Polish Journal of Veterinary Sciences Vol. 14, No. 4 (2011), 679-681

DOI 10.2478/v10181-011-0103-y

Short communication

Real-time PCR detection of *Mycoplasma felis* in domestic cats suffering from chronic conjunctivitis (Poland)

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Abstract

Real-time PCR directed to intergenic spacer (IGS) noncoding region between 16S and 23S rRNA genes was used for species specific detection of *Mycoplasma felis* in conjunctival scrapings. Samples were collected from 57 cats suffering from chronic conjunctivitis in 2008-2010 (Wrocław, Poland). Samples from 36 cats (63.2%) were shown to be positive for *Mycoplasma felis*. Our research gives a first insight in the occurrence of *Mycoplasma felis* among domestic cats in Poland suggesting that this pathogen may constitute an underestimated cause of chronic conjunctivitis.

Key words: *Mycoplasma felis,* domestic cats, chronic conjunctivitis, real-time PCR

Introduction

The chronic stage of conjunctivitis, lasting months or years, seems to be an important problem in our vet practices as well as in other countries. The role of *Mycoplasma felis* in ocular disorders in cats still remains unclear and debatable. *M. felis* can be isolated from clinically healthy cats thus some authors consider them as a part of the common microflora of the conjunctiva sacs and do not associate them with pathogenesis of conjunctivitis. Alternatively, *M. felis* is described as a pathogenic species, isolated from feline conjunctivitis (Sjödahl-Essen et al. 2007, Maggs 2008). Until now, *M. felis* in cats suffering from ophtalmological problems has never been confirmed in Poland. The aim of this study was Real-time PCR detection of *Mycoplasma felis* in domestic cats suffering from chronic conjunctivitis.

Materials and Methods

Conjunctival scrapings (n=57) were obtained in years 2008-2010 from cats suffering from chronic conjunctivitis presented to the Department of Epizootiology with Clinic of Birds and Exotic Animals, Veterinary Faculty in Wrocław (Poland). Samples were prepared using the procedure described and recommended by Sykes (2005). Genomic DNA was extracted directly using the QIA Amp Ultra Sens Virus kit (Qiagen,

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This work was supported by grant 410/GW/ 2010

USA) following to the manufacturer's instruction. *M. felis* ATCC 23391 (LGC Standards, Poland) was used as a positive control. The reference strain was cultured in Mycoplasma broth base medium (Oxoid, England), recommended as a basic medium for the selective isolation of *Mycoplasma spp.* Genomic DNA was isolated using QIAamp DNA Mini Kit (250) (Qiagen, USA). Real-time PCR was performed using primers Myc 1 (5'-CACCGCCCGTCACACCA-3') and MfelR1 (5'-GGACTATTATCAAAAG-CACATAAC-3') designated by Chalker et al. (2004). Expected amplified product of the target sequences was 238 bp in length. For real-time PCR a IQ™ Sybr Green Supermix (Bio-Rad, Poland) was used. Various real-time PCR conditions were tested, including modifications of annealing temperature and duration cycle. Finally the 16S/23S rRNA IGS region was amplified with real-time PCR conditions of: 95°C for 3min, 36 cycles of 95°C for 45s, 60°C for 30s and 72°C for 30s.

Results and Discussion

Of the 57 specimens investigated, *Mycoplasma felis* was detected in 36 samples (63.2%) (Fig. 1a,b). Provided that expected product (238 bp) was detected at 14 cycle for positive control, the limit of detection of the pathogen in clinical samples was established at 17 cycle.

M. felis is one of over 150 identified as yet *Mycoplasma* species. Using DNA sequencing in cats with conjunctivitis and upper respiratory tract diseases Hartmann et al. (2010) identified *M. felis*, *M. canadense*, *M. cynos*, *M. gatae*, *M. lipophilum* and *M. hyopharyngis* species. Chalker et al. (2004) tested PCR primers based on the sequence within intergenic spacer between 16S and 23S rRNA genes for specific detection of *M. felis.* The method yielded comparable to the culture approach. Other *Mycoplasma* species that could be isolated from cats (*M. gatae, M. arginini, M. feliminutum*) did not interfere with *M. felis* detection. No current data on *M. felis* in cats population in Poland are available. Our report allowed the first estimation of the prevalence of *Mycoplasma* infection in the population of cats with chronic conjunctivitis. In this study, all samples determined as positive (n=36) were collected from the conjunctiva, in opposite to the study of Chalker et al. (2004), where *M. felis* was isolated mainly from bronchoalveolar lavages (n=6) and from the conjunctiva only in single case. Although reports from various countries address the prevalence of *Mycoplasma* spp. infection in cats, only a few identify the species of *Mycoplasma*. Prevalence was reported in USA (9.6%) (Low et al. 2007), Canada

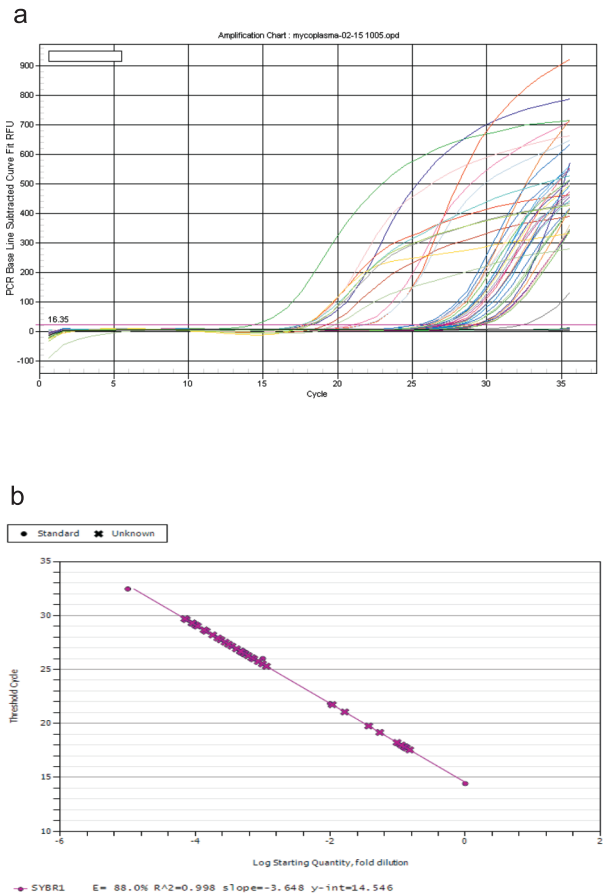

Fig. 1. The amplification curves (a) and standard curve (b) of the clinical specimens investigated by real-time PCR. Corresponding Ct values are presented on the vertical axis (b).

(11-27%) (Sandmeyer et al. 2010) or Germany (49%) (Hartmann et al. 2010). Our data suggest that a large number of cats in Poland could carry *M. felis*. To explain its role in etiology of the chronic conjunctivitis further studies are needed.

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