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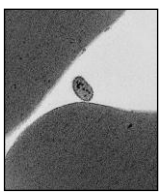
‚*Candidatus* Mycoplasma turicensis' infection: attempted reactivation and tissue loads in chronic carrier cats

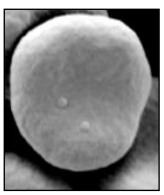
M. Novacco[a], F. S. Boretti[b], B. Willi[b], G. Wolf-Jäckel[a], M. L. Meli[a], B. Riond[a], and R. Hofmann-Lehmann[a]

[a]Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland; [b]Clinical of Small Animal Internal Medicine, University of Zurich, Switzerland

Aims of the study

The aims were to provoke and investigate reactivation of an experimental CMt infection and demonstrate potential sequestration sites





Electron microscopic imaging of CMt (Willi et al., submitted)

Introduction

Haemoplasmas

Haemotrophic mycoplasmas (aka haemoplasmas) are small cell-wall-free bacteria that attach to red blood cells and may cause fatal infectious anaemia in a variety of mammalian species (1). In cats, three haemoplasma species have been recognized: *Mycoplasma haemofelis* (Mhf), ‚*Candidatus* M. haemominutum' (CMhm) and ‚*Candidatus* M. turicensis' (CMt) (2, 3, 4).

‚*Candidatus* M. turicensis'

CMt has been discovered by our group in a Swiss pet cat with severe haemolytic anaemia and has been demonstrated worldwide in domestic and wild felids (4, 5). The pathogenesis and kinetics of CMt infection, particularly a possible tissue sequestration and reactivation of the infection, are still poorly understood.

Diagnosis

PCR systems have been developed that are the gold standard for the detection and differentiation of feline haemoplasma species (4, 6). No *in vitro* culture system has been established to propagate haemoplasmas outside their hosts. Thus, PCR assays have been applied to investigate the pathogenesis and haemoplasma tissue loads in experimental studies (4, 7).

Materials & Methods

Cats and attempted reactivation

Ten specified pathogen-free cats were used that had ostensibly recovered from CMt infection (16–21 months post infection). Five cats received intramuscularly high doses of methylprednisolon (10 mg/kg) in 3 consecutive weeks (first injection = Day 0) in an attempt to induce an immunosuppression and potential reactivation of CMt infection (group 1), while five cats served as controls (group 2).

Blood samples and CMt tissue loads

The cats were monitored by weekly blood collection. Tissue aspirations were performed by fine needle aspiration of kidney (K), liver (L), and salivary glands (Sg). Bone marrow aspiration was performed and saliva swabs were collected (for time points see Table 1).

Total nucleic acids extraction

Total nucleic acids (TNA) were extracted from 100 µl of EDTA-anticoagulated blood using the MagNa Pure LC Total Nucleic Acid Isolation Kit I (Roche Diagnostics) as described (4). For nucleic acid extraction from tissue samples, the DNA Micro kit (Qiagen) was used.

Real-time PCR

All TNA samples were analyzed by CMt TaqMan real-time PCR as described (4). To verify the presence of amplifiable DNA the feline glyceraldehydes-3-phosphate dehydrogenase (fGAPDH) gene was used (8, 9).

Results

Sufficient amounts of amplifiable DNA and absence of PCR inhibition were confirmed using fGAPDH real-time PCR. No difference was found in CMt blood loads between the two groups: all blood samples tested PCR-negative throughout the study, except for one sample (cat S1) collected at day 20. Two cats were PCR-positive in tissues (liver, salivary gland) at day 20 (Table1). Group 1 had significantly higher blood glucose levels (Fig. 1) and lower body weight than group 2 (P<0.0001; Fig. 2) after methylprednisolon administration.

Table 1: PCR results of tissue samples

	Group 1					Group 2				
Time point	**A2**	**X4**	**R2**	**S1**	**T1**	**A1**	**X5**	**R1**	**S2**	**T2**
Day -7	-	-	+ (Sg)	-	-	-	-	-	-	-
Day 20	-	-	+ (L, Sg)	-	+ (L)	-	-	-	-	-
Day 45	-	-	-	-	-	-	-	-	-	-

Fig. 1: Blood glucose

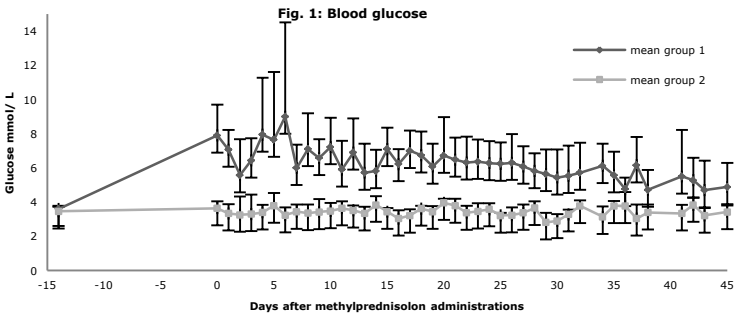
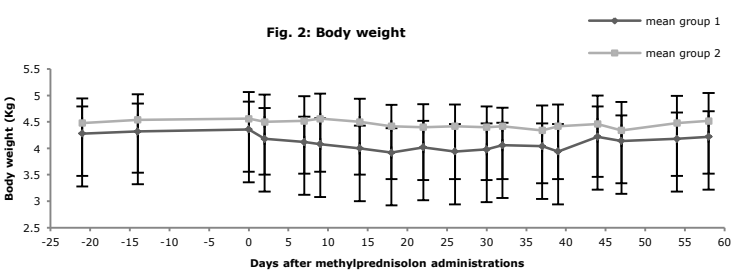


Fig. 2: Body weight



Conclusions

Experimental infections with feline haemoplasmas have been performed mainly with Mhf and CMhm; only very limited experimental data are available on CMt. The present study is the first to systematically document CMt copy numbers in tissues from haemoplasma chronic carrier cats and report CMt attempted reactivation under well-controlled, experimental conditions. The results of this study enhance the knowledge on the not well investigated carrier state of CMt infection and are of clinical relevance for the prognosis of CMt infected cats.

Summary

The present study reports for the first time the presence of CMt in tissues of chronically infected cats in the absence of bacteremia – although at low levels. The attempted immunosuppression did not lead to a reactivation of CMt infection

Acknowledgements

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