CITYU VETERINARY DIAGNOSTIC LABORATORY
城大動物醫療檢驗中心

CITY U VETERINARY DIAGNOSTIC LABORATORY

MESSAGE FROM THE DIRECTOR

Welcome to the 2nd edition of the CityU VDL newsletter. The laboratory continues to grow and develop and we are excited to offer our full range of services with the recent opening of the clinical pathology and serology sections.

Details of some of the tests offered are highlighted in this newsletter including; erythrocyte parasite testing, feline coronavirus testing options, a new option for cremation after post mortem, the latest on immunocytochemistry of cytology smears where lymphoma is suspected and more. Our test list is available on-line at https://www.cityu.edu.hk/ph/en/Facilities/VDL.html as well as our Facebook page https://www.facebook.com/HK.CityU.VDL/

Thanks to our growing list of clients for using our services. Our Hong Kong based pathologists are always willing to talk through cases either before sample submission to help select the best samples and after results are returned to assist with interpretation and additional testing options. We all enjoy assisting practitioners with case investigations and can be contacted by telephone or email for a free consultation.

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

IN THIS ISSUE

WHATS NEW AT CITYU VDL

POST MORTEM SERVICES

Cremation of the body and ashes return is now possible!

Recent changes in the Clinical Waste Disposal Ordinance for laboratory waste means animal bodies that had undergone a post mortem can now be returned to the owner after cremation.

If your client would like a post mortem examination undertaken, you can now advise them the animals' body can be returned to them in the form of ashes, as long as the body is cremated by an authorised cremation company.

Organising a cremation service provider and payment for the cremation is the responsibility of the owner and/or submitting veterinary clinic. The cremation company can pick up the body directly at CityU VDL.

If return of the ashes is not required, the animals' body will be disposed of via incineration by the clinical waste service.

BABESIA TESTING OPTIONS

CityU VDL is excited to offer 2 new tick fever PCR panels for dogs and cats: Comprehensive Tick Fever Panel and the Babesia Panel. Our previous Tick Fever Panel is now renamed to Tick Fever Screening.

TICK FEVER SCREENING & BABESIA PANEL

Our Pan *Babesia* and *Ehrlichia* PCR panel is called "Tick fever screening" and includes DNA sequence alignments for:

Babesia canis vogeli	*Babesia conradae*
Babesia canis canis	*Babesia sp. Coco*
Babesia canis rossi	*Ehrlichia canis*
Babesia gibsoni	*Babesia microti*

This test is used to screen for the presence of any *Babesia spp* or *Ehrlichia* infections. If DNA for *Babesia spp* is detected with the Tick Fever Screening panel, the sample can be used to run the Babesia Panel to check for the most common *Babesia* infections: *B. gibsoni*, *B. canis vogeli*, and *B. canis canis*.

If none of these pathogens are identified by the Babesia panel, sequencing of the DNA can be undertaken to make a definitive identification. This approach should ensure no *Babesia* species outside of the common ones are missed and any new infectious Babesia can be identified.

COMPREHENSIVE TICK FEVER PANEL

CityU VDL also offers a Comprehensive tick fever panel. Four individual PCRs are undertaken to definitively identify the following pathogens:

B. canis canis	*B. gibsoni*
B. canis vogeli	*Ehrlichia canis*

BABESIA PANEL

Besides using the Babesia Panel for screening for the presence of the three common babesia species, this panel has also been used to check and confirm the efficacy of treatment at 60 and 90 days post therapy. A 10% discount on this test is available if the case number of the initial testing for tick fever is included in the submission form.

RECENT BABESIA CASE EXAMPLES:

By Dr. Fraser Hill

A one-year-old male Poodle was presented to a veterinary clinic with necrosis of the tip of the penis related to prolapse of the penis. During a full clinical evaluation, routine in-house complete blood counts (CBC) revealed anaemia and thrombocytopaenia.

