

## Molecular Detection of *Mycoplasma felis* Infection in a Cat with Respiratory Symptoms

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**Abstract:** A 6-month-old male cat was presented for investigation of depression, loss of appetite, dehydration, pale conjunctival mucous membrane, weight loss, fast heart and respiratory rates, nasal discharge and cough. Nasal swabs collected from the studied cat. As the results of bacterial culture with nasal swabs, it was suspected with *Mycoplasma* spp. Also, *Mycoplasma* species was detected by the PCR reaction with *Mycoplasma* genus primers. At species PCR assay, the specimens evaluated for the presence of *M. felis*, *M. arginini*, *M. gateae*, and *Acholeplasma laidlawii* and the result was visualization of bands from 238 bp in agarose gel 1.5% showing *M. felis* amplicons in samples. In conclusion, we detected *M. felis* in a cat with respiratory disease. PCR was able to detect successfully *M. felis* infection in cats.

**Key words:** PCR, *Mycoplasma*, *M. felis*, respiratory, cat.

### Introduction

*Mycoplasmas* are important pathogenic organisms and members of the class Mollicutes, which contains approximately 200 known species. *Mycoplasmas* are the smallest known prokaryotes (1,6,7,16,21).

*Mycoplasma* species are not generally considered to be present in the lower airways of healthy cats (16,21). Studies in the USA and Australia have suggested that approximately 22% of cats with lower airway disease may have a concurrent *Mycoplasma* species infection (8,21).

*Mycoplasma (M.) felis*, frequently isolated from the feline conjunctiva, respiratory tract, and urogenital tract (2,4,7,9,17, 18), is strongly suspected as an etiologic agent in feline conjunctivitis, respiratory disease, and polyarthritis (4,7,9,10,17,21).

The purpose of the present study is to report a case of *M. felis*-associated respiratory symptoms in a young cat.

A 6-month-old male cat was obtained from the Animal Facilities of the Center for Animal Resources Development, Wonkwang University Korea. The animal experiment in this study was conducted according to ethical procedures of Wonkwang University IACUC. Over the previous 6 days, the cat had shown depression, loss of appetite, dehydration, pale conjunctival mucous membrane, weight loss, fast heart and respiratory rates, nasal discharge and cough. The cat was given a health examination. Abdominal sonography and radiography did not demonstrate any abnormal lesions. Nasal swabs were collected from the studied cat. Sampling was performed from the nares with a dried sterile swab. The tip of the collection swab was inserted into the nares and rolled

five times in each nostril. Nasal swabs were submitted for bacterial culture, while nasal flush samples with 5 ml of sterile saline were used for DNA extraction.

For bacterial culture, nasal swabs were first streaked onto a modified Hayflick agar. Plates were incubated under carbon dioxide nebulisation for 10 days, and then a gas pack humidified with 6 ml water was added before samples were incubated in an incubator at 37°C. Evaluation of plates for *Mycoplasma* species colonies was performed on days 2, 4, 7 and 10 with a stereomicroscope.

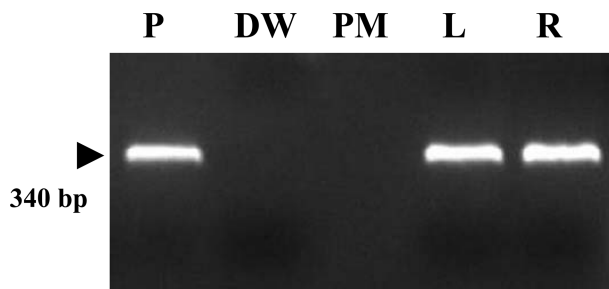
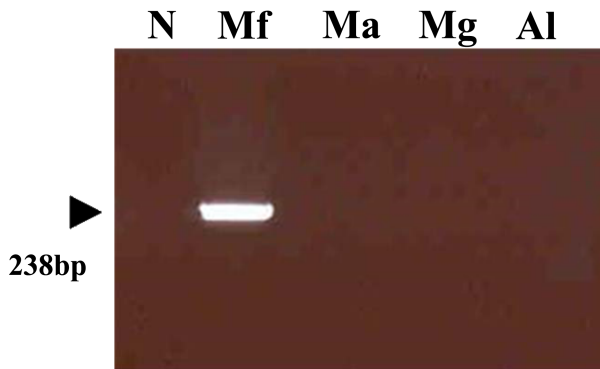
As the results of bacterial culture with nasal swabs, colonies with fried-egg appearance were apparent on PPLO agar after 4-7 days of incubation, and measured about 110 to 200 um in diameter. Based on the morphological characteristics of the cultured bacteria, it was suspected with *Mycoplasma* spp.

