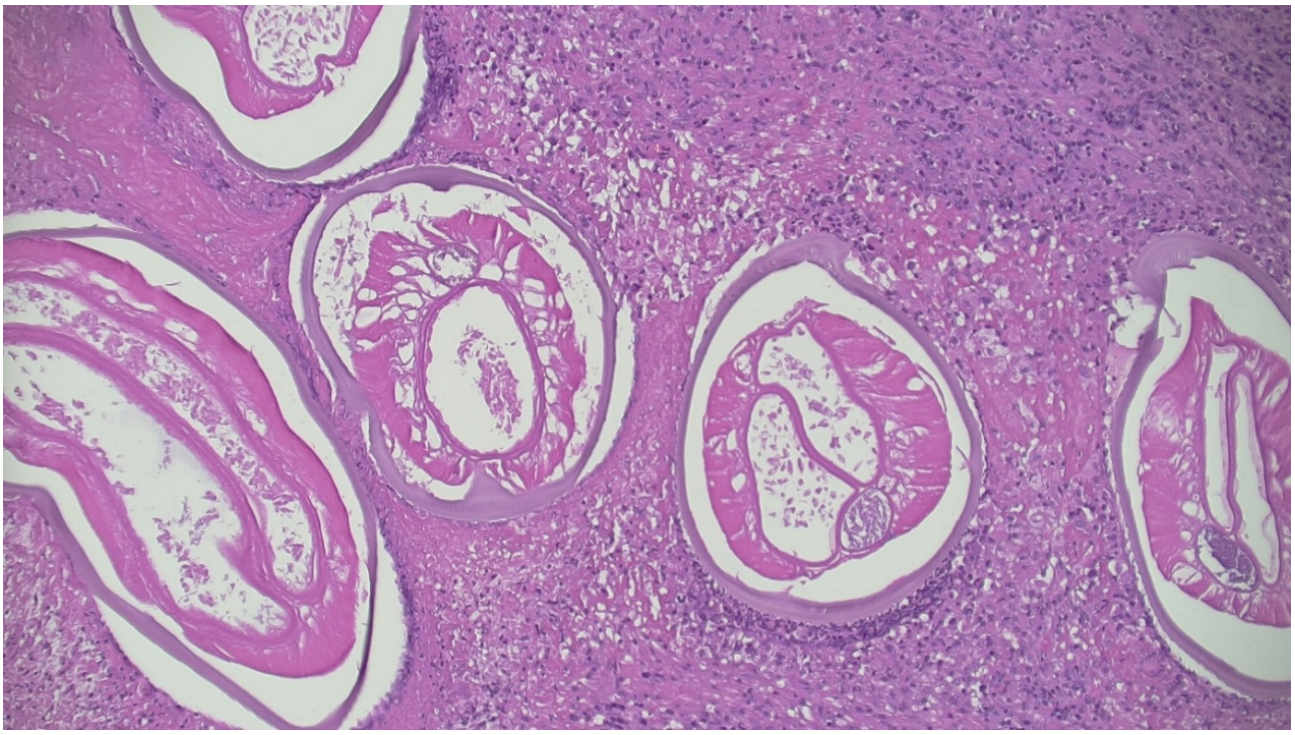
(1) Künzel, F., & Joachim, A. (2010). Encephalitozoonosis in rabbits. Parasitology research, 106(2), 299-309.
(2) Rodríguez-Tovar, L. E., Villarreal-Marroquín, A., Nevárez-Garza, A. M., Castillo-Velázquez, U., Rodríguez-Ramírez, H. G., Navarro-Soto, M. C., Trejo-Chávez, A. (2017). Histochemical study of Encephalitozoon cuniculi spores in the kidneys of naturally infected New Zealand rabbits. Journal of Veterinary Diagnostic Investigation, 29(3), 269-277.
(3) Künzel, F., Gruber, A., Tichy, A., Edelhofer, R., Nell, B., Hassan, J., Joachim, A. (2008). Clinical symptoms and diagnosis of encephalitozoonosis in pet rabbits. Veterinary parasitology, 151(2-4), 115-124.
(4) Giordano, C., Weigt, A., Vercelli, A., Rondena, M., Grilli, G., & Giudice, C. (2005). Immunohistochemical identification of Encephalitozoon cuniculi in phacoclastic uveitis in four rabbits. Veterinary ophthalmology, 8(4), 271-275.
(5) Felchle, L. M., & Sigler, R. L. (2002). Phacoemulsification for the management of Encephalitozoon cuniculi-induced phacoclastic uveitis in a rabbit. Veterinary ophthalmology, 5(3), 211-215.

Feline Heartworm Disease (Dr May Tse)

A three-year-old male neutered Domestic Short Hair cat presented with weight loss and vomiting. On physical exam there was a well encapsulated mass present in the right scrotal area the owner had noticed over the past two months. The cat was desexed at two years old. The mass was submitted to CityU VDL for histopathology. The mass (as show below) is composed of dense infiltrates of eosinophils, macrophages, and fewer lymphocytes admixed with clusters of cellular debris and fibrin. In the core of the inflammatory cells and debris are several to multiple, cross and tangential sections of adult nematodes consistent with Dirofilaria immitis (heartworm).



The cat is an atypical host for heartworms. Mosquitoes are the vector for transmission. Cats may become infected with heartworm and are typically microfilaremic or afilaremic due to the low number of adults (often one) and high frequency of filarial male-only infections, hence this can result in false negative blood tests for microfilariae and adult heartworm antigens. Tests for detection of antibodies to heartworm can be useful in recent infections with high sensitivity.

Heartworms in cats may migrate to other parts of the body, such as the brain, eye and spinal cord, systemic arteries and subcutaneous tissue. Inflammation can result when the adult worms die in the tissue of the cat (most likely the cause of the scrotal mass in this animal). Clinical signs in cats include coughing, dyspnea, vomiting, and neurological signs, or sudden death. The clinical signs of weight loss and vomiting in this case is most likely associated with heartworm infection, warranting further clinical investigation. Heartworm infection is endemic in dogs in Hong Kong. Even the so-called “indoor” cats may also be considered at risk. Monthly heartworm preventives are a safe and effective option for cats living in Hong Kong.

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4. American heartworm society. (2018). Heartworm in cats. Retrieved from https://www.heartwormsociety.org/heartworms-in-cats

CityU VDL thanks the submitting veterinarians for these interesting cases.

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