香港城市大學
City University of Hong Kong

VETERINARY DIAGNOSTIC LABORATORY

CITYU VETERINARY DIAGNOSTIC LABORATORY

MESSAGE FROM THE DIRECTOR

Welcome to the first edition of volume five of the newsletter.

Despite COVID-19 infections affecting CityU VDL staff, either with a primary infection or as a close contact, the laboratory has stayed open throughout the fifth wave of the pandemic. Thanks to all the hardworking staff at CityU VDL who have rallied to keep all sections open and worked extra time to keep processing samples so we can report results.

Thank you for your support of CityU VDL as you manage the challenge of COVID-19 infections in your practices.

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

IN THIS ISSUE

SNIPPETS

- Bromide for pharmaceutical use is dispensed and described as potassium bromide. It dissociates in the blood and the active agent is bromide and this is the compound tested for. Hence, while the test is actually bromide analysis, it is a measure of the therapeutic concentrations of the potassium bromide given.

- **How do I submit a sample for anaerobic culture?**

  Samples should be collected in a strictly aseptic manner and submitted to the laboratory using specialised collection receptacles that provide an anaerobic micro-environment e.g. BBL Port-A-Cul tubes for swabs and tissues and blood culture bottles for fluids. If a specialised receptacle is not available, a standard gel tube or a small sterile container for tissue may be used; however, it is important that these samples arrive at the lab promptly. Samples for anaerobic culture should be kept at room temperature and not refrigerated.

TESTING TIPS

Faecal parasite testing of dogs now available

After validation at CityU VDL, we can now offer parasite screening in faeces of dogs by PCR. The PCR can detect the presence of eggs of a number of gastrointestinal parasites of dogs including *Toxocara canis, Ancylostoma caninum, Uncinaria stenocephala, Trichuris vulpis* and *Dipylidium caninum.* For a screening test, results are provided as detected or not detected. If parasites eggs are detected and individual parasite identification is required, individual PCRs would be required.

Samples to collect: Fresh faeces in sample pot

Turnaround time: 3-5 days

Antimicrobial sensitivity testing - **By Dr Andrew Ferguson**

When CityU VDL performs **A**ntimicrobial **S**usceptibility **T**esting (AST), it follows the standards and guidelines established by Clinical Laboratory Standard Institute (CLSI).

The goal of CLSI is to promote accurate and reproducible AST results, along with standardised reporting and interpretation. A CLSI sub-committee for veterinary antimicrobial susceptibility testing has established guidelines for veterinary diagnostic laboratories including: testing methods, bacterial density, media and drug types, drug dilutions, incubation times, quality control requirements, and interpretative criteria.

The make-up of the antimicrobial panels that CityU VDL offers takes into account these guidelines and also takes into account the type of bacteria and the location of infection. CLSI guidelines are not available for all bacterial species as breakpoints have not been established for certain veterinary pathogens and in some instances, we extrapolate from human pathogens.

We have discovered very high levels of resistance in some bacteria grown from samples submitted to CityU VDL and in these cases we recommend performing a **M**inimum **I**nhibitory **C**oncentration (**MIC**) assay. MIC testing is more accurate and reliable than the standard disc diffusion, may clarify where there is an inconclusive result, and offers an extended panel of antimicrobials. You can request to perform an MIC at the same time as you submit the sample for culture or alternatively, you may add on an MIC test if your reported results indicate an organism has a high level of resistance (noting that the request to the lab for an add-on MIC needs to be made within 48hrs of the report release).

Turnaround time: 2-5 days, depending on speed and amount of bacteria grown.

Tritrichomonas foetus infections of cats in Hong Kong diagnosed by PCR

Tritrichomonas foetus is a trichomonad protozoan infection spread by faecal oral infection between cats. Once ingested, it localises in the large intestine and clinical signs of chronic diarrhoea are manifested. All ages of cats can become affected but most of the cases identified at CityU VDL are in cats less than one year of age (figure 2).

If a cat has chronic diarrhoea, collect a faecal sample for PCR testing to quickly identify if *T foetus* is present or not.

