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Establishment and characterization of a low-dose *Mycoplasma haemofelis* infection model

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ABSTRACT

Hemotropic mycoplasma are small, cell-wall-free bacteria that can infect various mammalian species, including humans. They cannot be cultured in vitro; therefore, animal models play an important role, e.g. for pathogenesis studies. *Mycoplasma haemofelis* (Mhf) is the most pathogenic of the three feline hemotropic mycoplasma species; it is known to induce severe hemolytic anemia in infected cats. The aims of this study were to establish and characterize a low-dose Mhf transmission model. Five specified pathogen-free cats were subcutaneously exposed to 1000 copies of Mhf per cat corresponding to 0.05 μL of infectious blood with 2×10^7 copies/mL as determined by real-time PCR. All cats became PCR-positive within 34 days post-exposure and reached a maximum blood Mhf load of 10^9 copies/mL, similar to previously reported high-dose infections. In a selected sample of modified Wright-stained blood smears, small epicellular coccoid structures on the surface of the red blood cells were identified by light microscopy. Additionally, using an Mhf rDnaK ELISA, seroconversion was demonstrated in all cats within 4–5 weeks after Mhf exposure. Four out of five cats developed anemia. While three cats showed only mild clinical signs of hemoplasmosis, one cat developed severe anemia and required antibiotic treatment. Our study demonstrated that minimal contact with Mhf infectious blood was sufficient for transmission of the infection and the induction of hemoplasmosis. This low-dose Mhf infection might more accurately mirror the natural route of infection, i.e., by arthropod vectors or aggressive interaction among cats. We therefore recommend this protocol for use in future animal model studies.

1. Introduction

Hemotropic mycoplasma are small, cell-wall-free bacteria that can infect various mammalian species. They are located on the surface of red blood cells and can induce hemolytic anemia. Three different hemotropic mycoplasma species are known in domestic cats: *Mycoplasma haemofelis* (*Mhf*), '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' (CMt) (Foley et al., 1998; Foley and Pedersen, 2001; Neimark et al., 2001; Willi et al., 2005). Feline hemotropic mycoplasmas are described worldwide with varying incidence rates in the wild and domestic cat populations (Sykes et al., 2008; Tasker et al., 2003b; Willi et al., 2007b, 2006b). *Mhf* is the most pathogenic species in cats and can induce severe hemolytic anemia (Berent et al., 1998; Tasker, 2010). Infected cats may typically present pallor, apathy, weight loss, fever and splenomegaly (Tasker, 2010).

Sensitive PCR assays are used to establish a definite diagnosis and to clearly distinguish between the different

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hemoplasma species (Tasker et al., 2003a; Willi et al., 2005). The route of transmission is largely unknown, but blood-sucking arthropods (fleas, ticks) appear to play a role. DNA of feline hemoplasmas was detected in the cat flea, *Ctenocephalides felis* (Barrs et al., 2010; Kamrani et al., 2008; Lappin et al., 2006; Shaw et al., 2004; Willi et al., 2007a; Woods et al., 2006), and in some *Ixodes* ticks (Schabereiter-Gurtner et al., 2003; Taroura et al., 2005). However, an experimental transmission study using feline hemoplasmas and *C. felis* yielded disappointing results: only one of six inoculated cats developed transient PCR positivity (Woods et al., 2005). Moreover, ingestion of *Mhf* positive *C. felis* by naïve cats was not an important means of *Mhf* transmission (Woods et al., 2006). Aggressive interactions between cats and blood transfusion have also been related to the transmission of hemotropic mycoplasmas (Gary et al., 2006; Museux et al., 2009; Willi et al., 2006a). In a previous study, we addressed the question whether CMt is likely to be transmitted via social or aggressive contact among cats; none of the cats exposed to infectious saliva became CMt positive, even though the same dose of CMt in blood led to successful transmission when inoculated subcutaneously (Museux et al., 2009). This led us to conclude that – at least for CMt – transmission by social contact seems less likely than transmission by aggressive interaction with blood exposure. In other experimental transmission studies, per oral, intravenous, and intraperitoneal inoculations of large volumes of infectious blood have been used to induce infection (Berent et al., 1998; Sykes et al., 2007; Tasker et al., 2009; Wolf-Jackel et al., 2010). However, these routes of transmission may be rather inadequate models for natural transmission, with the exception of infection by blood transfusion. Based on the results of the above mentioned CMt study (Museux et al., 2009), we hypothesized that subcutaneous inoculation and the use of small quantities of infectious blood would lead to Mhf infection and, if so, probably mimic the natural mode of transmission of feline hemotropic mycoplasmas more adequately than the high dose exposures.

