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City University of Hong Kong



CITYU VETERINARY DIAGNOSTIC LABORATORY

MESSAGE FROM THE DIRECTOR

Welcome to the fourth edition of volume four of the newsletter.

In this edition, we highlight new *Anaplasma* tests and the recent confirmation of *Hepatozoon canis* in dogs in Hong Kong.

Also showcased are isolation of *Mycoplasma* from dog urine, and the frequency of infectious disease diagnosis in Hong Kong dogs from our case files.

Best wishes for the Christmas and New Year festivities ahead.

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

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SNIPPETS

- Serum cortisol is predominantly used as part of dynamic paired testing for hyperadrenocorticism or hypoadrenocorticism. However, basal cortisol can be a useful stand-alone screening test to eliminate hypoadrenocorticism/Addison's disease from a differential list. In these situations, it may be appropriate to proceed with an ACTH stimulation test to fully assess the adrenal reserve.
- Antinuclear antibody: This serum test detects the presence or absence of antibody to various nuclear antigens. It is mostly indicated in cases of suspected systemic lupus erythematosus.
- When a bone marrow aspirate is sent to the laboratory, please remember to send an EDTA blood sample for a concurrent CBC. Interpretation is most useful clinically when both are carried out at the same time.

TESTING TIPS

Anaplasma PCR tests now available

CityU VDL is pleased to offer a new molecular test for *Anaplasma* species diagnosis.

After CityU VDL Clinical Pathologist Dr Daniela HERNANDEZ MUGUIRO saw platelet inclusions consistent with *Anaplasma platys* in a Golden Retriever in Hong Kong (figure 1), and further cases were then identified, the molecular team at CityU VDL developed a PCR test to screen for both *A. platys* and *A. phagocytophilum*.

This new PCR test is now available at CityU VDL either as an individual test or as part of the “Tick Fever Screening” or the “Comprehensive Tick Fever Panel”.

The new “Tick Fever Screening” panel has been expanded and now tests for the presence of *Babesia* species, *Ehrlichia* species **and** *Anaplasma* species. Both *Anaplasma platys* and *A. phagocytophilum* are detected, but not differentiated.

The new “Comprehensive Tick Fever Panel” is more comprehensive and tests for each individual *Babesia* species including: *Babesia canis canis*, *Babesia canis vogeli*, *Babesia gibsoni*, *Ehrlichia canis*, **and now** *Anaplasma* spp.

Anaplasma spp. can also be requested as an individual test, if you suspect disease or want to screen for the effectiveness of treatment.