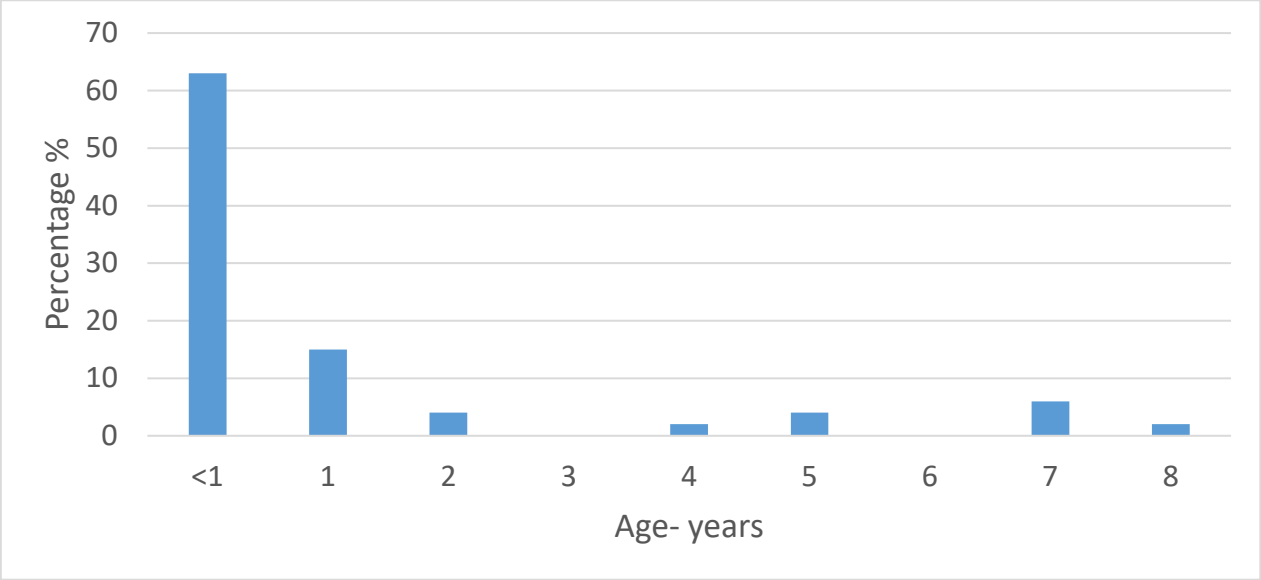

Figure 1: Frequency of age of *T foetus* diagnosis by PCR

Analysis of the data suggested there was no specific breed prediliction, although the majority of cases were seen in British and Domestic short haired, reflecting their popularity in Hong Kong (figure 3).

