



# Abbey Veterinary Services

DIAGNOSTIC HISTOPATHOLOGY AND CYTOLOGY

Clinicopathological Newsletter

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## CASE OF INTEREST

### A case of Cutaneous Leishmaniasis in a crossbred dog.

By Richard Fox, Veterinary Pathologist

Case donated by Lucy Genovese, Veterinary Pathologist.

A four year old crossbred dog was presented with a clinically swollen digit on the right foreleg (digit 2). The lesion was characterised by excessive scaling and moderate alopecia with mild hyperaemia and small areas of ulceration. The lesion had been treated with systemic 'antibiotics' whilst residing in Spain with no evidence of a clinical response. The lesion had been present for 3 months and the owner had correlated the formation of the lesion with running through long grass. Initial thoughts were of a foreign body reaction but given the history of a lesion not responsive to 'antibiotics' the lesion was excised. No other lesion were evident on the skin at that point and there was no evidence to support any systemic abnormalities.

A toe was submitted with a 1 cm raised mass lateral to nail. A section of haired skin taken through this mass displayed several changes. There was an ill defined nodular infiltrate of cells extending from the superficial dermis into the deep dermis. The overlying epidermis was markedly hyperplastic and there was marked compact orthokeratotic and parakeratotic hyperkeratosis (Fig. 1). Hair follicles displayed mural hyperplasia in the superficial dermis but mild atrophy in the mid to deep dermis. Hair shafts were rarely observed.

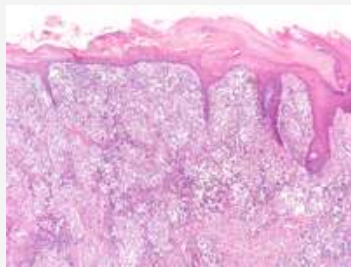


Figure 1. Histological section of haired (alopecic) skin with marked epidermal hyperplasia, hyperkeratosis and a dermal cellular infiltrate. (x5 obj.). HE Stain.

Large numbers of densely packed epithelioid macrophages with multifocal dense infiltrates of plasma cells were identified. There were lesser small lymphocytes and neutrophils scattered throughout (Fig 2.).

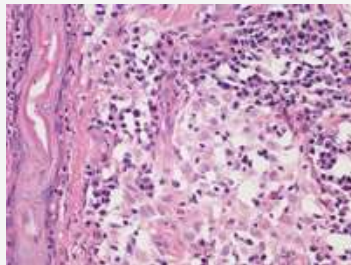


Figure 2. Histological section of a groups of macrophages (pale cells) and plasma cells (dark round nuclei with eosinophilic cytoplasm) adjacent to a hair follicle without a hair shaft. (x40 obj.). HE Stain.

Within the macrophages, throughout the lesion, there were small 1 micron diameter basophilic structures with a small body at one end. These structures were contained in a faint clear vacuole (Fig 3.).

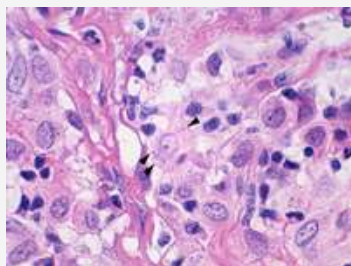


Figure 3. Histological section of macrophages containing leishmanial organisms (arrow heads) with a faint polar dot (kinetoplast)(x10 obj.). HE Stain.

Canine leishmaniasis (CanL) caused by *Leishmania infantum* (syn. *L. chagasi*, in Latin America), which is transmitted by the bite of phlebotomine sand flies, is endemic and affects millions of dogs in Europe, Asia, North Africa and South America. This organism is endemic in the Mediterranean region. It is an obligatory intracellular, diphasic protozoa of the genus *Leishmania*.

Despite the lack of pathognomonic manifestations, the commonest clinical signs are cutaneous alterations (cutaneous form), local or generalized lymphadenomegaly, loss of body weight, liver and spleen enlargement, glomerulopathy, ocular lesions, epistaxis, onychogryphosis and lameness. Atypical forms include monoclonal gammopathy, chronic colitis, haemostatic alterations and disorders of the cardiovascular, respiratory and musculo-skeletal systems (visceral form). In this way, *Leishmania* infection shares many of the clinical and pathological features with other canine diseases.