Samples to collect: EDTA blood

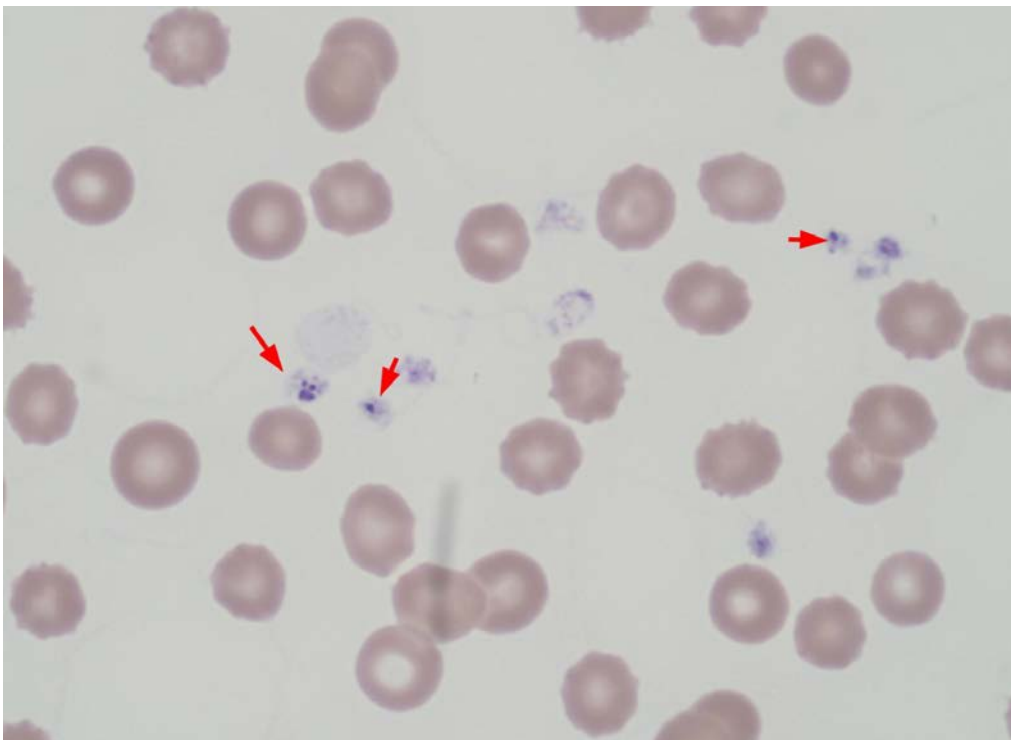


Figure 1: *Anaplasma platys* organisms within the platelets of an infected dog in Hong Kong

Investigating bone tumours: remember the ALP test

Imaging and pathology combine well when investigating tumours of bone in animals.

Once any imaging studies have been undertaken, share these results with the pathologist to maximize the information available and increase the diagnostic accuracy.

Ultrasound guided cytology taken at the same time as biopsy samples allow combined analysis and an option for ALP staining of the cytology specimen.

Diagnosis of osteosarcoma by ALP staining of cytology slides is now an option at CityU VDL. Dr Daniela Hernandez Muguero and the clinical pathology team have worked up the application of this test in Hong Kong. If you suspect an osteosarcoma in an animal, collect multiple samples for cytology and indicate your suspicions on the submission form. Some of the slides can then be retained unstained, for possible later ALP staining. If the cytology is suggestive of osteosarcoma and sufficient and suitable cellular material is present on the slides this test could be undertaken. Standard immunocytochemistry pricing applies.

This approach is highly recommended for investigation of bone tumours and can maximize the diagnostic accuracy.

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. Maintaining the appropriate ratio of blood to anticoagulant is especially important for hemostasis testing.

Recent Findings

Mycoplasma: a rare cause of canine urinary tract infection

Mycoplasmas are small fastidious bacteria commonly found associated with mammalian mucous membranes as part of their normal microbiome. They tend to act as secondary pathogens alongside other more pathogenic bacteria or viruses or when the host immune defences are overcome (e.g. with immune suppressive medication, neoplasia, indwelling catheter use and other devices). *Mycoplasma canis* is a known but infrequent cause of urinary tract infection in dogs, occurring mainly when there is compromised immune function. Whilst other Mycoplasmas are extremely difficult to culture in the laboratory, *M. canis* can be grown on regular media, (with just a little difficulty), and is thus easier to diagnose.

CityU VDL had its first case of canine Mycoplasma urinary tract infection (UTI) recently. Urine from an 8-year-old, female, de-sexed Bichon Frise grew unusual colonies on routine agar plates. Further purification and testing by our skilled technologists led to an identification of Mycoplasma species, most likely *M. canis*. The attending veterinarian clarified that the urine was collected from a

subcutaneous ureteral bypass (SUB). Repeat cultures of urine collected from the SUB were again positive for *M. canis* while a voided urine sample did not grow any Mycoplasma.

Antimicrobial sensitivity testing is difficult to perform in Mycoplasmas. Best options for treatment of *Mycoplasma* infections include doxycycline and the fluoroquinolones. In this instance, the patient was successfully treated with oral enrofloxacin.

This case demonstrates the importance of considering Mycoplasmas in those dogs where there are clinical signs suggestive of a UTI and urine sediment examinations support this diagnosis, but cultures fail to grow bacteria.

Thanks to the attending veterinarian for this interesting case and for assisting with relevant information.