A CBC undertaken at CityU VDL confirmed a regenerative anaemia as the red blood cell count was $1.5x10^{12}$/L (reference range: 5.7-8.5), haematocrit 12% (reference range: 41-58) with 12.6% reticulocytes (reference range: <1.5) and six nucleated red blood cells per 100 white blood cells. There was also thrombocytopaenia as the platelet count was $40x10^{9}$/L (reference range: 186-545, no platelet clumps were detected in the tail of the blood smear on examination) and neutrophilia with a left shift as the neutrophil count was increased to $14.3x10^{9}$/L (reference range: 2.7-9.4) and band neutrophils increased to $2.2x10^{9}$/L (reference range: 0-0.1).

Regenerative anaemia with thrombocytopaenia is suggestive of either blood loss or hemolysis. External blood loss was judged to be minimal from the penis, and so haemolysis was suspected. In Hong Kong, along with immune mediated haemolytic anaemia, erythrocyte parasitism should be considered as a differential, so testing for *Babesia* was undertaken by PCR. *Babesia gibsoni* infection was confirmed on a PCR test. Response to treatment would confirm if the *Babesia* was the cause of the regenerative anaemia. Inflammation was most likely related to the tissue damage and necrosis of the penis.

In another case, a 12 year old neutered mongrel dog was presented to a veterinary clinic for investigation of anorexia and urinary incontinence. The dog was pale and weak so a CBC was undertaken at CityU VDL. Regenerative anaemia was confirmed as the red blood cell count was $1x10^{12}$/L (reference range: 5.7-8.5), haematocrit 8% (reference range: 41-58) with 18.1% reticulocytes (reference range: <1.5) with 18 nucleated red blood cells per 100 white blood cells. A platelet count was $95x10^{9}$/L (reference range: 186-545). In this case there were platelet clumps in the tail of the smear, and thus the dog did not have real thrombocytopaenia and the platelet numbers were considered adequate. Again regenerative anaemia, in the absence of external blood loss, suggested erythrocyte parasitism and should be checked for. A PCR test confirmed the presence of *Babesia gibsoni.*

Courier Services at CityU VDL

CityU VDL is pleased to offer a twice a day courier service pick-up of samples to most areas of Hong Kong from Monday to Friday. We aim to provide a fast a reliable sample delivery from your clinic to our laboratory at City University of Hong Kong. All our couriers are highly trained professional drivers. They are well trained to handle your samples safely and professionally. Our environmental friendly vehicles are equipped with a thermoelectric cooler, allowing us to keep samples that require refrigeration in a temperature controlled environment. We also have designated boxes for specific sample types, guaranteeing your cytology slides will never come into contact with formalin-filled biopsy pots.

Implementing Personalized Medicine in your Veterinary Clinic

Personalised medicine is still a new concept in veterinary medicine but it has already been widely used in human medicine. This approach treats each animal as a unique individual according to their specific traits and risks relating to their breed, genetics, sex, age, weight, location, and lifestyle and then designs a lifelong plan for the animal that meets their specific needs over their lifetime. This means we are no longer treating a dog as a dog, but as an individual based on their unique traits. This allow the veterinary team to focus on prevention and early detection of disease leading to more effective prevention, management and better clinical outcomes for each individual animal.

The Key Steps to Implementing Personalised Medicine in Your Veterinary Clinic

First, build an individual risk profile using the animal's signalment, geographic location, pre-exisiting conditions, genetic makeup, and lifestyle input from pet owner (via a questionnaire). Once you have all this data, you can combine them and come up with a list of possible risks the individual animal may be predisposed to. Once a list is made, you can prioritise them due to severity or prevalence. Now that you have a list of diseases this individual animal may be predisposed to, you can decide how you can screen for them, how often to test them for, and at what stage in life. This will be the **life plan** of the animal.

Such screening tools can include phenotypic tests such as:

- Urinalysis
- Fecal analysis
- CBC and biochemistry profile
- Thyroid screening
- Clotting tests
- Heart evaluation

Or genotypic tests such as

- DNA screen (for heritable disease and breed identification)

Identifying disease risks early on can generate a new revenue stream relying on in-house and laboratory diagnostic tests and professional services (rather than merchandising and dispensing medications).

Personalised medicine is a client-centric approach to driving preventive care. It allows pet owners to be a partner in wellness planning and prevention. It also allows them to plan for the cost of care over the long haul. This will lead to improved customer satisfaction and differentiate your clinic from other clinics who may not be practicing personalized medicine.

