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 1st edition of volume four of the newsletter.

CityU VDL has been operating for four years providing local high quality veterinary diagnostic services. A huge range of information has been built up and this year we will begin sharing the unique diseases Hong Kong experiences while tailoring our diagnostics to local conditions.

Look out for an exciting virtual veterinary conference coming up in June this year with a significant contribution by VDL staff.

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

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## SNIPPETS

Along with the common and expected cases we also see new and unusual findings. Recent identifications include new species of fungi in the Pythium group and detecting *Anaplasma platys.*

Having locally based and highly qualified veterinary pathology specialists based in Hong Kong keeps us alert to the possibilities of new pathogens or the detection of existing ones.

Over the past few years CityU VDL has identified *Leptospira interrogans* serogroup Hebdomadis, *Cryptococcus neoformans*, *Hepatozoon canis* and *Ehrlichia canis.*

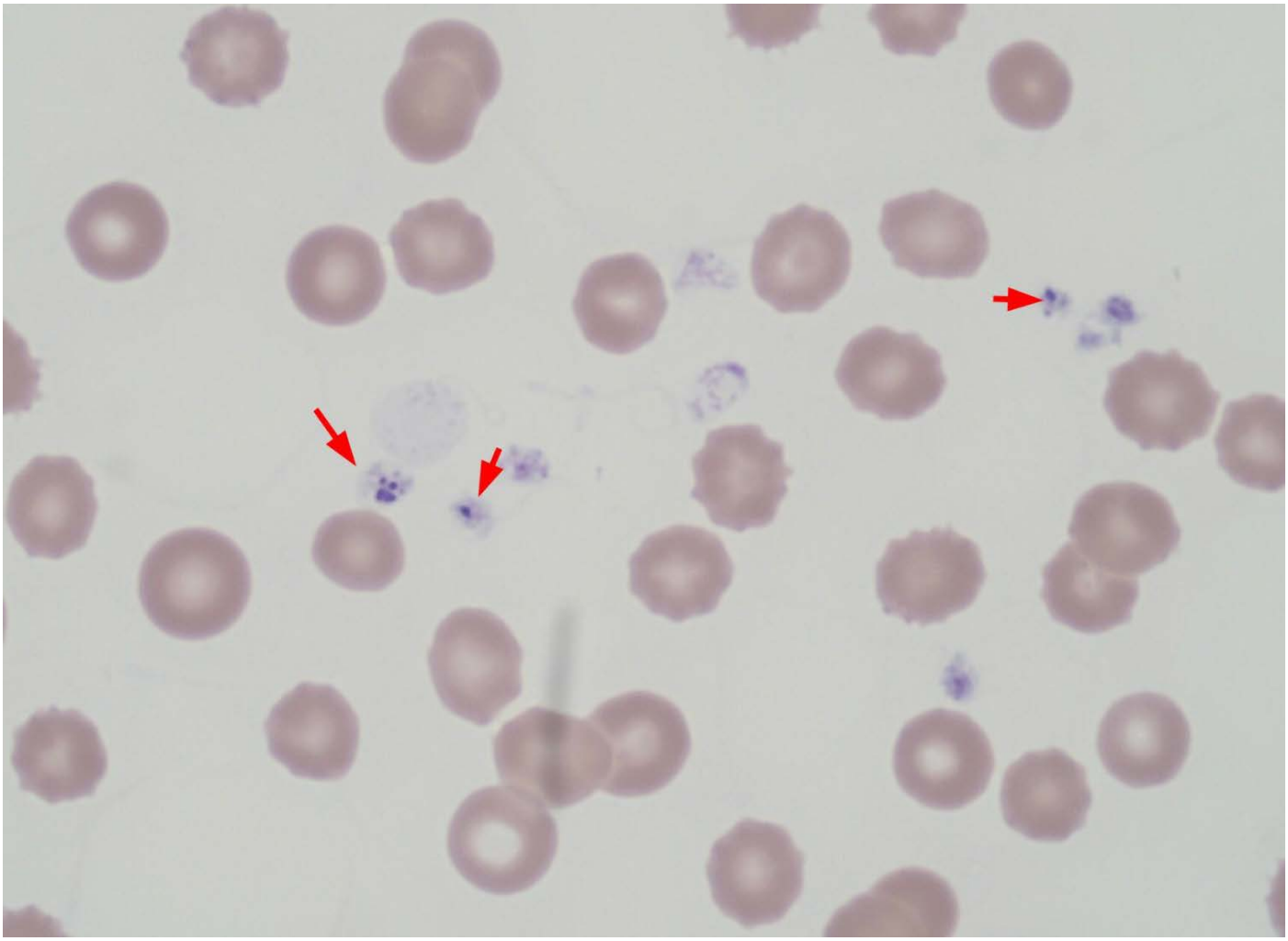
Utilising all our own skills often makes the first detection and then diagnostic tests from our contacts around the world can be called in to make confirmations.

