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Short communication

Humoral immune response to a recombinant hemoplasma antigen in experimental '*Candidatus* Mycoplasma turicensis' infection

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ABSTRACT

'*Candidatus* Mycoplasma turicensis' is a feline hemoplasma species that was isolated in a cat with hemolytic anemia. PCR has been widely used to investigate and diagnose '*Candidatus* Mycoplasma turicensis' infection, but so far, little is known about the humoral immune response in infected cats. Recently, enzyme-linked immunosorbent assays (ELISA) were developed to monitor anti-feline hemoplasma antibodies. The aim of the present study was to investigate the humoral immune response in cats experimentally infected with '*Candidatus* Mycoplasma turicensis' and to monitor the influence of the pre-administration of methylprednisolone and subsequent antibiotic treatment. Serum and plasma samples from 15 specified pathogen-free cats infected with '*Candidatus* Mycoplasma turicensis' were analyzed by ELISA. Seroconversion was demonstrated in all cats, and the antibodies remained detectable until the end of the study (up to 100 weeks post-exposure). In some cats, the ELISA seemed more sensitive and better able to demonstrate exposure to '*Candidatus* Mycoplasma turicensis' than PCR. The peak antibody level occurred after the peak of the bacterial blood loads. The methylprednisolone administrations were associated with increased antibody levels, while antibiotic treatment, particularly with doxycycline, resulted in a decrease in antibody levels. Additionally, preliminary data indicated that three of four seropositive cats were protected from bacteremia after a subsequent challenge. In conclusion, the ELISA was found to be a useful tool to investigate the humoral immune response in hemoplasma-infected cats and a desirable addition to PCR to study the pathogenesis of hemoplasma infections.

1. Introduction

'*Candidatus* Mycoplasma turicensis' ('*Candidatus* M. turicensis') was first described in a pet cat with hemolytic anemia (Willi et al., 2005). The course of '*Candidatus* M. turicensis' infection was assessed using sensitive real-time PCR (Museux et al., 2009; Tasker et al., 2009; Willi et al., 2005). In addition, seroconversion was monitored by Western blot assay in 13 experimentally '*Candidatus* M. turicensis'-infected cats (Museux et al., 2009). The Western blot assay was based on a recombinant *Mycoplasma haemofelis* (*M. haemofelis*) protein which was found to be a truncated version of the *M. haemofelis* heat shock protein DnaK, an analog to the HspA1 antigen of *Mycoplasma suis* (*M. suis*) (Hoelzle et al., 2007a, b). Subsequently, the complete DnaK was recombinantly produced and used in an enzyme-linked immunosorbent assay (ELISA) (Wolf-Jackel et al., 2010). This and a similar ELISA have been applied to quantify anti-DnaK antibodies in experimentally hemoplasma-infected cats (Barker et al., 2010; Novacco et al., 2011; Wolf-Jackel et al., 2010). However, to date no data are available regarding the humoral immune response during '*Candidatus* M. turicensis' primary experimental infection. Thus, the goal of this study was to measure the humoral immune response to DnaK in 15 '*Candidatus* M. turicensis'-infected cats. In

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addition, the antibody levels were monitored in cats that received methylprednisolone, antibiotic treatment or underwent reinfection with '*Candidatus* M. turicensis'.