For more information on our **LifePlan** and **DNA Screening**, please contact us at 3442-6538.

WHAT IS YOUR DIFFERENTIAL DIAGNOSIS?

by Dr. Allan Kessell

In this section, our pathologist will present to you a case submitted to us at CityU VDL with the relevant clinic history and preliminary tests results provided by the referring clinic. We will then allow you to come up with your own list of differentials. The laboratory results and definitive diagnosis will then be presented to you on a different page where you can then check and see if your differentials are correct. This is a fun way to stimulate some thinking.

The following case is a **post mortem case** submitted to us at CityU VDL.

CLINICAL HISTORY

A local Hong Kong veterinarian rang the CityU VDL reception requesting a post mortem. The veterinarian was put through to the duty pathologist at the VDL for discussion of the case. The animal involved in this enquiry was an 8 year old entire female Siberian Husky dog that had visited his clinic the day before. The animal had come to the clinic for her yearly vaccination, which was administered after the owner reported that the animal was active and in good health, and after a full physical examination revealed no abnormalities. The animal returned home that afternoon, and was subsequently taken for a walk, fed dinner and was happy and well when the family retired to sleep that evening. However the animal was found dead the next morning on its bedding, which was inside the house.

Arrangements were made for CityU VDL to collect the body for a full gross post mortem that day.

Formulate a differential diagnosis that would fit with this history and signalment, and turn to **page 7** to find out the results of the post mortem examination and subsequent investigation.

CASE OF THE MONTH

by Dr. Jeanine Sandy

CLINICAL HISTORY:

A 10 year old, female, toy poodle was presented to a referral veterinary clinic with progressive ascites associated with hypoalbuminaemia. The dog was emaciated.

Hypoproteinaemia was noted on in-house biochemistry (albumin and globulins were both low) as well as, hypocholesterolaemia.

Question:
What would be your differential diagnoses in this case?

DIFFERENTIAL DIAGNOSIS AND SUGGESTED FURTHER TESTING:

<u>Protein/albumin loss</u>

1) Protein-losing nephropathy (PLN) – Biochemistry; check for azotemia, Urinalysis and/or urine protein/ creatinine (UPC) ratio.
2) Protein-losing enteropathy (PLE). Ultrasound to look for mechanical obstruction; intussusception, foreign body, abdominal masses/enlarged lymph nodes. Biopsy to assess gastrointestinal disease.
3) Haemorrhage – CBC/biochemistry; evidence of anaemia? Assess sites of chronic bleeding - haematuria/gastrointestinal bleeding. Faecal examination to look for parasites.

<u>Insufficient protein production</u>

4) Hepatic disease? CBC/Biochemistry; assess liver enzymes. Ultrasound, liver biopsy.
5) Starvation. Sometimes requiring difficult questioning of the client.

A UPC was performed which was normal. No evidence of haemorrhage or anaemia in this case.

Endoscopic biopsy of stomach and duodenum performed.

HISTOLOGY:

Approximately 40% of duodenal villi had moderate to marked dilation of lacteals, with dilated lacteals filled with pink protein and low numbers of monocytes. However other duodenal samples showed no significant changes, indicating this lacteal change had a patchy distribution within the duodenum (figure 1).

