ISSUE 36

SPRING 2021

NATIONWIDE LABORATORIES SPRING NEWSLETTER

TIME FOR NEW BEGINNINGS

NEWS

"Mammary tumours in dogs and cats" by S. Dawson gives some useful recommendations

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"Histological Classification and Immunohistochemical Study of Feline Colorectal Epithelial Tumors" by Mizuho Uneyama, James K. Chambers, Ko Nakashima, Kazuyuki Uchida, Hiroyuki Nakayama.
This study was published in the March 2021 edition of the journal Veterinary Pathology. It examined colorectal epithelial tumours in cats and was carried out at the University of Tokyo

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"Getting the best from your pathologist: skin biopsy technique" by T. Whitbread

READ MORE →

"Metastatic feline mammary cancer: prognostic factors, outcome and comparison of different treatment modalities – a retrospective multicentre study" by Gonçalo Petrucci, Joaquim Henriques, Hugo Gregório, Gonçalo Vicente, Justina Prada, Isabel Pires, Luís Lobo, Rui Medeiros, Felisbina Queiroga

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Message from David Charvill

Dear friends,

Welcome to our spring newsletter. As always, we are here for you. Our experienced team of pathologists, laboratory scientists, couriers and customer service advisors are working with you and your samples. We have started the "Voice of Our Customer" feedback program to make sure that all your requirements are met, so please get in touch and let us know what we can do for you. We are continuing to offer you CPD and materials to help you to get the most out of your lab. This spring we have launched a virtual interactive hub at THE Vet Exhibition with FREE CPD sessions and a CPD certificate. The project will last for a year. We will be changing the theme and the content every month. Follow our updates on social media and visit our stand at any time from any connected device. We are supporting the veterinary community by sponsoring BSAVA Virtual Congress (which is still available On Demand), BVDSG and BSVP online meetings etc. As the country is slowly coming out of lockdown and the practices are performing more than essential services, we would like to remind you about personalised care packages based on DNA profiling which we can offer to your patients. Thank you very much for your custom. We are very proud to support amazing work you are doing for nation's animals. Keep well and stay in touch!

David Charvill,
Director of Laboratory Services

British Veterinary Dermatology Study Group

SPRING MEETING

In March NWL sponsored the BVDSG (British Veterinary Dermatology Study Group) Spring meeting, with a focus on bacterial skin disease. Excellent presentations were given by speakers Tim Nuttall, Anette Löffler and Finola Leonard on topics including the skin microbiome, antimicrobial stewardship and primary factors in pyoderma.

The importance of protecting the diversity of the skin microbiome was highlighted, particularly in atopic dogs where disease flares are associated with dysbiosis as they are in humans – notably a reduction in diversity and shift towards staphylococci. In humans, loss of intestinal microbiome diversity is associated with a multitude of chronic conditions including allergies, IBD, type II diabetes, cardiovascular disease, autoimmunity and behavioural disorders. An individual's microbiome is influenced by many factors including age, diet, lifestyle, season, geography and environment. Pet owning people have better diversity than non-pet owners, as do those living in rural compared to urban environments. The skin microbiome is a major contributor to cutaneous immune function and is also vital in skin barrier function.

In staphylococcal pyoderma genetic studies have shown that colonising and infecting strains of staphylococci are the same, rather than acquired exogenous pathogens and we could therefore reconsider staphylococcal superficial pyoderma as 'staphylococcal dysbiosis'. Management of dysbiosis in dogs with atopic dermatitis requires treatment of the underlying disease, aiming for proactive treatment rather than regular reactive therapy for flares. Systemic antibiotic therapy (particularly pulse therapy) should be avoided unless there is sound clinical justification, and this can be spared through the use of anti-inflammatory and anti-atopic medications, along with topical antiseptics where indicated. Topical therapy is highly effective in superficial pyoderma, and anti-inflammatory/immunomodulating therapy and topical antiseptics have been shown to not adversely affect the skin microbiome. Systemic antibiotic therapy treats the whole microbiome (cutaneous and gut), potentially with catastrophic effects on diversity. In addition, every time we use systemic antibiotics we are selecting for resistance and pruritus remains one of the most common reasons for antimicrobial prescribing in small animal veterinary practice. Cytology can be a vital adjunct in therapeutic decision making, along with culture and sensitivity testing where indicated. Culture is particularly warranted in life threatening or deep infections, infections with suboptimal response to empirical therapy, cases with rod shaped bacteria on cytology or where cytology does not correlate well with the clinical picture, and infections where resistance is more likely (e.g.: previous courses of antimicrobial therapy, non-healing wounds, patients where nosocomial infection is possible).