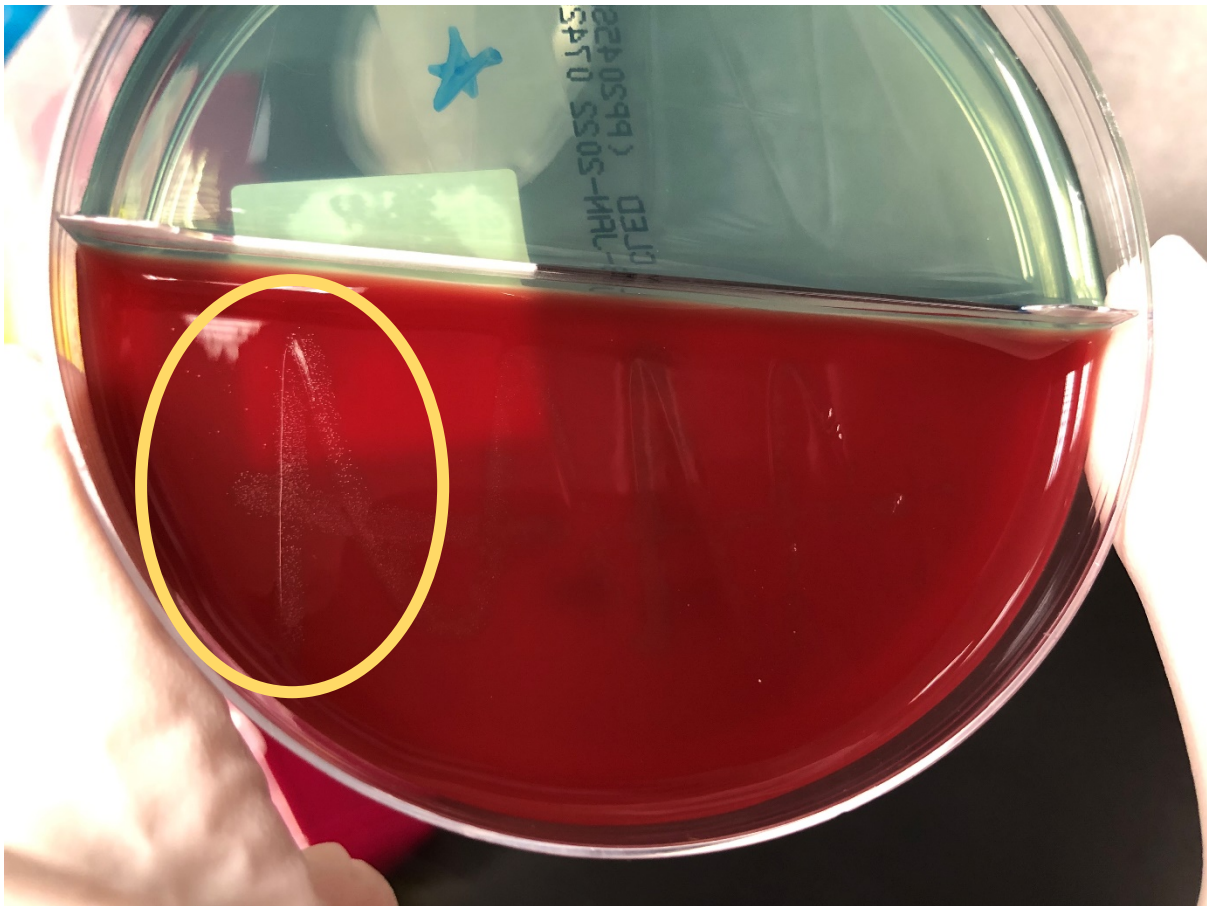


Figure 2: Mycoplasma colonies on the blood agar plate within the yellow oval

Hepatozoon canis in a dog

Fixed tissue was received from the mammary area of a female, desexed, 6-year-old Schnauzer dog to investigate an apparent mass. On histopathology examination the tissue was confirmed as inflamed lymph node reacting to the presence of protozoal meronts and micromerozoites in the tissues (figure 3).