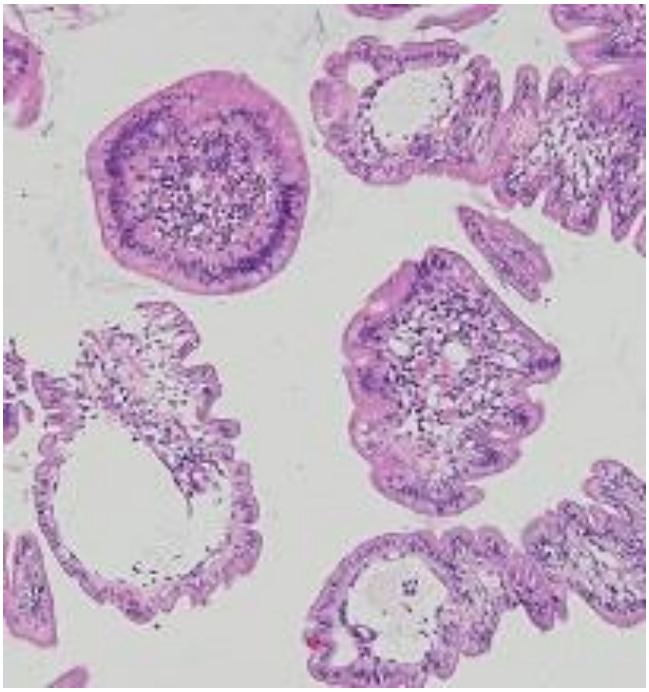

Figure 1. Duodenal biopsy x10 objective, H&E.

HISTOLOGICAL DIAGNOSIS:
Duodenal lymphangectasia.

DISCUSSION:

Lymphangectasia is present within the duodenum and exhibits a characteristic patchy distribution within the small intestine. Ileal sections are often the site of choice to confirm lymphangectasia, but sampling of the ileum cannot be achieved via endoscopy, and requires laporatomy derived specimens, which are more invasive.

Lymphangectasia is reported in dogs and less commonly, horses. Clinically affected dogs often present with ascites, hydrothorax, weight loss despite normal appetite, a change in the quality of stools, with increased fluid content. The low cholesterol in this case is consistent for lymphatic obstruction and malabsorption as well as loss into the lumen. Albumin and globulins can both be reduced, and calcium is often low due to the reduction of albumin.

Fluid analysis on body cavity fluids generally reveals a transudate. Lymphopenia is often present due to loss within lymphatic fluids from ruptured lacteals. Malabsorption within the small intestine occurs due to lack of lacteal drainage from the villi, leading to loss of protein and cholesterol. Low cholesterol results in decreased calcium. On endoscopy, lymphangectasia is really the only time that intestinal villi become visible macroscopically, due to dilation of lacteal channels broadening the structure of the villi, which appear white due to chyle and lymph within the lacteals. On laparotomy, scattered white and firm nodules, approximately 5-10 mm in size can be seen scattered over serosal surfaces and draining lymph nodes may be enlarged due to granulomatous inflammation triggered from spillage of lymphatic fluids from obstructed lymphatic vessels.

The underlying cause of lymphangectasia is most commonly unknown in dogs but occasionally, impedence to lymphatic/vascular drainage is present from a tumour, abscess, or granuloma.

Treatment focusses on addressing the underlying cause, (if one can be found), the use of anti-inflammatory medications, and dietary modification. Resolution is rare and in most cases, remission of clinical signs is the best that can be achieved. Even in patients where remission can be attained, the longest reported survival time after diagnosis of intestinal lymphangectasia is 2 years.

ACKNOWLEDGEMENT

Special thank you to the veterinarian who forwarded this interesting case to us and allowing us to publish it in our newsletter

IMMUNOCYTOCHEMISTRY ON CYTOLOGY SMEARS

New tests are now available to immunotype neoplastic cells on cytology smears. If lymphoma is suspected by the pathologist on review of a cytology aspirate, markers for T and B lymphocytes are available at CityU VDL. The pathologist will recommend additional immunocytochemical stains (ICC) to be undertaken in their report so you can discuss this option with your client and let us know whether to proceed.

Feline Infectious Peritonitis: a summary

By Dr. Allan Kessell

Feline coronavirus (FCV) is closely related to canine coronavirus, and it is speculated that feline coronavirus arose from that virus by mutation and/or recombination during the decade of the 1950s.

Feline enteric coronavirus (FECV) refers to the enteric biotype present in most cat populations, whilst the FIPV is the virulent biotype causing FIP. FECV (and therefore FIP) is present in all cat populations around the world and is common in catteries and shelters and is common in multi-cat households. Transmission of FECV is faecal-oral. The virus can be shed for variable periods of time: in some cats, the virus is cleared within 6 months, whilst in others, shedding can persist past 18 months. However re-infection is common.