There may be a role for prebiotics and/or probiotics in supporting and optimising the skin microbiome, and further investigation into the interactions between the immune system, microbiome and skin barrier will increase our understanding and help optimise management of these cases.

Helen R Campbell
BVM&S FRCPath MRCVS

Do I need to send a history with my cytology and histology samples?

Dear Valued Customer,

The answer is YES! But short and relevant.

The pathologist is here to try and help reach a diagnosis, so the clinician can progress to possible further tests, treatment and ultimately resolve the problem.

In order to get the best and most relevant pathology interpretation from your sample, please always send us the following:

- Age
- Species
- Breed
- Sex
- Site of sample or lesion
- Brief description of the lesion

We then come to the clinical history. This needs to be RELEVANT. A short paragraph as notes is ideal. Please do NOT send computer printouts. They are usually too long and include irrelevant details. For this reason they are often counterproductive for getting the best results from your pathologist. This is NOT a situation when more is better.

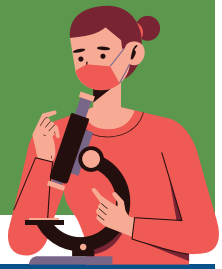
A list of your clinical differential diagnoses is extremely useful, as the pathologist can then speak to those differentials.

You may also like to include short relevant notes on some of the following:

- Clinical signs and duration.
- Any previous therapy administered.
- Any previous lesions, other relevant pathology that has previously been identified.
- Description of lesion with size.
- Any unusual findings encountered during sampling.

So, send us your samples!

Yours sincerely,
NWL Pathology Team



BRUCELLOSIS IN IMPORTED DOGS – AN IMPORTANT UPDATE

READ MORE

It is important to highlight the recent increase in cases of *Brucella canis* infection diagnosed in the UK. Since summer 2020, the APHA has been notified of more than 40 cases of canine brucellosis. Almost all cases have been imported dogs, the vast majority from Romania. *B. canis* is not considered endemic in the UK and historically it has only rarely been diagnosed in imported dogs.

B. canis is predominantly associated with reproductive failure, but other clinical signs include discospondylitis and uveitis, and infection can be subclinical. Zoonotic infection of *B. canis* is infrequently reported in humans but may be under-diagnosed, due to often vague clinical signs and lack of testing. Infected dogs pose a significant risk to owners, veterinary staff and laboratory staff processing pathology samples, particularly in individuals with certain underlying health conditions.

In order to identify cases and minimise the risk of infection and transmission to other dogs or humans the following measures are recommended:

- **Highlight risk to clients** importing dogs from Eastern Europe (particularly Romania) or those travelling with their pets to this region. Encourage potential owners to request pre-export testing and consider pre-breeding testing.
- **Highlight risk to the veterinary team** and routinely ascertain the origin and travelling history of dogs (and their parents) when they are registered and seen.
- Ensure **appropriate testing** is performed and **PPE** is used in suspected clinical cases.
- **Notify diagnostic laboratories clearly on submission forms** of the import/travelling history when submitting samples from these patients to enable us to take appropriate precautions, regardless of whether they have clinical signs of Brucellosis. Ensure sample packaging is appropriately **biosecure**. This also helps protect us against other potential pathogens in addition to *B. canis*.

NationWide Laboratories proudly supports @FyldeBeeProject

Local Fylde Bee Project brings our community together to support the bees. Bees pollinate around 80% of wild flowers in Europe. There are over 20,000 known species in the world. Around 270 species have been recorded in the UK. It is predicted 40-70% of species could go extinct without action. At our site in Poulton we have planted bee friendly mix of seeds native to the UK. We are going to be keeping a diary and sharing the information on @FyldeBeeProject Facebook page, LinkedIn and Twitter. Please follow our social media for more updates.

SAMPLE TUBE TIPS

Dear Valued Customer,

Please remember to **mark sample tubes** with the animal name and if it is fluid (not blood) to mark the tube clearly (e.g. Abdo fluid or pericardial fluid) and for ProBNP mark the tube clearly as EDTA Plasma.
Thank you for your help!

Yours sincerely,
NWL Team

WELCOME TO VITEK

We have started to use our new Vitek equipment for microbiology, so you might see that the reports you receive have a new updated format. If you have any questions please contact our team at 01253 899215.

Topic of month 1 is Cytology for vets and nurses.