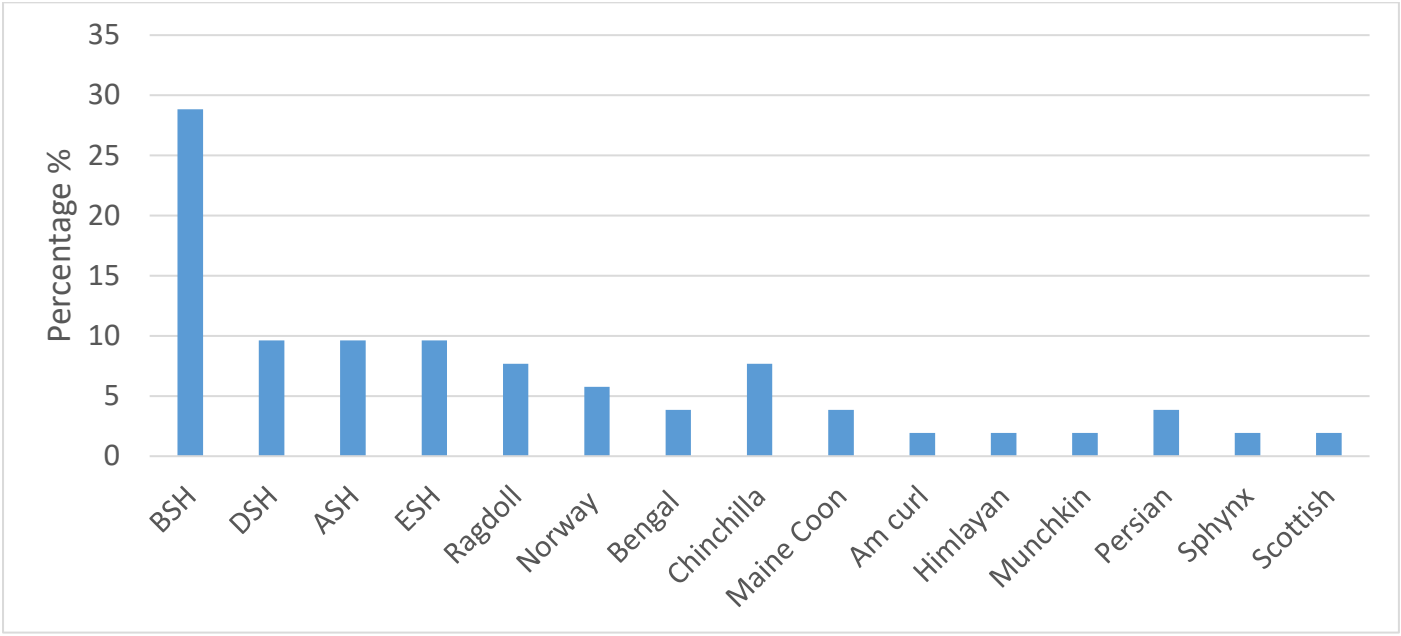

Figure 2: Frequency of PCR diagnosis of T foetus by breed

Infections were confirmed all year round, although there were peaks of diagnoses in spring and summer.

After therapy with ronidazole is instituted, it is recommended to repeatedly PCR test the faeces of treated cats for up to 20 weeks to confirm elimination of infection. In multiple cat households, regular removal of faeces, plus cleaning and disinfection of litter boxes is recommended to prevent spreading the infection.