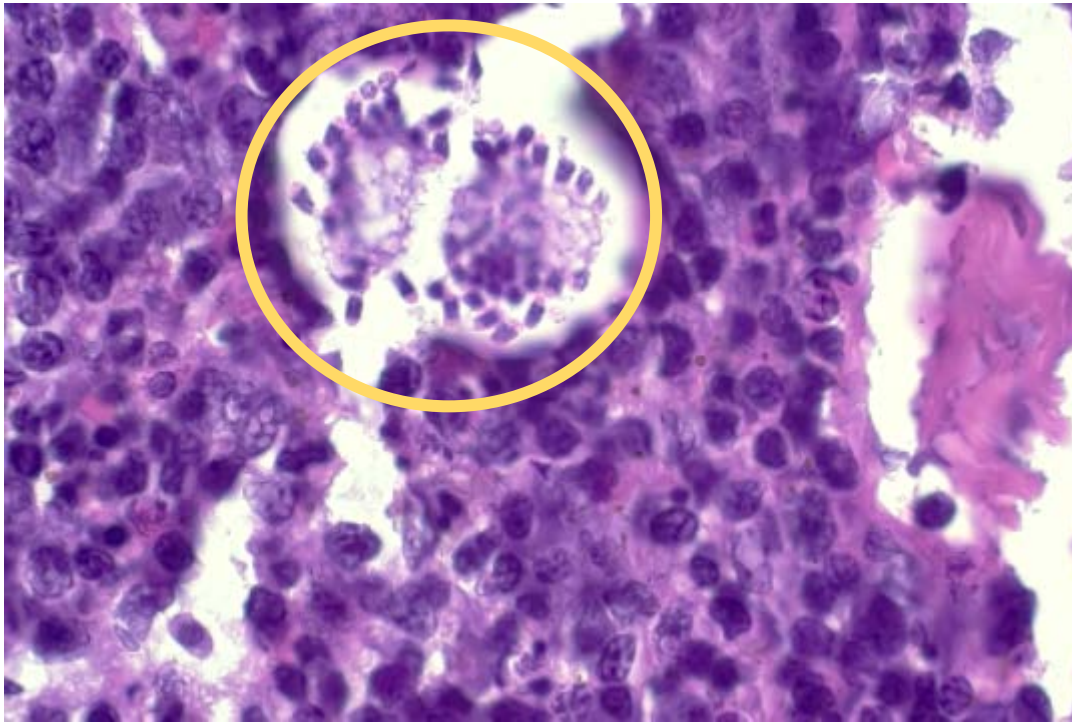


Figure 3: A cluster of micromerozoites within a lymph node (circled)

Gamonts were observed within neutrophils (Figure 3) on smears of subsequent blood samples from the dog, supporting a diagnosis of *Hepatozoon canis* infection. EDTA blood was referred to Auburn University College of Veterinary Medicine for PCR identification of the pathogen. The PCR test results confirmed the protozoa were *Hepatozoon canis*. Most dogs infected with *H. canis* have no or only mild clinical signs, and show low levels of parasitemia. Immunosuppression or concurrent disease (including other tick-borne diseases) may lead to clinical disease. Dogs may develop hepatitis, glomerulonephritis, pneumonia, severe anaemia, fever, and cachexia. Dogs with low parasitaemia have a good prognosis overall. This is the second recognition of this parasite in dogs in Hong Kong, and should be considered in dogs exposed to ticks.