FIPV is highly tissue and cell associated, and spread of the virus from cat to cat is uncommon. Rather, the FIPV variant is believed, in most cases, to arise in individual cats by mutation/s in the resident FECVs that allow survival and replication of the virus within macrophages. These types of mutations are common, and in one study, occurred in up to 20% of FECV infected cats. These mutant viruses do not progress to produce FIP in most cases due to a rapid and strong cellular immune response. In those cases that do progress to FIP, macrophages carrying the FIPV extend past the mesenteric lymph nodes to serosal surfaces, mesentery and other abdominal organs, sometimes pleura and occasionally meninges. Most affected cats are <2 years of age, but FIP may be seen in cats of any age.

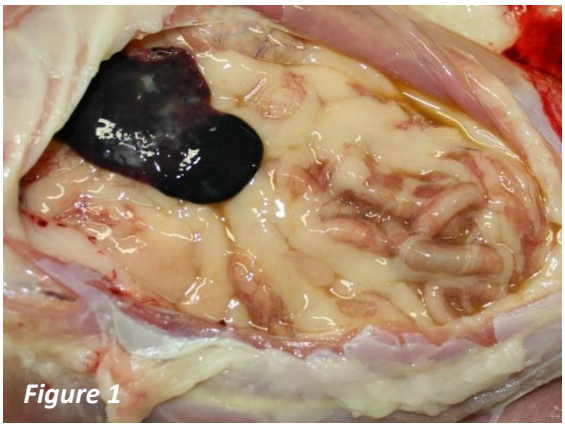

Figure 1

It is common to classify FIP into 2 forms – wet and dry. In the more common effusive “wet” form, the immune response is characterised by an exuberant humoral response and a pyogranulomatous vasculitis and cavity effusions (see figure 1), whereas in the dry granulomatous form, there is some cell mediated immunity but not enough to clear the organism (see figure 2). Usually one or the other is predominant clinically, although transitions from one form to the other are recorded. Incubation periods of weeks to months have been recorded with clinical onset preceded by a long history of vague ill health and stunted growth.

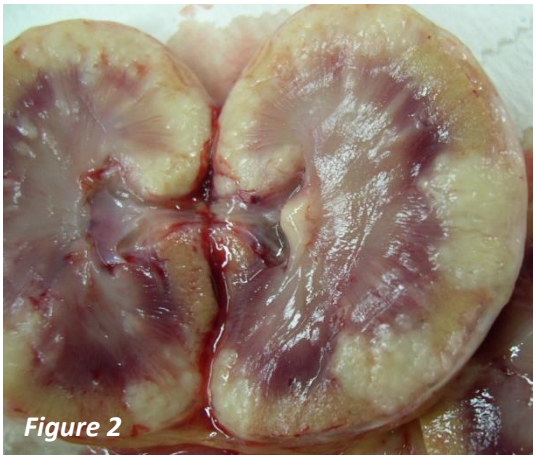

Figure 2

Definitive diagnosis of FIP remains difficult. Diagnosis does generally require a knowledge of history and signalment combined with supportive clinical-pathological findings (mild non regenerative anaemia, leucocytosis with lymphopenia, hyperproteinaemia with low albumin/high globulins, modified transudates with high protein in the wet form) before FIP is strongly suspected. The demonstration of the characteristic lesions at gross necropsy and histopathology of fixed tissues used to be the gold standard for diagnosis, but to this, has been added the ability to demonstrate the organism by immunohistochemistry (IHC) within the macrophages in pyogranulomas/granulomas. This is now the new gold standard. Immunocytochemistry can also be used on effusions in cats with the wet form - it is less sensitive than IHC, but just as specific. Two recent studies suggest that a positive FCV PCR on abdominal or thoracic effusions in cats with other supportive findings is also likely a gold standard test.

WHAT IS YOUR DIFFERENTIAL DIAGNOSIS?

… Continuing from page 4

GROSS PORT MORTEM RESULTS:

There was little post mortem autolysis, and the animal was in good body condition with good fat reserves in the subcutis, around the kidney, within the mesentery and in mediastinum of the thoracic cavity. The only observable external abnormality was a small amount of blood within the mouth. On internal examination the pericardial sac was distended with approximately 50mls of whole blood, and the heart appeared compressed.