The aims of the present study were to establish a low-dose *Mhf* transmission model thereby imitating the natural mode of *Mhf* transmission (i.e. transmission by blood sucking vectors or exchange of small quantities of blood via aggressive interaction among cats) and to characterize the course of the infection.

2. Materials and methods

2.1. Animals and experimental design

Five adult male specified pathogen-free SPF cats (KCY2, ZKA2, AKL4, JCT2 and KCU1) were included in the present investigation. Three cats were 3 years of age (KCY2, JCT2, KCU1), and two cats were 6 years of age (ZKA2, AKL4). The five cats were of blood type A. All animal experiments were performed in accordance with Swiss law and were officially approved by the veterinary office of the canton of Zurich (TVB 159/2010). The cats were kept in a group under ethologically and hygienically ideal conditions, as described (Geret et al., 2011). Prior to the start of the study, the SPF status of the cats was verified, as described previously (Museux et al., 2009).

All cats were challenged subcutaneously with 1×10^3 copies of *Mhf* as determined by 16S rRNA real-time Taqman® PCR at day 0 (Fig. 1). Infectious blood was diluted in a final volume of 100 μL (see below). EDTA-anticoagulated blood and serum samples were collected for PCR analysis, hematology, clinical chemistry, and serology from the five recipient cats prior to and regularly after the *Mhf* inoculation. Clinical condition, body temperature and body weight were recorded at each sampling date. Cats were monitored for 90 days post-*Mhf* exposure. *Mhf*-infected cats that developed severe anemia (PCV < 10%) and/or were in a poor general condition were treated with doxycycline orally (10 mg/kg/day, Grünenthal GmbH, Mitlödi, Switzerland) for 14 days, prednisolone orally (2 mg/kg every 12 h, gradually withdrawn, Streuli Pharma AG, Uznach, Switzerland) for 10 days and fluid therapy (Ringer's lactate solution, Fresenius Kabi (Schweiz) AG, Stans, Switzerland).

2.2. Preparation of inoculum

Heparinized infectious blood from cat QLA5 (Wolf-Jackel et al., 2010), preserved in dimethyl sulfoxide (20%, v/v) in liquid nitrogen, was used to prepare the inoculum for the five experimental cats. The blood contained 2.2×10^7 copies of *Mhf*/mL, as determined by 16s rRNA TaqMan® real-time PCR. It was thawed at 37 °C and diluted in phosphate-buffered saline to a final concentration of 1×10^3 *Mhf* copies in 100 μL. The inoculum was kept on ice until use. The cats were injected subcutaneously in the region of the neck with 100 μL within 5 min of inoculum preparation.

2.3. Hematology

White blood cell differentials and complete hemograms were performed using a Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan) (Weissenbacher et al., 2011). Packed cell volume (PCV) values between 33% and 45% were considered to be within the reference range as determined in our laboratory using identical methods and blood samples from 63 clinically healthy cats; anemia was defined as a PCV value below 33%. Blood samples for hematology were collected prior to and at days 1, 2, and 6 after *Mhf* inoculation, biweekly until week 7 and weekly until week 13. In addition, blood smears were prepared manually and were Wright stained using a Hema-Tek 1000 (Bayer AG, Zurich, Switzerland). Selected blood smears at time points of high bacteremia (weeks 5–12) were evaluated for *Mhf* by light microscopy.

2.4. Clinical chemistry

Serum chemistry was performed using a Cobas Integra 800 system (Roche Diagnostics, Rotkreuz, Switzerland) and included bilirubin, glucose, blood urea nitrogen (BUN), creatinine, protein, albumin, cholesterol, triglyceride, alkaline phosphatase, amylase, aspartate aminotransferase, alanine aminotransferase, lipase, sodium, chloride,