Recent Cases

Anaplasma platys and *Hepatozoon* spp. canis in a dog

- By Dr Daniela Hernandez Muguiro

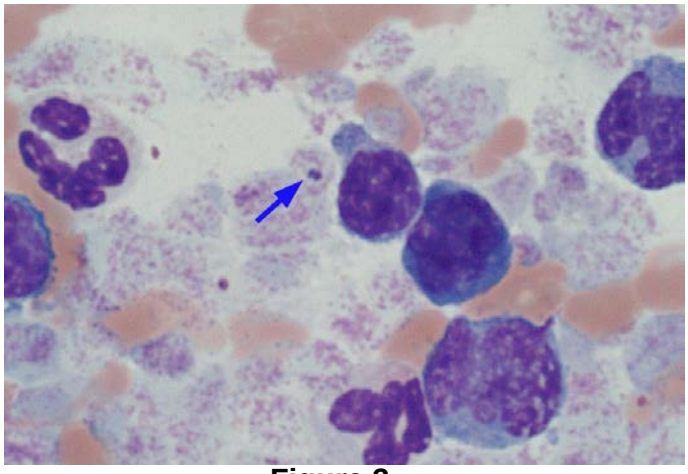

Figure 3

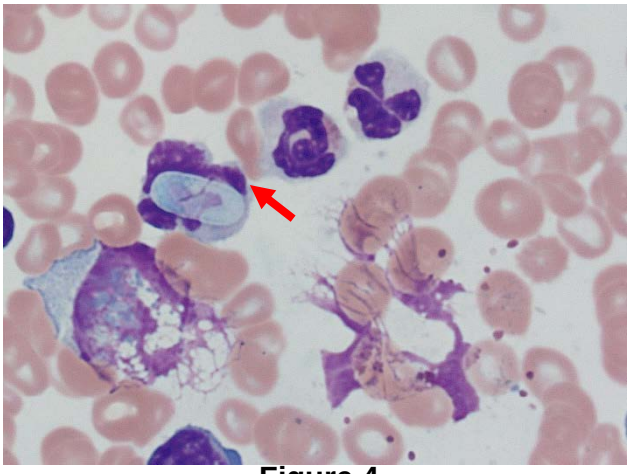

Figure 4

Figures 3 and 4 are from a buffy coat smear from 1-month-old, female, mongrel dog. EDTA blood was submitted for a "Comprehensive tick-fever PCR panel" that tests for *Anaplasma* spp., *Babesia canis*, *Babesia vogeli*, *Babesia gibsoni*, and *Ehrlichia canis*. The PCR test results were positive for *Anaplasma* spp. Out of interest, direct and buffy coat smears from the EDTA blood were prepared and examined. Rare small morulae consistent with *Anaplasma platys* (figure 3, blue arrow) within platelets were seen on the buffy coat smear. Additionally, rare *Hepatozoon* spp. sausage-shaped gamonts within leukocytes, either a neutrophil or monocyte, were found (figure 4, red arrow). No parasites were seen on the direct blood smear despite careful searching. Both *A. platys* and *Hepatozoon* spp. are transmitted by ticks. *A. platys* is transmitted by *Rhipicephalus sanguineus* during feeding. There has been reports of *A. platys* detected in *Haemaphysalis longicornis* and *Rhipicephalus haemaphysaloides* ticks suggesting they play a potential vector role for *A. platys*. A few cases of canine hepatozoonosis have been seen now in Hong Kong, which have been secondary to *Hepatozoon canis*. Dogs become infected with *H. canis* after eating infected *Rh. sanguineus* ticks.

A. platys causes a cyclic parasitaemia characterized by a concurrent thrombocytopaenia (2-3 weeks post-infection) that may be severe (< 20 x 10^9/L). The thrombocytopaenia lasts 3-4 days, before rapidly increasing. This tend to occurs at 1-2 weeks intervals. Infected dogs may not show clinical signs, or clinical signs may be mild, with minimal or no evidence of hemorrhage. Fever, lethargy, lymphadenopathy, uveitis, and bleeding diathesis including epistaxis, petechiae, and ecchymosis, can occur. Although clinical disease may not be severe, co-infection with *A. platys* and *E*. canis may lead to more severe anemia. Therefore, if it important to detect asymptomatic carriers for *A. platys*. Most dogs infected with *H. canis* have no or only mild clinical signs and show low levels of parasitaemia. Immunosuppression or concurrent disease, (including other tick-borne infections), may result in clinical disease. Dogs with severe disease and parasitaemia may develop hepatitis, glomerulonephritis, pneumonia, fever, cachexia, and severe anaemia. It is recommended that all dogs with *H. canis* infection be treated, because parasitaemia may increase over time or may be triggered by concurrent disease.

Although PCR is a useful tool for the diagnosis of tick-borne infections, this case highlights the importance of blood examination too. Blood smear and buffy coat examinations may help determine the degree of parasitaemia, type of parasite, and help detect co-infections; as was seen in this case.

Fast growing *Mycobacteria* in a cat - **By Dr Fraser Hill**

Mycobacterial infections in cats occurs in three forms:

- Tuberculosis: caused by *Mycobacterium bovis* or *M. tuberculosis*
- Leprosy: caused by *M. lepraemurium, M. malmoense,* or *M. mucogenicum*
- Opportunistic: caused by mycobacteria found in the environment such as *M. fortuitum*, *M. smegmatic, M. phlei* and others

Most cats present with cutaneous disease in all the forms, while tuberculosis can also become systemic and is zoonotic.

Recently, a 6-year-old, male-castrate, exotic shorthaired cat presented for veterinary examination because of a discharging abscess and swelling in the left inguinum. The affected tissue was resected and sent for histopathology examination at CityU VDL.

Haematoxylin and eosin sections revealed marked pyogranulomatous inflammation centred around vacuoles in the adipose tissue. Faint, long, tangles of bacteria could be seen in the cleared spaces and when Ziehl-Neelsen stains were applied to the section, these confirmed the presence of acid-fast bacteria consistent with Mycobacteria (figure 5). Bacterial culture identified a moderate growth of rapidly growing *Mycobacteria abscessus*.