A virtual veterinary conference has been arranged for June 16-18, 2021. This is an exciting opportunity to learn about the latest in clinical and diagnostic testing.

See https://www.veterinaryeducationtoday.ca/hong-kong for details.

# Identification of *Anaplasma platys*

By Dr Daniela Hernandez Muguiro



The image above is from the peripheral blood (Wright's stain, 100x oil objective) of a 10 year female spayed Golden Retriever presented for acute weakness and anaemia. Inclusions were noted in low numbers of platelets during blood smear examination. These inclusions were concerning for *Anaplasma platys* infection. Serology testing (4DX) was negative for antibodies against *Anaplasma* spp. However, infection with *Anaplasma platys* was confirmed via PCR performed at the CityU VDL (*Anaplasma* spp.), followed by DNA sequencing at a referral laboratory. The dog was negative for other Anaplasma species and *Ehrlichia canis* infection. *Anaplasma platys* specifically infects platelets and causes cyclic thrombocytopenia in dogs. Cyclic parasiteaemia and thrombocytopaenia usually occur concurrently at one to two week intervals. Platelets remain below 20 x $10^9$/L for 1-2 days before rapidly increasing. Platelets were low-normal in this dog (200 x$10^9$/L, RI: 186-545 x$10^9$/L), which suggest the end of the interval of thrombocytopenia. Of note in this case, is the negative serologic testing suggesting negative infection. However, seroconversion for Anaplasma may take up to 14 days. Therefore, clinicians should be aware that negative serology does not rule out infection. If acute infection is clinically suspected (e.g. thrombocytopaenia, anaemia, hyperglobulinemia, fever, lymphadenopathy and/or tick exposure) blood smear examination and/or PCR testing may help identify acute and/or active infection.

# Introducing Dr Arthur Ching

## Molecular and Serology Scientist

Dr Arthur Ching is now the molecular and serology scientist at CityU VDL. Dr Ching has been working in the section for four years and has replaced Dr Christina To. Dr Ching has a BSc in Biology and PhD degree from the Chinese University of Hong Kong. He is also a registered MLT Part I. He has more than 10 years of clinical experience in Molecular diagnostics with significant research experience in molecular biology prior to that. Dr Ching will lead the section ably assisted by Miss Chu Lai On.

# TESTING TIPS

## Diagnosis of Hyperadrenocorticism

**Which screening test for hyperadrenocorticism (HAC) to perform**

Choosing between the different screening tests to diagnose canine HAC can be confusing. The 2012 ACVIM consensus statement about the diagnosis of spontaneous canine HAC, postulated the LDDST as the screening test of choice for the diagnosis of HAC in the dog. Ultimately however, the screening test of choice will depend on what the clinician is looking for and the financial capabilities of the owner. Table 1 summarizes some of the features of the screening test for the diagnosis of HAC in the dog, which may help the veterinarian decide which test to perform.

**Table 1. Characteristics of the screening tests for the diagnosis of canine HAC**

| UCC ratio | LDDST* | ACTHst |
|---|---|---|
| High Sensitivity (good to r/o HAC) | High Sensitivity for both PDH and ATs | Good Sensitivity for PDH, poor for ATs |
| Low Specificity (21%) | Low Specificity (more affected by stress) | Reported highest Specificity, but studies contradict |
| Random single urine | Longer test (8 h) | Inexpensive and shorter (1-2 h) |
| No baseline for monitoring | No baseline for monitoring | Baseline data prior therapy and therapeutic response |
| No useful for diagnosis of iatrogenic HAC | No useful for diagnosis of iatrogenic HAC | Diagnosis of iatrogenic HAC |
| Does not differentiate between PDH and ACT | May differentiate between PDH and ACT | Does not differentiate between PDH and ACT |
| UCC ratio | LDDST* | ACTHst |

*ACVIM 2012 consensus; screening test of choice, unless iatrogenic HAC is suspected

**Monitoring medical treatment**

There are several medical options for the treatment of canine HAC, though trilostane and mitotane (less so) are the most widely used. Monitoring trilostane treatment will be briefly described here.