Cutaneous lesions are in most cases generalised and produce and exfoliate (scaling) dermatitis. This is usually non-pruritic and accompanied by alopecia. Nodules and multifocal ulceration may occur in

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## JOURNAL Articles(with e-links)

1. T. M. Blacking, H. Wilson and D.J Argyle. Is cancer a stem cell disease? Theory, evidence and implications. *Veterinary and Comparative Oncology* (2007) 5(2)pp76-89. [Link](#)

In this review article the authors describe recent research centered on the concept that one or more groups of cells within a cancer population are efficient at producing tumours whilst other groups of cells lack this ability. The evidence suggests that the groups of cells with this ability have stem cell-like characteristics.

Most cancer research has focused on a model of multistage carcinogenesis in which progressive genetic changes lead to malignant transformation. In this model any cell in the body has the potential for malignant transformation. Recently attention has become directed towards an alternative model in which the tumour is maintained by a cancer stem cell (CSC) and composed mostly of daughter cancer cells and a small number of CSC; it being the CSC that drives tumour growth and expansion.

Adult stem cells are characterised by a capacity for self renewal. They are undifferentiated but capable of multilineage differentiation. They cycle slowly, are clonogenic and capable of asymmetric division. It is still uncertain if a CSC is a normal tissue stem cell which has undergone malignant transformation or a more differentiated cell which has acquired stem cell-like characteristics as a result of mutation or dedifferentiation.

There are several implications for cancer therapy. If a population of CSC propagates a tumour then these need to be eliminated if a cure is to be achieved. Conventional strategies targeting rapidly dividing cells may not be effective. Stem cells can enter periods of quiescence in which they will be resistant to therapies aimed at cycling cells.

Other therapies may preferentially target CSC. For example, forcing CSC down a pathway of symmetrical division so that 2 committed daughter cells are produced. This would deprive a tumour of its self renewal potential. Such treatments may have little effect on the differentiated progenitor and daughter cells, leaving the bulk of the tumour intact. Further implications are that remission may lag behind the destruction of the CSC population, creating difficulties in assessing response to therapy. In these circumstances overall survival may be a better assessment than clinical response, which may require long study times.

Reviewed article by L. Genovese

2. Favrot C, Welle M, Heimann M, Godson DL, Guscetti F. Clinical, histologic, and immunohistochemical analyses of feline squamous cell carcinoma in situ. *Vet Pathol.* 2009 Jan;46(1):25-33. [Link](#)

Actinic keratosis (AK) and Bowenoid in situ carcinoma (BISC) are two distinct forms of in situ squamous cell carcinoma in felines. They usually occur on different locations and present with specific clinical and histologic features. However, in some cases, these diseases cannot be distinguished either clinically or histopathologically.

The aim of the present study was to determine the accuracy of diagnosis based on clinical or histologic criteria alone, and whether immunohistochemistry for papillomavirus or p53

concert with exfoliation as the disease progresses. Nodular skin disease is less common but in this case may have represented an initial site of infection.

The laboratory diagnosis of CanL still poses a challenge, despite progress made in the development of several direct and indirect methods. PCR-based methods applied for *Leishmania* detection are more reliable for determining the presence and identification of the parasite not only in active cases, but also for monitoring parasitological cure after chemotherapy. PCR for the detection of *Leishmania* DNA can be carried out with a broad range of clinical specimens: whole blood, buffy coat, bone marrow, lymph node, skin or conjunctiva swabs/tissue. A single negative PCR result in a clinically suspected dog is not enough to rule out infection. Studies evaluating PCR from different tissues of infected dogs have shown variable and sometimes conflicting results.

Other direct and indirect tests including ELISA, Immunodiffusion Assay and Direct Agglutination Test have also shown good sensitivity and specificity some of which are available as a commercial diagnostic test.