- CPD webinar: "Basic cytology for practitioners" by Trevor Whitbread.
- "HOW TO" podcast on on fine needle aspirates, impression smears and scrapings.
- Downloadable guide on selected aspiration techniques.
- LabFacts.
- Bitesize overview of recent scientific papers.

And much more! Come to see us virtually.

DON'T LET REALITY HOLD YOU BACK!

CANINE SOFT TISSUE SARCOMA: CASE STUDY

An 8-year-old male Labrador presented with ongoing history of lameness. Clinical examination revealed marked swelling of the right elbow. A mass was noted close to the elbow joint and was suspected to affect joint mobility.

Cytology examination of a synovial fluid sample from the right elbow was performed. This revealed low cellularity composed predominantly of large mononuclear cells (occasionally vacuolated), with lower numbers of small mononuclear cells and spindle cells. Large mononuclear cells had round nuclei with prominent single or multiple small nucleoli and moderate amounts of mid-basophilic cytoplasm. There was mild anisocytosis and anisokaryosis and occasional mitotic figures were present. Occasional binucleated or multinucleated spindle cells were also noted.

Sampling of structural lesions with biopsy for histopathology examination was advised to rule out neoplasia.

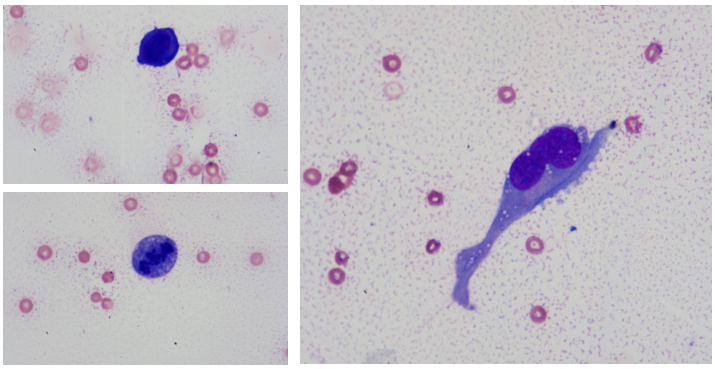
Biopsies were submitted from joint capsule and adjacent muscle of right forelimb.

Throughout the connective tissue there were infiltrates of plump spindle cells forming short interweaving bundles. The cells had oval nuclei with irregular outlines and reticulate chromatin patterns. Cells contain small nucleoli. They had low to moderate amounts of fibrillar cytoplasm with indistinct borders. There was mild anisocytosis and within this population there were mitotic figures. Sections from the joint capsule consisted of dense fibrocollagenous connective tissue with scattered isolated blood vessels and the same plump spindle cells forming interweaving bundles or whorls and aggregates.

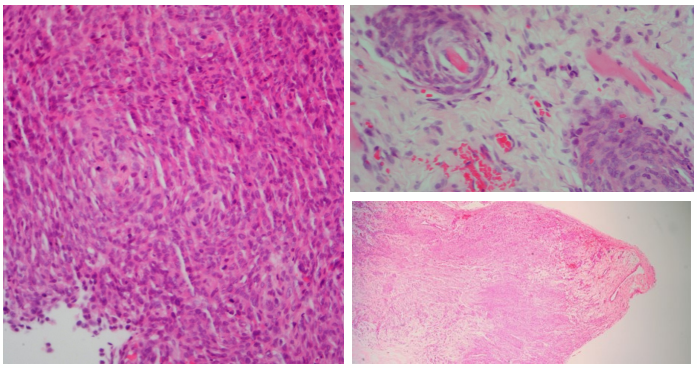
A diagnosis was made of a canine soft tissue mesenchymal tumour (sarcoma) which appears to be of low to intermediate grade. Differentials include fibrosarcoma, perivascular wall tumour or peripheral nerve sheath tumour.

Historically, synovial cell sarcomas have been reported commonly in dogs. However, more recently studies using immunohistochemistry have shown that many different types of mesenchymal tumours can develop in and around joints in dogs. The majority are not of synovial cell origin. This particular tumour is considered to be of connective tissue origin. Soft tissue sarcomas (mesenchymal tumours) tend to behave in the same way regardless of the exact cell of origin. Given the cytological features, mitotic activity, and absence of necrosis this may be a low-grade tumour, although on examination of larger areas of the tumour this grade may increase. Such tumours are locally infiltrative and frequently recur following surgery due to difficulty in achieving adequate excision margins at awkward surgical sites and the ability of these tumours to dissect between fascial planes. Metastasis however is uncommon or develops late in the course of the disease, often by haematogenous rather than lymphatic spread.