**Post-ACTHst serum cortisol concentration**

Monitoring treatment is typically done by assessment of the clinical response and serum cortisol concentrations post-ACTHst, though an official consensus does not exist. Additionally, the proposed optimal target cortisol concentrations for dogs undergoing treatment are varied. The target cortisol concentrations proposed here are per the manufacturer recommendations (Dechra®). The recommended target post-ACTH cortisol concentrations are is 40-150 nmol/L. The first monitoring occurs between days 10-14 of treatment consisting of a clinical exam, chemistry panel, CBC, and ACTHst 4 h post-trilostane. Trilostane should be given with food (routinely and on days of recheck) to enhance absorption (fasting invalidates the test results). Depending on the clinical response and post-ACTH cortisol concentration, doses are adjusted (return to day 1 of treatment) or treatment is continued at the current dose for later rechecking. If no dosing adjustment and clinically well, then rechecking should be at 1 to 3 months, at 3 months, and then every 3 to 6 months.

**Pre-trilostane serum cortisol concentration**

Monitoring treatment via the pre-trilostane cortisol concentration can be considered if monitoring treatment via the ACTHst is not possible due to either financial constraints or unavailability of exogenous ACTH. It is important to note however, that the scientific evidence to justify the use of this monitoring protocol is sparse. So far only two studies have evaluated this approach, both of which were funded by Dechra®, the manufacturer of the drug Vetoryl ®.

Monitoring treatment using the pre-trilostane cortisol relies heavily on the clinical response as reported by the owners and clinical findings by the veterinarian. Using the pre-trilostane cortisol concentration for monitoring should be reserved for dogs that are clinically well. Dogs that are exhibiting signs of cortisol deficiency should have a complete evaluation, including an ACTHst, performed. The dog is first rechecked at day 10, and if clinically well, maintained on the current dose until day 28. At day 28, the dog is clinically evaluated and the pre-trilostane serum cortisol determined. Clinical signs of HAC and pre-trilostane cortisol above cut-off value warrant dose adjustment and the process starts again (return to day 1). Otherwise, if the dog is well-controlled, the treatment is continued at the current dose and rechecking every 3 months. If at any point the dog is clinically unwell (clinical signs of hypoadrenocorticism), treatment should be stopped, and a standard ACTHst and electrolyte determination are indicated. The dose is adjusted and symptomatic treatment is given as necessary. The target pre-trilostane cortisol concentrations are 40 - 138 nmol/L (1 h pre dose).

## MICROBIOLOGY NEWS: Change in Fluoroquinolone Testing

Ciprofloxacin and Ofloxacin are second-generation fluoroquinolones, used heavily in human medicine, due to their increased antibacterial activity.

Ciprofloxacin has increased activity against *Pseudomonas aeruginosa,* and is widely used as a topical antibiotic in ear and eye infections. However, its use as a systemic antibiotic (oral/injectable) is not recommended in veterinary species, as its pharmacokinetic profile does not support its usage (Clinical and Laboratory Standards Institute, VET01S, 5$^{th}$ edn).

Ofloxacin is not commonly used as an oral/injectable antibiotic in veterinary species, however, as human formulations exist, veterinarians do have ready access to this formulation. The reference ranges for antimicrobial sensitivity testing for ofloxacin are based on human pharmacokinetics and are not reflective

of the clinical situation in veterinary species. Therefore, testing for ofloxacin could lead to wrong interpretations and result in improper selection of antibiotic.

CityU VDL will continue to use ciprofloxacin and ofloxacin in our topical panels but not in the STANDARD and URINARY antibiotic testing panels.

## CityU VDL introduces five new convenient panels

Panels of biochemical tests are cost-effective and convenient for monitoring specific organ systems, response to therapy or prior to anesthesia.

A range of five new biochemical panels have been introduced at CityU VDL to give you more options when working with your patients.

**The panels include:**

**Liver Panel:** For general health screening. To screen for, evaluate and monitor liver disease, and/or injury. To monitor adverse effects of certain drugs. To evaluate liver function.

**Renal Panel:** For general health screening. To screen for, evaluate and monitor renal disease, and/or injury. To monitor adverse effects of certain drugs.

**NSAID Panel:** To screen for, evaluate and monitor NSAID-related adverse effects.

**Pre-anesthesia Panel:** Recommended for animals undergoing anesthesia. To assess overall health and ensure the patient is a good candidate for anesthesia.