Nasal flush samples were submitted to extract genomic DNAs for PCR assay. DNAs were extracted using the bead beater-phenol extraction method (12). In order to detect *Mycoplasma* spp., genus PCR amplification of the V3 region of the 16S ribosomal DNA performed with consensus primers GC-341F (5'-CGC CCG CCG CGC GCG GCG GCG GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG) and 534R (5'-ATT ACC GCG GCT GCT GG), which were based on the sequences reported by Weisburg *et al* (20). Thereafter, PCRs were performed to identify the species with species-specific primers in Table 1 (5,19). The template DNA (50 ng) and 20 pmol of each primer were added to a PCR mixture tube (Maxime PCR PreMix; iNtRON, Korea) containing 1 U of Taq DNA polymerase, 250 μM each deoxynucleoside triphosphate, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM MgCl<sub>2</sub>, and the gel loading dye. The volume was adjusted with distilled water to 20 μl. The reaction mixture was subjected to denaturation at 94°C for 5

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**Table 1.** Primer sets used in this study

Target	Primer	Nucleotide sequence (5' → 3')	Product size (bp)
<i>M. felis</i>	F	CACCGCCCGTCACACCA	238
	R	GGACTATTATCAAAAGCACATAAC	
<i>M. arginini</i>	F	GCATGGAA TCGCATGATTCCT	545
	R	GGTG TTCTTCCTTATATCTACGC	
<i>M. gateae</i>	F	ACACCATGGGAGCTGGTCAT	400
	R	CTTCTCGACTTCAGACCAAGGCAT	
<i>Acholeplasma laidlawii</i>	F	TAATCCTGTTTGCTCCCCAC	505
	R	AGCCGGACTGAGAGGTCTAC	

**Fig 1.** Amplification of 16S rDNA gene by *Mycoplasma* genus PCR was identified on a 1.5% agarose gel electrophoresis. Lane: P, positive control; DW, distilled water; PM, *Pasteurella multocida* DNA; L, Left nostril swab sample; R, Right nostril swab sample.**Fig 2.** *Mycoplasma* species PCRs were performed to identify the species with species-specific primers. Lane N: distilled water, Mf: *M. felis*, Ma: *M. arginini*, Mg: *M. gateae*, Al: *Acholeplasma laidlawii*.

min followed by 30 cycles of 95°C for 1 min, 55°C for 45 s, and 72°C for 1 min and a final extension step of 72°C for 10 min with Thermocycler (model PTC-100, MJ Research, USA), and samples were kept at 4°C until analysis. After amplification, a 5 µl aliquot of each PCR was separated by electrophoresis on 1.5% agarose gels followed by ethidium bromide staining and UV transillumination.

In order to identify it as *Mycoplasma* spp, a genus PCR analysis method using the 16S ribosomal DNA was employed. The 16S rDNA gene (340 bp) was specifically amplified by

PCR with *Mycoplasma* genus-specific primers (GC-341F and 534R). The target nucleic acid fragments were specifically amplified by consensus PCR with 16S ribosomal DNA primers. As the result, *Mycoplasma* species was detected by the positive PCR reaction (Fig 1). At the end of the PCR assay, it was evaluated for the presence of *M. felis*, *M. arginini*, *M. gateae*, *Acholeplasma laidlawii* and the result was visualization of bands from 238 bp in agarose gel 1.5% showing *M. felis* amplicons in samples. However, other *Mycoplasma* species were appeared as negative (Fig 2).

*Mycoplasma* spp have been reported as a cause of feline pneumonia and pyothorax (2,4,9,10,17). Randolph *et al* (15) recovered *Mycoplasma* spp in 21.4% of tracheobronchial lavage samples from cats with pulmonary disease, using both a Hayflick medium with 15% horse serum and 20% porcine serum agar plates, and broth incubated at 35.5°C at 8% CO<sub>2</sub> and 100% humidity (15). Foster *et al* (2004) identified *Mycoplasma* spp in 22.4% of cases, based on negative Gram-staining of BAL cytology and colonial morphology in aerobic culture of sheep blood agar at 37°C.

*M. felis* is a prokaryotic organism from the class Mollicutes which lacks a cell wall and survives on mucosal surfaces where it acts as both primary and commensal opportunistic pathogen (1). *M. felis* infects both domestic and wild cats as well as horses (11,22). Zoonotic transmission from cats to humans has also been reported through a cat bite and increased feline exposure (3,14). Experimental infection of kittens with 10<sup>7</sup> CFU in both the nostrils and conjunctiva reliably produced disease (9,17).

*M. felis* is one of several species of mycoplasma recovered from cats; other *mycoplasmas* associated with cats are *M. gateae* and *M. arginini* (16,21). *Acholeplasma laidlawii* has also been isolated from cats (16,21). *M. felis* has been implicated in few disease processes, most notably conjunctivitis. Respiratory disease and arthritis have also been associated with *M. felis* infection as the etiologic agent (6,11,13). However, the specific pathogenesis of *M. felis* in these disease processes remains unclear.

The fact that this is a rare reported case of *M. felis* associated respiratory symptoms warrants additional examination of the potential role of *M. felis* in respiratory disease. Whether this infectious agent is merely a rare opportunist in a uniquely susceptible host or a more common etiologic agent in feline respiratory diseases remains to be determined.

In conclusion, we detected *M. felis* in a cat with respiratory disease. PCR was able to detect successfully *M. felis* infection in cat s.

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