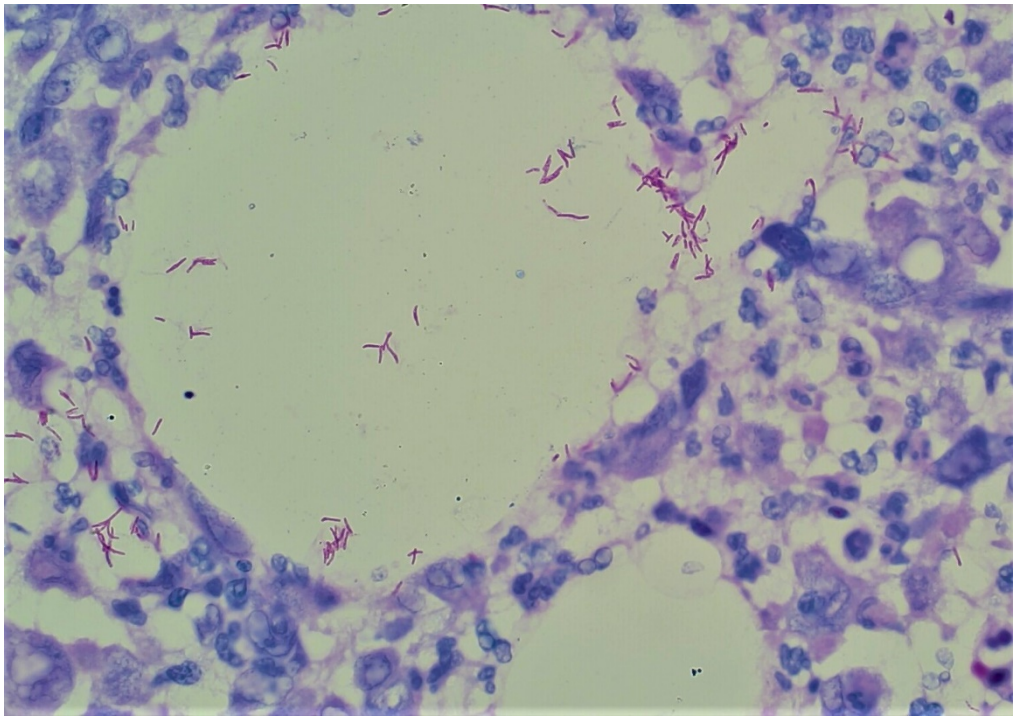

Figure 5: Large numbers of elongate acid fast mycobacteria (staining red to pink) within a vacuole in the adipose tissue of a cat surrounded by inflammatory cells

These findings are consistent with a diagnosis of opportunistic mycobacteriosis (also known as atypical mycobacteriosis or non-tuberculosis mycobacteria). This infection is caused by facultative pathogenic, saprophytic mycobacteria found in soil, water and vegetation. Disease usually occurs following contamination of wounds, particularly if infection is primarily in adipose tissue, and the inguinum is a common site of infection in cats. While the disease is not zoonotic, immunosuppressed people can develop skin lesions if scratched by infected cats, or their own wounds become contaminated. Atypical mycobacteriosis has been reported in humans in Hong

Kong associated with fish handling and aquarium cleaning (Ho *et al,* Hong Kong Med J, 12, 1, 2006). Although there is anecdotal evidence of mycobacterial infections in cats, no published records could be found. If you have diagnosed mycobacterial infection in cats in Hong Kong, could you send the details to fraser.hill@cityu.edu.hk, as it would be interesting to know the frequency of infection here.

Diagnosis can involve cytology, histopathology and culture. PCR testing of the organism with sequencing can assist with identification and help target therapy.

If you suspect mycobacterial infection in a cat, once you have taken a biopsy, fix one third in formalin for histopathology, keep one third fresh for culture and one third frozen for PCR testing.

Staff changes at CityU VDL

Senior Laboratory Technologist

Introducing our new senior laboratory technologist, Miss Tsz Ying Cheung. Tsz Ying is an MLT I with extensive experience in microbiology and is relishing learning all the techniques of the various sections at CityU VDL while guiding the technologist team forward.

Figure 6: Miss Tsz Ying Cheung

Microbiology

One of our pathologists, Dr Andrew Ferguson, is now leading the highly skilled team of microbiology scientists and technologists.