**Total Protein Panel:** For general health screening. To help diagnose or monitor certain liver and renal disorders, or dysproteinemias. To check general nutritional status.

The details of the panels are provided in the table below:

| Panels and Profiles | Test Includes | Sample Required | TAT |
|---|---|---|---|
| Pre-anesthesia Panel | Na, K, Cl, Bicarb, Anion Gap, Creatinine, Ca, Glucose, ALT | 1.3mL clotted whole blood in serum tube | 24hrs |
| Liver Panel | Alb, AST, Glu, ALT, Urea, ALP, GGT, total bilirubin, cholesterol | 1.3mL clotted whole blood in serum tube | 24hrs |
| Renal Panel | Na, K, Cl, Bicarb, Anion gap, Urea, Creatinine, Alb, Ca, P | 1.3mL clotted whole blood in serum tube | 24hrs |
| Total Protein Panel | TP, Albumin, Globulin, A/G Ratio | 1.3mL clotted whole blood in serum tube | 24hrs |
| NSAID Panel | ALP, ALT, Urea, Creatinine, USG | 1.3mL clotted whole blood in serum tube + 1mL urine | 24hrs |

# PATHOLOGIST BLOOD SMEAR (CBC) REVIEW PROTOCOLS

At CityU VDL all the blood smears are reviewed by our technologists. Cases that fulfill certain criteria are further reviewed by the clinical pathologist. Alternatively, there is the option of requesting **a CBC with clinical pathologist review**.

**Criteria for clinical pathologist review:**

**Leukocytes:**

- Pancytopenia
- Degenerative left shift with no toxic change or if questioning cell identification
- Toxic change with no left shift
- Left shift (>1% bands) with no toxic change in a horse or cow.
- WBC > $30x10^9$/L in large animals
- WBC > $50x10^9$/L in small animals
- Histiocytes seen
- Mast cells seen – any in a cat, >3 in a dog
- Absolute lymphocytosis in an animal >2yrs
  - If there is an absolute lymphocytosis and the animal is less than 2yrs old, and the lymphocytes are small mature forms, there is no need to send for review since it is likely an epinephrine response.
  - If the animal is over 2 yrs or there are any atypical lymphocytes seen, then send it for review.
- Blast cells or other atypical cells:
  - Blasts: round cells with fine chromatin with or without nucleoli.
  - Other leukocytes with abnormal morphology which may include:
    - Abnormal granulation or inclusions
    - Large cells – e.g. giant neutrophils
- Very low neutrophil count.
- Toxic change only in bands

**Red blood cells:**

- Anemia with HCT ≤ 15, or ≤ 20 in the horse and dog
- Any nucleated RBCs in a horse
- Large numbers (>20) of nucleated RBCs in other species in the absence of regeneration
- Hypochromasia
- Macrocytes in anemic horses
- Spherocytes
- Agglutination
- Parasites other than leukocytozoon, microfilaria and hemoproteus
- Ovalocytes in a dog
- Moderate to many siderocytes or very prominent siderocytes
- Heinz bodies in any species but a cat
- Large Heinz bodies in a cat

**Platelets:**

- Very low platelets - <$50x10^9$/L when the count is not matching the smear

Any time the counts are not matching the smear.

# STAFF PROFILE

## Clinical pathology section

- **Dr Daniela Hernandez Muguiro (BVSc, MV, Dip ACVP, Registered Specialist Veterinary Clinical Pathologist)**
- **Ms Fannie Tsang (MSc, BSc, MLT I, Senior Technologist)**
- **Miss Wynne Tse (MSc, BSc, MLT I, Technologist)**
- **Ms Markay Lardizabal (BMLS, RMT, MLT II, Technologist)**

Led by registered specialist clinical pathologist Dr Daniela Hernandez Muguiro, the clinical pathology team includes Ms Fannie Tsang, Miss Wynne Tse and Ms Markay Lardizabal.

Dr Hernandez Muguiro (BVSc, MV, Dip ACVP) studied veterinary science at the Universidad de Guadalajara in Mexico before undertaking clinical pathology residency studies at the National Autonomous University of Mexico and the College of Veterinary Medicine, Cornell University. Dr Hernandez Muguiro successfully completed the American board examinations in veterinary clinical pathology in 2018.

Ms Fannie Tsang (MSc, BSc, MLT I, Senior Technologist) received her Bachelor degree in 2008 from The Hong Kong Polytechnic University and Master degree in 2011 from The Chinese University of Hong Kong and has over 12 years of clinical diagnostic experience in laboratory diagnostics.