Dissection of the heart revealed a darkly mottled 2 x 1 x 1 cm darkly mottled mass within the wall of the right atrium. There was a 4mm rupture in the pericardial surface over the mass to which was attached a small blood clot (figure 1).

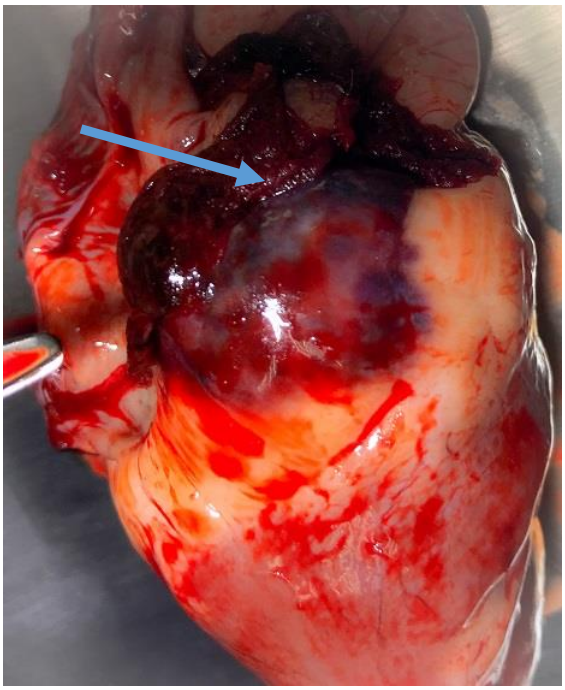

Figure 1. Notice the mottled haemorrhagic mass within the wall of the right atrium. Note blood clot attached to epicardial surface of mass.

GROSS MORPHOLOGICAL DIAGNOSIS:
Suspected Right Atrial Haemangiosarcoma

In order to confirm the gross morphological diagnosis, the duty pathologist recommended histopathology to be done of the mass. The veterinarian agreed and the sample was sent to the histopathology team.

HISTOPATHOLOGY:

The mass in the right atrium had a large central area of haemorrhage, surrounded by multiple, randomly orientated fascicles of neoplastic spindle cells. The neoplastic cells had largely replaced myocardial fibres within the right atrium and extended into the epicardial adipose tissue (figure 2). Tumour cells were generally closely packed but in some areas, small aberrant vascular channels were apparent (figure 3). Neoplastic cells contain round to oval to elongated vesicular nuclei that contained 1-2 nucleoli. Mitoses were frequent, often >5/HPF.

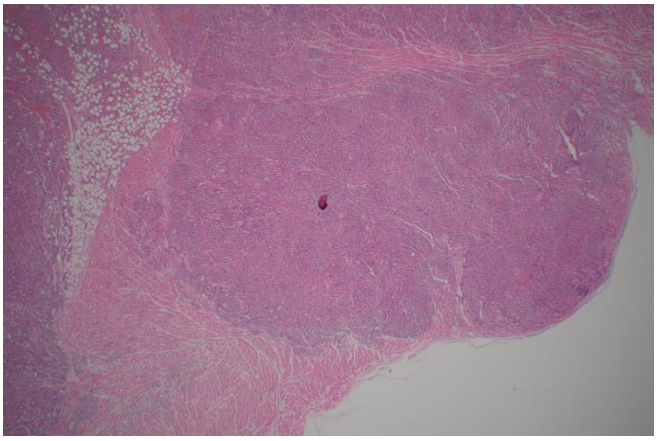

Figure 2. Notice that the darkly stained neoplastic tissue replaces the full thickness of the atrial muscle here. There is some spread of the tumour into the epicardial adipose tissue (at left).

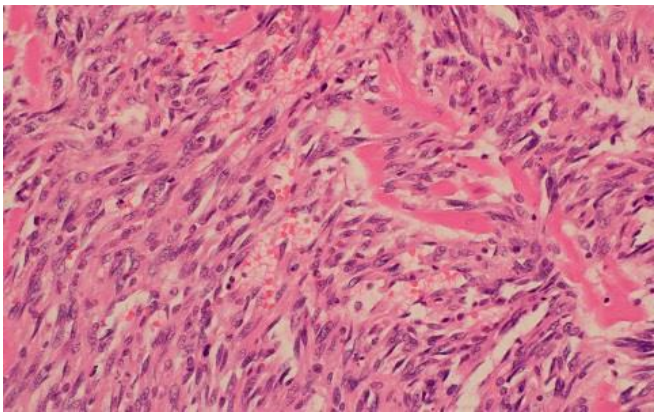

Figure 3. High power view of neoplastic cells. Note scattered vascular channels throughout.