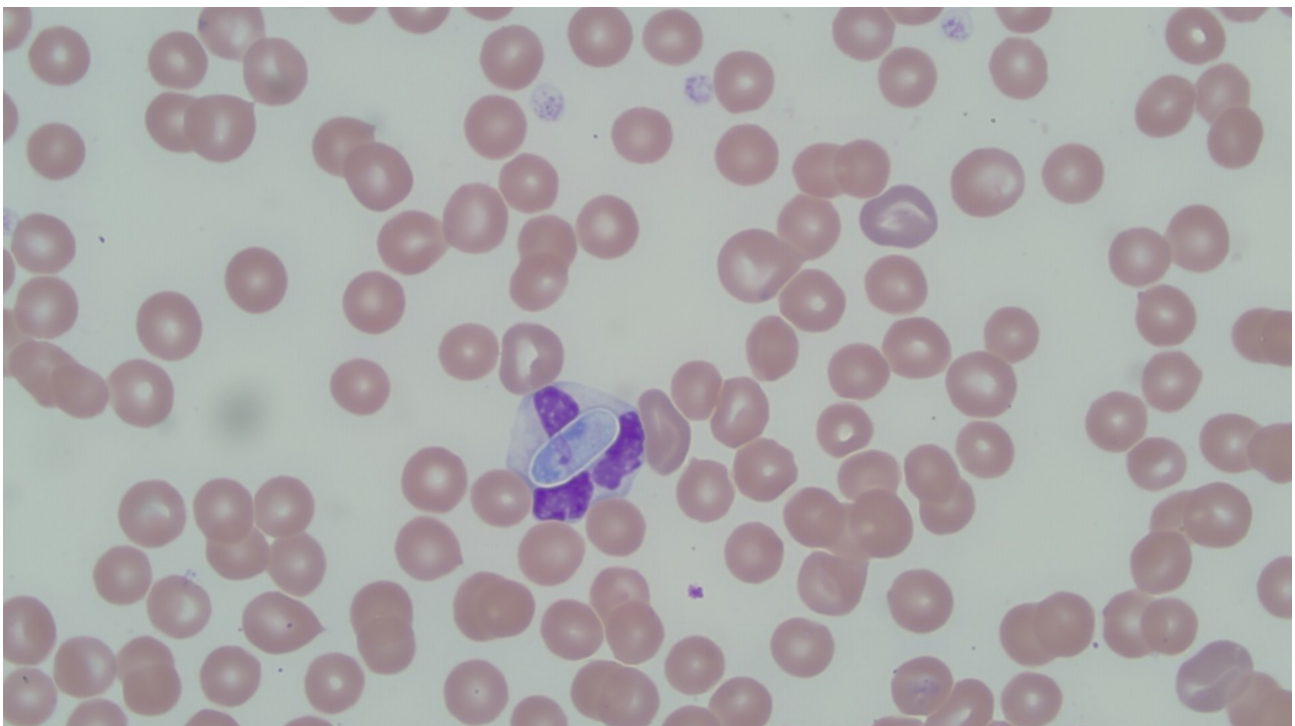
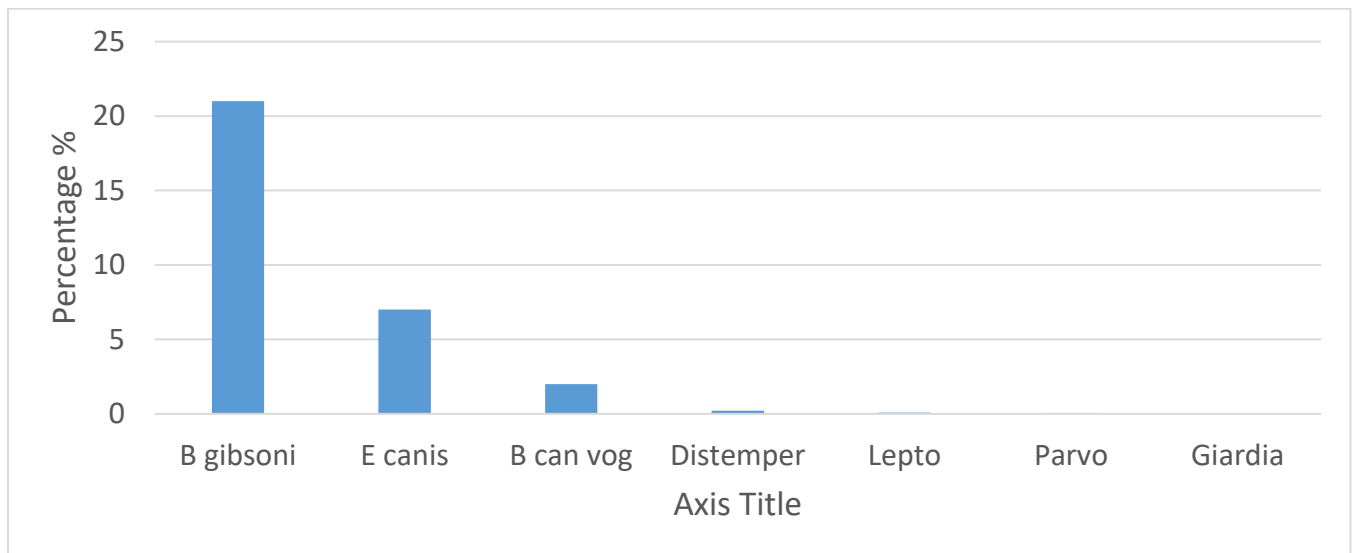