References:

1. Maia C, Campino L. Methods for diagnosis of canine leishmaniasis and immune response to infection. *Vet Parasitol.* 2008 Dec 20;158(4):274-87
2. Alvar J, Cañavate C, Molina R, Moreno J, Nieto J. *Adv Parasitol. Canine leishmaniasis.* 2004;57:1-88.
3. Infectious nodular and diffuse granulomatous and pyogranulomatous diseases of the dermis. in *Skin Diseases of the Dog and Cat, 2nd edition (2005)*, Gross, Ihrke, Walder and Affolter pp. 312-319.

can improve the accuracy of diagnosis. A series of in situ squamous cell carcinoma cases (n = 45) were selected according to their location and initial histologic classification and subsequently classified as AK (n = 22) or BISC (n = 23) according to the clinical criteria and were re-evaluated histologically by 2 dermatopathologists.

All BISC cases and most of the AK cases (n = 15) were confirmed histologically. In 7 cases clinically classified as AK, this diagnosis was not unanimously confirmed histologically because of the presence of overlapping features. P53 immunoreactivity was observed in 11/14 (79%) confirmed AK cases and in 4/22 (18%) BISC cases, while papillomavirus antigen was not detected in any confirmed AK case but was detected in 11/23 (48%) BISC cases.

It was concluded that BISC can usually be reliably diagnosed histologically. The histologic diagnosis of lesions clinically suggestive of AK might sometimes be difficult. Results of immunohistochemistry for p53 and papillomavirus antigen were supportive for a role of sun exposure and papillomavirus in the pathogenesis of AK and BISC, respectively.

**LATEST NEWS**

**Anti-freeze poisoning in cats**

Abbey Veterinary Services have received a number of submissions containing renal tissue (mostly post-mortem material) from cats presenting with signs of renal failure in recent weeks (stumbling, vomiting and depression, seizures, ). Cats often present, initially, with increased urination but in later stages, urinary output is decreased.

Histology has revealed numerous intra-tubular crystals, embedded into the tubular epithelium with necrosis of tubular epithelium and some with epithelial loss, and some with regeneration (sub-acute).

These samples are typical for crystal induced nephrosis and at this time of year we are noticing a typical seasonal increase in cases. We usually suspect suspect that this is oxalate induced nephrosis and we therefore assume that this is due to the use of ethylene glycol in anti-freeze products.

**SIDE STORY**

**Permanent identification to be required for Canine Health Schemes**

Excerpt from the BVA.

The British Veterinary Association (BVA) has welcomed the Kennel Club's confirmation that, from January 2010, all dog owners and breeders wishing to participate in the Kennel Club/BVA Canine Health Schemes (CHS) for eye disease and hip and elbow dysplasia will be required to have their dogs permanently identified with either a microchip or tattoo.

"In November, following discussion at our Ethics & Welfare Group, we called, along with an independent review of the breeding of dogs, for the permanent identification of all registered pedigree dogs. The inclusion of this requirement for participation in the Canine Health Schemes is hopefully the first step."

For further information on this topic visit [www.bva.co.uk](http://www.bva.co.uk).

**CYTOLOGY TIPS**

**Avoiding the most common problems - quick tips**

- **No (or few) cells obtained:** submit more slides (4-6) from multiple collection attempts. Can be due to inadequate negative pressure.
- **Blood Contamination:** Usually caused by the use of too large a needle ( >21 gauge) or excessively prolonged aspiration although in tissues which are highly vascular (spleen, liver and kidney) this is hard to avoid.
- **Poorly prepared slides:** High yields are useless if the deposits are too thick etc. The smear should be made immediately after expulsion of material onto the slide and should be smeared gently, evenly and as thinly as possible without inducing rupture or smearing artifact (too hard leads to smearing artifact - ruptured cells). If the quantity of material is excessive it can be split into two preps.

**MESSAGES**

**SEMINARS**

We have recently been asked to give clinicopathological and pathological seminars by groups of practices and specialist groups.

If you have a request for us to give a talk on a particular subject, especially if you are have a specialist interest or are a member of a specialist referral centre we would like to hear from you.

We have a team of very experienced pathologists with a broad knowledge of disease in a wide variety of animals. Many of our pathologists are accustomed at presenting talks. Just Let us know!

**OUR DETAILS**

Abbey Veterinary Services  
89 Queen Street  
Newton Abbot  
Devon  
U.K.  
TQ122BG

[admin@abbeyvetservices.co.uk](mailto:admin@abbeyvetservices.co.uk)

Tel: +44 (0)1626 353598  
Fax: +44 (0)1626 335135

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