Miss Wynne Tse (BSc, MSc, MLT I) received her Bachelors and Master degree from the Hong Kong Polytechnic University in Medical Laboratory Science. She has 10 years of clinical diagnostic experience in laboratory diagnostics.

Ms Markay Lardizabal (BMLS, RMT, MLT II) received her degree in Medical Laboratory Science and is a registered Medical Technologist in the Philippines and Hong Kong. She has over 7 years of clinical diagnostic experience in laboratory diagnostics

All the technologist team hold the Medical Laboratory Technologist license and are able to readily perform all clinical pathology and cytology tests.

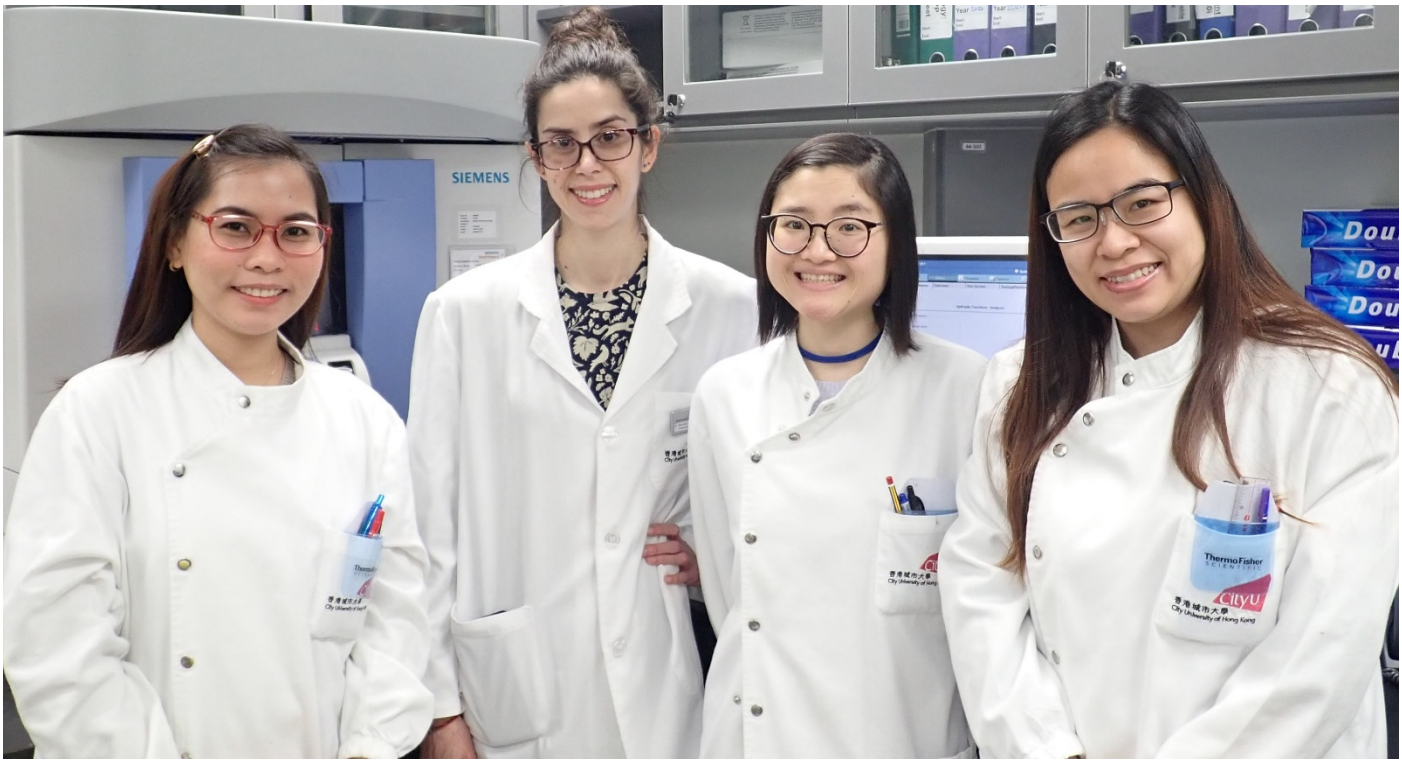



Figure 4: The CityU VDL clinical pathology team includes Ms Markay Lardizabal, Dr Daniela Hernandez Muguiro, Miss Wynne Tse and Ms Fannie Tsang

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