Figure 4: Gamonts of *Hepatozoon canis* within a neutrophil

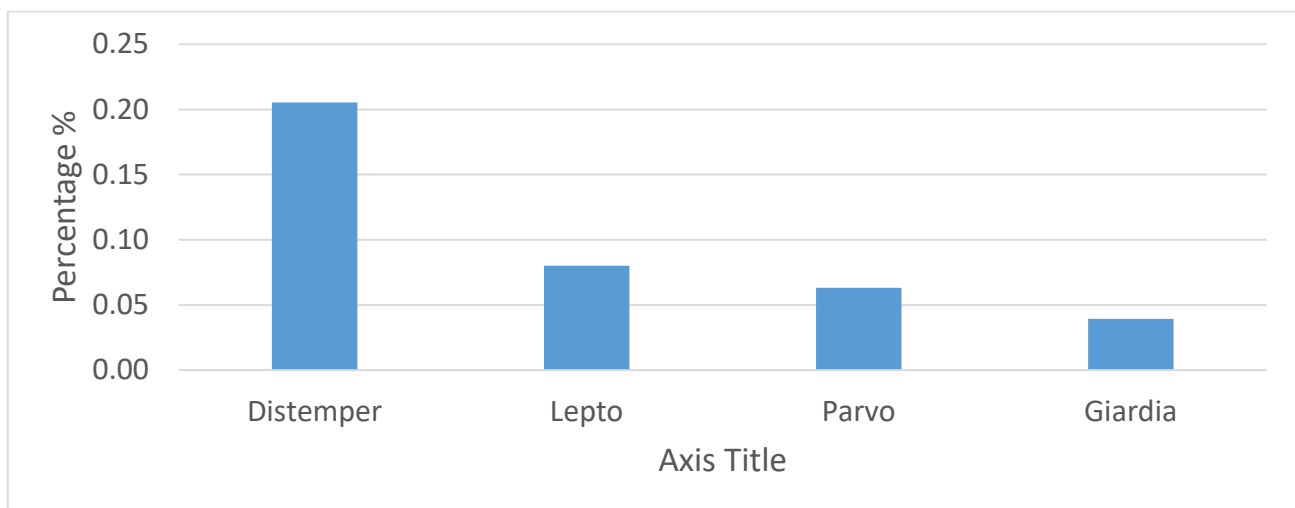
Infectious disease of dogs in Hong Kong diagnosed by molecular (PCR) methods at CityU VDL

Analysis of the data for canine samples submitted to CityU VDL for molecular testing over the past four years revealed some interesting trends. The most common infectious disease diagnosis by PCR in dogs are tick borne diseases. Data on the percentage of infectious diseases are shown in Graph 1 illustrating the dominance of Babesia and Ehrlichia infections.



Graph 1: Frequency of positive PCR diagnoses of canine infectious disease in Hong Kong from samples at CityU VDL

If tick borne disease are excluded, the next most common infectious diseases diagnosed by molecular testing are distemper, leptospirosis, parvovirus infection and Giardiaisis. This data is shown in graph 2.



Graph 2: Frequency of positive PCR diagnoses of non-tick borne canine infectious disease in Hong Kong from samples at CityU VDL

These sample numbers only represent samples received at CityU VDL. Diagnosis of some of these infections can be achieved in-clinic and that data is not included in this analysis.

Christmas treats are great for humans but not cats and dogs

A range of food and flowers associated with Christmas add cheer and joy to the Christmas season but some holiday treats and traditions can be hazardous to pets.

Here are a few to keep in mind.

Christmas lilies (*Lilium regale*):

Lilies from the genera *Lilium* (true lilies) and *Heemerocallis* (day lilies) have been associated with renal failure in cats (but not dogs) due to the presence of toxic steroidal glycoalkaloids. All parts of the plant are toxic, including the pollen, so keep them well away from cats.

Christmas food containing raisins eg mince pies, cakes and pudding:

Eating raisins or grapes is associated with acute renal failure in dogs (and possibly cats). The toxin is unclear but thought to be part of the skin and flesh of the grape or raisin.

Chocolate:

Chocolate contains theobromine and this can lead to toxicity in dogs of varying severity depending on the amount consumed.

Onions (*Allium* species):

Both onions and garlic (and food containing them) have sulphur-containing oxidants. Heinz body formation and methaemoglobin increases in red cells can lead to haemolysis about 72 hours after consumption.

Ham:

Ham contains high concentrations of salt and can result in salt toxicity in dogs if excessive quantities are consumed.

Remind your clients to feed their pets recommended pet food only and not share humans Christmas goodies. For toxicity investigations, history and access to these types of foods or lilies is a critical component in achieving a diagnosis. Serum chemistry and complete blood counts can be useful to give important clues but specific toxin analysis is not possible.

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