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Effect of chronic FIV infection, and efficacy of marbofloxacin treatment, on *Mycoplasma haemofelis* infection

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Abstract

The purpose of this study was to investigate the effect of chronic feline immunodeficiency virus (FIV) infection, and efficacy of marbofloxacin treatment, on *Mycoplasma haemofelis* infection. Six cats chronically infected with FIV-Glasgow8 (Group X) and six FIV-free cats (Group Y) were infected with *M. haemofelis* on Day 0 by intravenous blood inoculation. From Day 0 until Day 86 post-infection (pi), blood samples were collected for *M. haemofelis* and FIV provirus quantitative real-time PCR and haematology. Three of the six cats in each of Groups X and Y were randomly selected to receive marbofloxacin treatment (2 mg/kg PO q24 h) from Day 16 to 43 pi, with the remaining cats being untreated controls with no antibiotic treatment. The *M. haemofelis* copy numbers and haematological data were compared between Groups X and Y, and between marbofloxacin-treated and control cats using a Mann–Whitney *U*-test.

M. haemofelis infection was associated with development of macrocytic hypochromic anaemia. In some cats, marked variation in *M. haemofelis* copy number over time (>100,000-fold difference within 48 h in some cats) and/or cycling of copy number was seen. No correlation was found between FIV provirus copy number and *M. haemofelis* copy number or haematological variables. No significant effect of chronic FIV infection on *M. haemofelis* copy number kinetics or haematological changes due to *M. haemofelis* infection was found, other than MCHC ($P = 0.03$). Marbofloxacin treatment was associated with a significant decrease in *M. haemofelis* copy number ($P = 0.002$), although consistent clearance of infection was not demonstrated.

This study reveals the presence of marked fluctuations in *M. haemofelis* copy number kinetics in vivo and a significant response to marbofloxacin antibiotic treatment.

Keywords: Haemoplasma; *Mycoplasma haemofelis*; Haemobartonella; Feline immunodeficiency virus; Quantitative real-time PCR

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1. Introduction

Three distinct species of feline haemoplasmas have been described: '*Candidatus* Mycoplasma haemominutum', *M. haemofelis* and the novel '*Candidatus* M. turicensis' (Foley and Pedersen, 2001; Neimark et al., 2001, 2002; Willi et al., 2005, 2006). '*Candidatus* M. haemominutum' infection does not usually result in significant anaemia (Foley et al., 1998; Westfall et al., 2001) but experimental infection with *M. haemofelis* often causes severe haemolytic anaemia (Berent et al., 1998; Foley et al., 1998; Westfall et al., 2001).

An association between retroviral infection and haemoplasma infection has been suggested in some studies. It was found that feline immunodeficiency virus (FIV) infected feral cats in the USA were more likely to be haemoplasma infected than non-FIV infected cats (Luria et al., 2004) although UK studies of feral cats failed to show an association between FIV infection and haemoplasma infection (Yamaguchi et al., 1996). It is also believed that co-infection with FIV can influence the pathogenesis of feline haemoplasma infection. One experimental study reported more severe clinical signs due to haemoplasma infection in FIV infected cats compared to non-FIV infected cats (Reubel et al., 1994), but in naturally infected cats (Harrus et al., 2002) no significant difference in haemoplasma disease severity was found in cats infected with FIV compared to FIV-free cats. No studies have yet been performed to specifically evaluate the effect of FIV infection on the pathogenesis of *M. haemofelis* infection.

Doxycycline and enrofloxacin are effective treatments for *M. haemofelis* infection (Berent et al., 1998; Dowers et al., 2002; Foley et al., 1998; Tasker et al., 2003b), although studies have used a maximum of 2 weeks treatment or have not included untreated controls. Negative PCR results, for up to 6 months, were obtained in one study (Dowers et al., 2002) in 25% treated cats following either 2 weeks of doxycycline or enrofloxacin treatment. An additional case report (Braddock et al., 2004) documented negative PCR results for over 1 year after a 6 week course of doxycycline. However, a elimination treatment protocol for *M. haemofelis* has not yet been validated. Marbofloxacin, a fluoroquinolone related to enrofloxacin, has not yet been evaluated for *M. haemofelis* treatment.

The establishment of a sensitive and specific quantitative real-time PCR assay for *M. haemofelis* has allowed successful monitoring of *M. haemofelis* copy number kinetics in vivo in a limited number of cats (Tasker et al., 2003a). The aim of the current study was to use real-time PCR to evaluate the effect of chronic FIV infection on the kinetics of *M. haemofelis* infection. Additionally, haematological parameters and the efficacy of 4 weeks of marbofloxacin in the treatment of *M. haemofelis* infection were evaluated.

2. Materials and methods

2.1. Study design and protocol

Twelve adult barrier-maintained cats were used in this study. Six cats had been experimentally infected with FIV-Glasgow8 (Callanan et al., 1992) for between 23 and 32 months and were clinically asymptomatic at the start of the study (Group X). The other six cats were known to be FIV-free (Group Y). Groups X and Y each comprised three male neutered and three female entire cats. Groups X and Y were housed separately for the duration of the study.

Blood samples (EDTA-anticoagulated) were collected on Day −7 of the study for haematological examination (including reticulocyte count), packed cell volume determination (PCV), blood typing (RapidVet-H blood typing cards, DMS Laboratories Inc., NJ, US), *M. haemofelis* real-time PCR, FIV provirus real-time PCR and FIV antibody ELISA testing (Idexx Laboratories, Wetherby, UK). Blood samples were also collected on Day 0, just before inoculation with *M. haemofelis*, for haematological examination, PCV, *M. haemofelis* real-time PCR, and FIV provirus real-time PCR. Experimental infection of all 12 cats with *M. haemofelis* was carried out by obtaining 24 ml of heparinised blood from a barrier-maintained donor cat chronically infected with *M. haemofelis*. Two milliliters of heparinised blood were given intravenously to all 12 cats, via pre-placed cephalic intravenous catheters, within 10 min of collection from the donor. The blood type of the donor cat was predetermined to be compatible with all recipients.

EDTA blood samples were collected from all 12 cats three times a week from Day 0 to 86 post-infection (pi):