The team includes staff with between 3 and 25 years of experience in all aspects of microbiology dealing with the wide range of clinical and research samples received at CityU VDL.

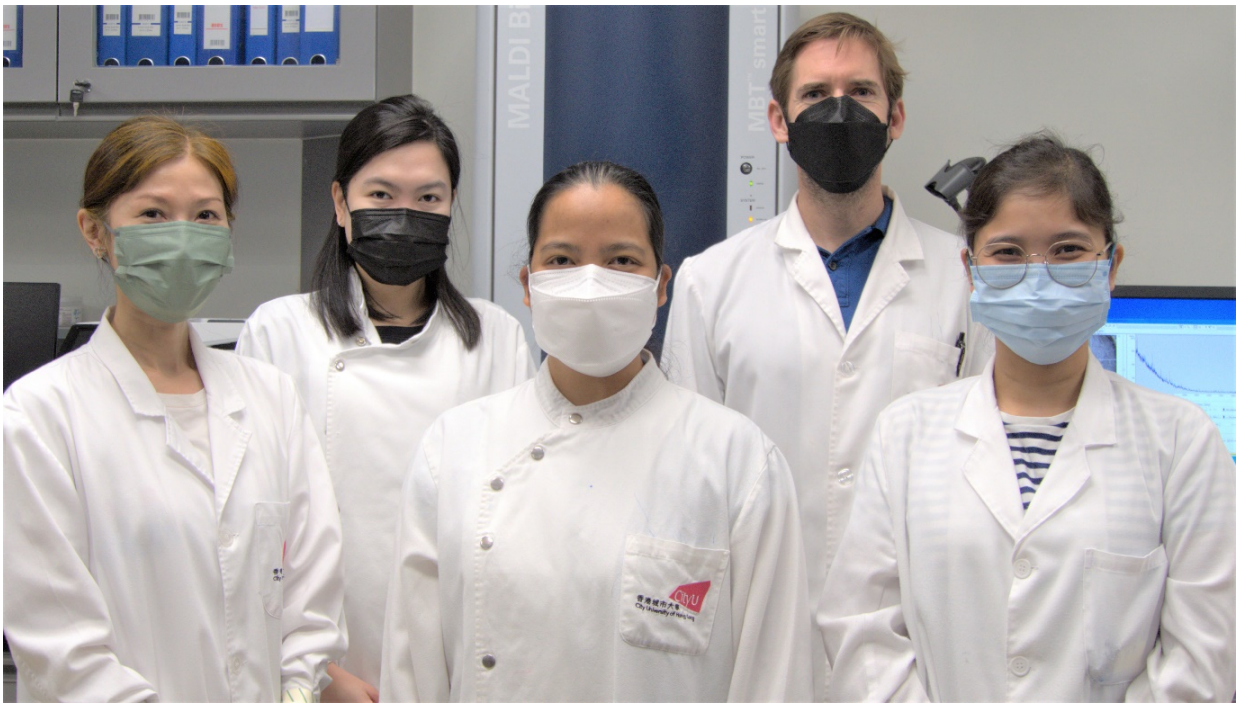

Figure 7: The microbiology team at CityU VDL includes: (from left to right), Ms. Hester Siu, Ms Rosil Dimalibot, Ms Kristel Lorenzo, Dr Andrew Ferguson and Miss Manilyn Rodriguez

Anatomic Pathology

CityU VDL is pleased to welcome Professor Kerstin Baiker to the team. Prof Baiker is a Clinical Professor for Veterinary Pathology at City University's Jockey Club College of Veterinary Medicine and Life Sciences.

Prof Baiker completed a residency in Anatomic Pathology at the Royal Veterinary College in London and became a Diplomate of the European College of Veterinary Pathologists in 2013. She is one of the very few certified specialists in forensic veterinary pathology in Europe.

Working one day per week on the anatomic pathology roster, Prof. Baiker brings a wealth of experience with her to apply to CityU VDL clinical cases.

Figure 8: Professor Kerstin Baiker

To contact our veterinary staff, call 3442-4849 and ask to be connected, or email:

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