Immunohistochemical (IHC) staining for Factor 8 confirmed the endothelial origin of these neoplastic cells (figure 4)

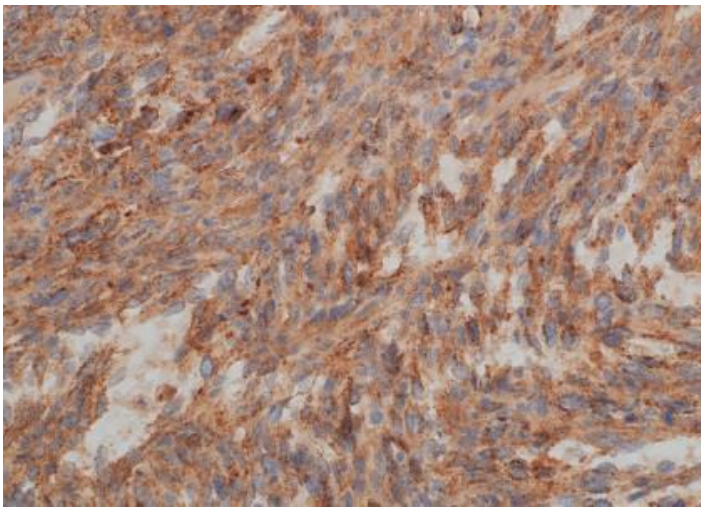

Figure 4. IHC staining for Factor 8 (specific for endothelial cells) is positive (brown granular staining within cytoplasm of neoplastic cells)

HISTOLOGIC DIAGNOSIS:
Right Atrial Haemangiosarcoma

COMMENTS:

The veterinarian was concerned that he had missed some abnormality on physical examination, or that the animal may have had a delayed reaction to the vaccination. Another possibility discussed was a possible poisoning (in light of recent media reports within Hong Kong), and the attending pathologist also mentioned the possibility of a right atrial haemangiosarcoma, as they can present with this sort of history.

Haemangiosarcomas (HSC) arise from the endothelial cells that line vascular channels. Most veterinarians are familiar with those primary tumours that arise within the spleen, and are associated with such a poor prognosis. Animals with a HSC in the spleen may present with clinical signs referable to rupture of the primary tumour into the abdomen and sudden anaemia, or they can present with clinical signs referable to the effects of one of the widespread metastases that can be seen with this aggressive tumour.

Primary HSC can arise within the heart, and more specifically within the right atrium. Cardiac HSC will usually present with the history seen with this case – sudden unexpected death after abrupt rupture and bleeding of the tumour into the pericardial sac, which results in tamponade and rapid death. Some cases may present with vague clinical signs if there has been low level leakage of blood into the pericardial sac, but that is must less common with this type of tumour. It is likely we could have found evidence of micrometastases elsewhere in this case, but only limited histopathology was requested to confirm the gross diagnosis.

This case illustrates a number of points - the usefulness for a direct conversation between the pathologist and veterinarian when considering differential diagnosis; discussions around the cost of the procedure/s that could be performed, the usefulness of a complete gross post mortem to investigate the cause of death, and the usefulness of follow up investigations which can be generated from samples taken at post mortem.

Owners can be understandably reticent to request post mortem examinations on their beloved pet. However recent changes in regulations in Hong Kong allowed for this animal to be forwarded to a cremation service and the ashes returned to the owner.

ACKNOWLEDGEMENT

Special thank you to the veterinarian who forwarded this interesting case to us and allowing us to publish it in our newsletter

Contact Us

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If you have any questions about our tests or want us to have a chat with you in person, please contact Dr. Ada Chu at 3442-6538 or adachu@cityu.edu.hk

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