Investigating vitamin D metabolism in cats with tuberculosis caused by infection with *Mycobacterium bovis* and *Mycobacterium microti*

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**AIMS OF STUDY**

To determine whether infection with *M. bovis* or *M. microti* affects vitamin D receptor levels and if this correlates to more severe clinical signs and disease progression. Understanding the role of the immune response, particularly of macrophages and vitamin D, may help to determine more effective methods for treating cats with mycobacterial infections, or improve diagnosis.

**METHODS**

Twelve feline tissues samples were divided into three groups: *M. bovis*, *M. microti* and ‘Healthy’. Four tissue samples in each category were used for this study. Immunohistochemistry was performed to detect the antigens of interest (macrophage specific molecule and vitamin D receptors [VDR]). Antigen retrieval was required to unmask the antigens. Two different methods were used: Heat-induced epitope retrieval (HIER) and Protease-induced epitope retrieval (PIER). Haematoxylin and eosin staining was performed to enable comparison of tissue morphology, including the identification of cellular infiltrates and granulomas. Ziehl–Neelsen (ZN) staining was used to identify acid-fast organisms, primarily mycobacteria.

**RESULTS**

In *M. bovis*-infected cats granulomas and/or areas of significant inflammatory infiltrates were observed which were associated with the presence of macrophages. These were often clustered with ZN+ organisms. The more macrophages present, the lower the number of bacteria. It was difficult to draw a solid link between expression of VDRs and the severity of disease in the *M. bovis*-infected cats.

In *M. microti*-infected cats a link between VDRs and the severity of disease could not be drawn and there was no clear correlation between macrophage and mycobacteria numbers.

In the healthy cats there were considerably less VDRs and macrophages than in the cats with mycobacterial infections. Low numbers of macrophages were seen and no ZN+ organisms were identified.

**CONCLUSION**

The mycobacteria-infected cats could have up-regulated the number of VDRs present in their tissues in order to increase the responsiveness to vitamin D. It is known that VDRs are up-regulated by 1,25(OH)2D and since infection increases the conversion of inactive 25(OH)D to active 1,25(OH)2D this could explain the increased VDR expression seen. Cytokines secreted by T cells during inflammation also affect VDR expression illustrating that regulation of the VDR level is a common mechanism used in the defence against pathogens.

To enhance this study a larger sample size should be used to increase the reliability of the results obtained. The effects of the different treatment regimes could be accounted for by using a larger sample size and cross referencing the results to the specific treatments. More investigation into vitamin D needs to be carried out in order to gain a better understanding of the affects vitamin D has on the immune response. Researchers have already discovered that higher levels of vitamin D are linked to better survival chances for hospitalised cats. We know that vitamin D plays a major role in the immune response but we need to know how significant that role is in mycobacterial infections, as this could lead to new treatment methods and help fight infections.

Development of a novel diagnostic assay for the diagnosis and monitoring of canine immune-mediated thrombocytopenia (IMT).

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**AIM**

The aim of this study was to develop a novel diagnostic assay for the diagnosis and monitoring of canine immune-mediated thrombocytopenia (IMT). This is a common condition affecting dogs for which there is no diagnostic test currently available in the UK. Previously described assays used for research purposes require immediate analysis of samples, therefore an additional aim was that the assay must be accurate even when samples are stored for 48 hours at room temperature to mimic postage of samples to a reference laboratory.

**METHOD**

A flow cytometry assay was developed utilising ‘direct to blood’ (DtB) immunofluorescence staining of whole blood at the point of collection. Participating small animal hospitals were provided with pre-prepared sample tubes allowing immunofluorescence staining anti-canine-CD61-Alexa Fluor
Investigation of sensitivity and specificity of DGGR lipase for diagnosis of acute and chronic pancreatitis in dogs

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Pancreatitis is a common, potentially fatal condition of dogs. The DGGR lipase catalytic assay is selective for lipase of pancreatic origin. The test is cheaper than Spec cPL and can be added to in-house biochemistry panels.

This study investigated the performance of the DGGR lipase assay for diagnosis of histopathologically confirmed canine acute and chronic pancreatitis. Agreement between DGGR lipase and Spec cPL assays was also assessed.

Dogs with histologically confirmed acute pancreatitis (n=2), chronic pancreatitis (n=7) and normal pancreatic tissue (n=7), that had stored (-80°C) serum samples available, were identified. Sections were reviewed by one author and disease status confirmed. The control population was selected based on clinical signs for which pancreatitis was a differential diagnosis. Azotaemic cases were excluded. DGGR lipase was measured in-house using a previously validated colourimetric DGGR lipase assay. Spec cPL was measured by IDEXX Laboratories.

Sensitivity and specificity of DGGR lipase and Spec cPL were compared using receiver operator characteristic (ROC) curve analysis. Agreements of each assay with histopathology, and with each other, were assessed using Cohen’s kappa coefficient (κ).

The DGGR lipase assay was 100% sensitive and 100% specific for cases of histologically confirmed acute pancreatitis when a cut point of >190 IU/L was used. Spec cPL was also 100% sensitive and specific for acute cases at a cut point of >200μg/L. There was a perfect agreement (κ = 1.00) between histologically confirmed cases of acute pancreatitis with DGGR lipase >190 IU/L and Spec cPL >200μg/L.

Sensitivity and specificity of the DGGR lipase assay at cut point >190IU/L was poor (29%) and excellent (100%) respectively for chronic pancreatitis. Sensitivity of Spec cPL was also low for chronic pancreatitis (29% at cut point >200μg/L). Agreement between histopathologically confirmed chronic pancreatitis with both lipase assays was fair (κ = 0.286) at cut points of >190 IU/L for DGGR and >200μg/L for Spec cPL.

Perfect agreement (κ = 1.00) was found between DGGR lipase (>190IU/L) and Spec cPL (>200μg/L).

The results of this study suggest that the DGGR lipase assay is very sensitive and specific for histopathologically confirmed acute pancreatitis, however it has poor sensitivity for chronic pancreatitis. The DGGR lipase and Spec cPL assays can be used interchangeably allowing a quicker, cheaper and more accessible diagnosis.

RESULTS

Samples were received from 24 dogs (10 healthy, 11 hospitalised and 3 IMT cases).

I) PSAIgG: IMT cases had significantly higher percentage of PSAIgG than healthy or hospitalised dogs (p = 0.02).

II) RP: Both IMT cases and hospitalised dogs had a significantly higher percentage of RP than healthy dogs (p = 0.0002).

III) Platelet Count: RP count increased as platelet count decreased.

RESEARCH

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The results of this study suggest that the DGGR lipase assay is very sensitive and specific for histopathologically confirmed acute pancreatitis, however it has poor sensitivity for chronic pancreatitis. The DGGR lipase and Spec cPL assays can be used interchangeably allowing a quicker, cheaper and more accessible diagnosis.

CONCLUSION

Dogs with IMT have a significantly higher percentage of PSAIgG than hospitalised or healthy dogs, and this assay is a potential diagnostic tool for IMT. Furthermore, the technique was robust with analysis >48 hours after sample collection and may therefore be suitable for samples sent by post. The DtB staining kit was simple and easy to use for practitioners and had the added advantage that only a very small volume (<100μl) of blood is required.

Further studies are needed to optimise the assay including analysis of a greater number of samples from varied cases. Additionally, further studies will investigate the effects of existing and novel immunosuppressive agents on PSAIgG and RP.

This project was funded by a BSAVA PetSavers Student Research Grant and a BBSRC Small Project Grant. Heather Birrell was funded by a scholarship from the Carnegie Trust.