Sandra Dawson,
BSc BVMS FRCPath MRCVS
Alina Bodnariu,
DVM MSc PhD FRCPath MRCVS



Cytology showing large mononuclear cell (top left), mitotic figure (bottom left) and multinucleated spindle cell (right).



Histology showing poorly defined, densely cellular areas of shorts interweaving bundles and whorls of neoplastic spindle cells. Mitotic figures present in the large image on the left.

PREGNANCY DIAGNOSIS IN DOGS AND CATS

Currently the **RELAXIN** test which can be performed (after 30 days gestation) on serum or heparin plasma is currently NOT AVAILABLE.

Progesterone is not a useful test for pregnancy.

In the dog, non pregnant bitches have significant levels of progesterone as part of their normal cycle (metoestrus), therefore progesterone alone cannot be used as a canine pregnancy test. It can be use to rule out pregnancy where very low levels are detected.

In the cat, progesterone analaysis will also only indicate if the cat is non pregnant (< 3 nmol/l).

After ovulation the progesterone rises Rises steadily until day ~ 21 post-ovulation: In non-pregnant diestrus queens, it begins to decline shortly thereafter remaining **partially elevated** for 35-40 days.

Thus other methods of diagnosis include:

1) **Palpation**: by ~**21 days**: Formation of small swellings along the uterine horns (deciduomata). These are palpable, assuming the animal is cooperative, at this time.

Fetal growth is rapid during early pregnancy, and these swellings double in diameter every 7 days.

After **day 35–38**, they become less distinct, and palpation becomes difficult until late pregnancy when fetal heads and rumps are palpable as firm, nodular structures in the ventral caudal abdomen.

2) **Radiography: day 42-45** gestation - Although the fetal skeleton begins to calcify as early as day 28, it is not detectable by routine radiography until approximately day 42–45 and is quite prominent by day 47–48. Radiography at this time is not teratogenic.

Late gestational radiography (>55 days) is the best method to determine litter size. Fetal dentition becomes visible at term, and its appearance can be used to confirm fetal development adequate for an elective caesarean section when ovulation timing is not available and breeding dates are vague or spread over many days.

3) **Ultrasonography**: Best at **25–35 days** gestation - is also useful in pregnancy determination and permits evaluation of fetal viability.

Before 21 days, "false-negative" results are seen. Doppler-type instruments allow one to "hear" the fetal heart, which beats 2–3 times faster than that of the dam. Placental sounds may also be heard. Ultrasonography is especially helpful in differentiating pregnancy from other causes of uterine distention (eg, hydrometra, pyometra, mucometra). Ultrasonographic measurements can be used to calculate gestational age.

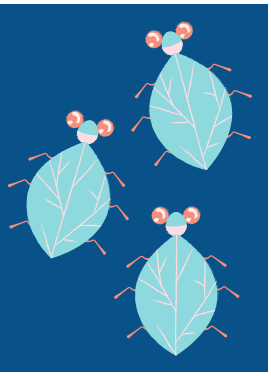
THANK YOU FOR FEEDBACK

Giles Constant, Melton Vets:
Very happy with the service not a single complaint. Our vets know that your Pathologists are happy to help.

Lesley Chamley, Quarry Veterinary Group:
I don't have to contact you much (thank goodness, means tricky cases!), however, I had to call twice in the last 2 weeks. The people who answered the phone were AMAZING! So helpful, knowledgeable and friendly - so, thanks!

Sue Whitehead, Oakhill Veterinary Centre:
We are delighted with NationWide Laboratories. The staff who answer our calls go out of their way to help. If an answer can't be found immediately there's always follow up later in the day. The courier facility is outstanding and has made all the difference to quick diagnostics, ultimately improving our service in the eyes our clients.

DR. THIERRY OLIVRY'S POINT OF VIEW ON STORAGE MITES



All mites (Acari) are arthropods of the arachnid class, and they are further subdivided into numerous orders based on the anatomy of their respiratory system openings. There are two main types of mites against which humans and pets can sensitize: the house dust (HDM) and the SMs. In temperate climates, the formers are generally of the Pyroglyphydae family, with Dermatophagoides pteronyssinus (Dp), D. farinae (Df), and Euroglyphus maynei representing the principal mite species. Among dozens of genera and species, the main SM of interest belong to either the Acaroidae (Acarus siro [As] and Tyrophagus putrescentiae [Tp]) or Glyciphagidae families (Glyciphagus domesticus [Gd] and Lepidoglyphus destructor [Ld]).