2. Materials and methods

2.1. Animals and experimental design

This study was conducted using 15 specified pathogen-free (SPF) castrated male cats that had been previously included in two experimental '*Candidatus* M. turicensis' transmission studies (Museux et al., 2009; Willi et al., 2005) (Table 1). The present study analyzed the EDTA-anticoagulated blood and serum samples collected during those two studies; in addition, the cats were observed during a follow-up period outlined below when additional samples were collected. All of the cats were kept in groups in a confined university facility under ideal ethological conditions. All experiments were performed according to Swiss law and were officially approved by the veterinary office of the canton of Zurich (TVB 147/2003 and 101/2007). The present study included ten juvenile cats (group A: cats 1 to 5; group B: cats 6 to 10; Table 1). They were infected subcutaneously with '*Candidatus* M. turicensis' PCR-positive blood at day 0 (Table 1). They had been previously exposed sequentially to '*Candidatus* M. turicensis' PCR-positive saliva and blood either oronasally or subcutaneously. The details on the outcome of the early exposures were reported elsewhere (Museux et al., 2009). In the present study, the observation period was prolonged to a total of 700 days after the first '*Candidatus* M. turicensis' exposure for group A and B cats. In addition, five adult cats from previous '*Candidatus* M. turicensis' transmission studies were included (Table 1). Three cats (cats X, Y, Z) had been inoculated intraperitoneally with '*Candidatus* M. turicensis' PCR-positive blood (Table 1). Cats X and Y were monitored for 60 days post exposure, whereas cat Z was followed for 120 days post exposure. Cat 11 was inoculated intravenously with '*Candidatus* M. turicensis' infectious blood from a pet cat with hemolytic anemia, and cat 12 received infectious blood intravenously from cat 11 (Table 1). Cats 11 and 12 were monitored for 170 and 130 days post exposure, respectively.

2.2. Methylprednisolone acetate administration

Cats X, Y, and Z and cat 11 each received two doses of methylprednisolone acetate prior to '*Candidatus* M. turicensis' inoculation (10 mg/kg of body weight, intramuscularly) in an attempt to immunocompromise the cats and amplify the bacterial blood loads or to increase the probability of transmission. Additionally, cat Z received prednisolone orally (4 mg/kg of body weight for 20 days) starting 14 days post-infection. Cat 12 did not receive any prednisolone, and the cats in groups A and B had received methylprednisolone during early exposure to saliva but not prior to or during the subcutaneous exposure to '*Candidatus* M. turicensis'.

2.3. Antibiotic treatment

Cats X, Y, and Z were treated with antibiotics. Cats X and Y were administered marbofloxacin at 2 mg/kg/d for 10 days (days 41–50 post exposure), and then the treatment was changed to doxycycline at 10 mg/kg/d for 14 days. Cat Z received only the doxycycline treatment for 14 days (days 41–54 post infection). The cats in groups A and B and cats 11 and 12 did not receive any antibiotic treatment.

2.4. 'Candidatus M. turicensis' reinoculation

One cat in group B (Cat 7) remained PCR-negative after the '*Candidatus* M. turicensis' exposure. This cat was again exposed to '*Candidatus* M. turicensis' PCR-positive blood subcutaneously 103 days after the first exposure (Table 1). At the same time, the four other cats in group B were also exposed to the same dose of '*Candidatus* M. turicensis' used for cat 7 (Table 1).

2.5. Serology

Antibodies to '*Candidatus* M. turicensis' were assessed in the serum and plasma samples from the 15 '*Candidatus* M. turicensis'-infected cats using an ELISA (Wolf-Jackel et al., 2010). A serum dilution of 1:100 and 50 ng of the recombinant *M. haemofelis* DnaK protein per well were used. The ELISA signal-to-noise ratio was calculated by dividing the post-infection by the pre-infection absorbance values for each individual cat (Wolf-Jackel et al., 2010). An ELISA signal-to-noise ratio ≥ 1.5 was considered to be serologically positive.

2.6. Nucleic acid extractions

Total nucleic acids (TNA) were extracted from 100 μl of EDTA-anticoagulated blood using the MagNaPure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Rotkreuz, Switzerland). During the extraction, negative controls

Table 1
Overview on animals and studies referred to in the present study.

Groups/cats	Prednisolone administration	Inoculum (total copies of '*Candidatus* M. turicensis')	Inoculation route	Antibiotic treatment	Reference
Group A: cats 1–5	No	6×10^3 copies	Subcutaneous	No	Museux et al. (2009)
Group B: cats 6–10	No	1×10^3 copies Reinoculation: 1×10^3 copies	Subcutaneous	No	Museux et al. (2009)
Cats X, Y, Z	Yes	9×10^5 copies	Intraperitoneal	Yes	Museux et al. (2009)
Cats 11, 12[a]	Cat 11 only	3.8×10^3 copies (cat 11) 1.6×10^4 copies (cat 12)	Intravenous	No	Willi et al. (2005)

[a] Cats previously named cat 1 and 2, respectively. Cats were renamed to cat 11 and 12 in the present study, to avoid confusion with cat 1 and 2 of group A.