Both HDM and SM thrive in humid conditions, with a relative humidity of 50–60% favoring the presence of Df, 70–80% that of Dp, and higher rates promoting SM development. The optimal temperature for the growth and survival of Tp appears to be 25-30°C. At optimal conditions, Tp can reproduce 500 times a month! Of course, such high humidity also promotes the growth of molds on which SM like to feed.

Because of their need for high relative humidity, one should expect a seasonal increase in HDM and SM growth in the environment—with a corresponding increase in their allergens—in months of highest humidity, months that will vary depending on where you live.

As such, one should look at the possibility of mite allergy beyond solely expecting nonseasonal allergic signs; seasonal exacerbations are possible at times of highest mite population growth.

Storage mites in the environment

Storage mites, such as Tp, appear to have a diverse range of habitats, as these mites have been recovered from grains, flours, oil-seeds, bread, cheese, hams, dried milk, dried eggs, dried fish, dried fruits, mushrooms, and pet foods; this mite tends to prefer fat- and protein-rich commodities.

For decades, we have presumed that pets contact HDM in their environment, while they might encounter SM in improperly stored dry pet foods. The reality is more complex, with mite populations in the environment likely depending upon the various climates and the detection method.

To illustrate such complexity and variability, let me highlight the results of three studies done in various geographical locations with three different methods.

A decade ago, R. Farmaki and colleagues collected dust samples from Greek households with mite-sensitive dogs with atopic dermatitis (AD), normal dogs, or without dogs. Samples were vacuumed from the owner's mattress and dog sleeping areas or couch for households without dogs. The dust mite quantity was estimated by floatation, and the mites were speciated by light microscopy. Dermatophagoides species were the predominant mites encountered in the households (40-64% for Df, 35-48% for Dp), while SM were detected much less commonly (4-20% of households). As expected, dust mite numbers were lower in the winter than in the summer months.

In a master's degree thesis from the University of Liege, D. Combarros used a mite trapping system (Acar'up – a textile with mite-attracting pheromones) to evaluate the presence of HDM and SM in atopic dog sleeping areas in Belgium. The procedure was repeated twice weekly for three weeks; mites were identified by microscopy. Surprisingly, the number of mites per dog bedding area was very high (7,600 to 24,600 mites/m2), with 76% of the mites identified as Tp, 12% s Df, and 10% as Dp; other SM species were not found.

Finally, using a PCR method, G. Reboux and colleagues recently reported that the concentrations of SM (As, Gd and Ld; there was no testing for Tp) were higher than those of HDMs in homes in Eastern France; SM concentrations were similar in bedrooms and kitchens, while HDM concentrations were higher in bedrooms, especially under beds than in kitchens.

... CONTINUED

Storage mites in pet foods

We recently published a critically appraised topic on storage mites in commercial pet foods; you can download this article openly here. The main conclusions reached after reviewing the evidence were as follows: SM, especially Tp, can grow in protein- and fat-rich dog food kibbles. Their population growth is higher when the initial contaminating mite density is high, when kibbles are crushed, or suboptimal storage leads to mold overgrowth. When purchasing commercial dry dog foods, SM contamination is undetectable, but, when present, the SM density is very low. When storing bags in temperate conditions indoors, one can expect little overgrowth—if any—of SM. In contrast, when keeping dry dog foods in high temperatures and humidity, for example, in a garage, Tp will enter and proliferate in the packages. In such conditions, SM can also contaminate sealable bags, which they will invade via faulty seals.
Finally, a recent study added some pertinent information on the influence of storage on Tp contamination in Taiwan. If first confirmed that bags of stored products (including pet foods) are least contaminated with Tp when the bags are sealed. The contamination of stored products with Tp was nil when the relative humidity was below 50%, or the temperature lower than 4°C (that of a refrigerator); the higher the humidity or the temperature, the higher the contamination with Tp.

While I heard colleagues recommend freezing pet food kibbles to prevent their contamination with SM, I could not locate evidence supporting this recommendation. Anyway, if a bag of pet food were contaminated with SM, freezing it might merely kill mites and prevent further population growth, but it would not reduce the mites' allergenicity and feces in that bag!

Clinical bottom line:

It is likely that, in some locations, dogs encounter SM—especially Tp—more often in their immediate environment (bedding) than in their pet foods. Commercial pet foods' contamination occurs mostly in suboptimal storage situations, especially when the temperature or relative humidity is high. Keeping dry commercial pet foods indoors in low humidity conditions and sealed bags should prevent their contamination with SM.

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