

## Molecular Detection of Feline Hemoplasmas in Feral Cats in Korea

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**ABSTRACT.** The purpose of this study was to determine if *Mycoplasma haemofelis*, ‘*Candidatus* Mycoplasma haemominutum’ exist in Korea. Three hundreds and thirty one feral cats were evaluated by using PCR assay targeting 16S rRNA gene sequence. Fourteen cats (4.2%) were positive for *M. haemofelis*, 34 cats (10.3%) were positive for ‘*Candidatus* M. haemominutum’ and 18 cats (5.4%) were positive for both species. Partial 16S rRNA gene sequences were closely (>98%) related to those from other countries. This is the first molecular detection of feline hemoplasmas in Korea.

**KEY WORDS:** feral cat, hemoplasma, PCR.

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Feline hemoplasmosis (previously haemobartonellosis) has been primarily associated with two hemotrophic *Mycoplasma* species, *Mycoplasma haemofelis* and ‘*Candidatus* Mycoplasma haemominutum’ [3, 5, 6]. Though recent reports from Switzerland have shown that the existence of a third feline hemoplasma species, namely, ‘*Candidatus* M. turicensis,’ [11–13], there is still little knowledge of its epidemiology other than Swiss pet cat population and the study of outside of Switzerland has been investigated to date [14].

For detection of feline hemoplasma infections, PCR assays are considered as a more sensitive and accurate technique than blood smear examination [1, 2, 4, 7, 8, 10]. This technique now makes it possible to diagnose either single or mixed infections accurately and is now considered to be the diagnostic test of choice for feline hemoplasma infection.

Thus the aim of present study was to use PCR assay in order to determine if *Mycoplasma haemofelis* and ‘*Candidatus* Mycoplasma haemominutum’ exist in naturally infected cats in Korea.

A total of 331 blood samples were analyzed from March to October 2006. Blood samples admitted to a Trap-Neuter-Return (TNR) program in Seong-Nam city in Korea, were collected and periodically transported to the laboratory with a cold pack for molecular survey. The DNA extraction was performed and purified DNA was stored at –20°C and used as the template for PCR amplification. Primers used in this study target the 16S rRNA gene (producing a 273 bp product from *M. haemofelis* and a 202 bp amplicon from ‘*Candidatus* M. haemominutum’) and the PCR assay was optimized as previously described [9]. *M. haemofelis* (Data-Base GenBank accession no. U88563) and ‘*Candidatus* M. haemominutum’ (Data-Base GenBank accession no. U88564) DNA was used as positive controls and a reagent negative control was included in each PCR run to monitor

for contamination. PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide and photographed using a still video documentation system (Gel Doc 2000, Bio Rad, U.S.A.). PCR amplicons were purified by GFX™ PCR DNA purification kit (Amersham Biosciences, UK) and ligated into the pGEM-T easy vector (Promega, U.S.A.) and transformed into TOP 10 competent cells. Plasmid DNA for sequencing was prepared using SV minipreps DNA purification system (Promega, U.S.A.) and sequenced by a dideoxy termination with an automatic sequencer (ABI PRISM® 3700 DNA Analyzer, U.S.A.). The sequence data was collected using ABI Prism Data Collection software (ver. 2.1), and analyzed by ABI Prism Sequence analysis software (ver. 2.1.1) and Chromas software (ver. 1.62) (Technelysium Pty., Ltd., Mt. Helensvale, Queensland 4212, Australia). Sequence homology searches were made at the National Center for Bio-technology Information (National Institute of Health) BLAST network service.

Out of the 331 cats, 34 (10.3%) were PCR positive for ‘*Candidatus* M. haemominutum’ alone, 14 (4.2%) were positive for *M. haemofelis* alone and 18 (5.4%) were positive for both species as shown in Fig. 1. Results of PCR reaction were confirmed by subsequent sequence analysis. Partial sequences of 16S rRNA gene of *M. haemofelis* and ‘*Candidatus* M. haemominutum’ in this study showed high percent similarities of 98.0–100 % and 99.0–100.0%, respectively, with those from the published sequences in different geographical origins (The GenBank database accession numbers for the 16S rRNA nucleotide sequences used for comparison in this study are as follows: *M. haemofelis* strains Yamaguchi: AY529632, Australian: AY150976, Switzerland: DQ157160, UK: AY150986, Israel: AY150975, Oklahoma: AF178677, Ohio-Florida: U88563, ‘*Candidatus* M. haemominutum’ strains Yamaguchi: AY529638, UK1: AY50983, UK2: AY271154, Switzerland: AY157149, California: U88564, South Africa: AY150979, Israel: AY150974). The nucleotide sequences

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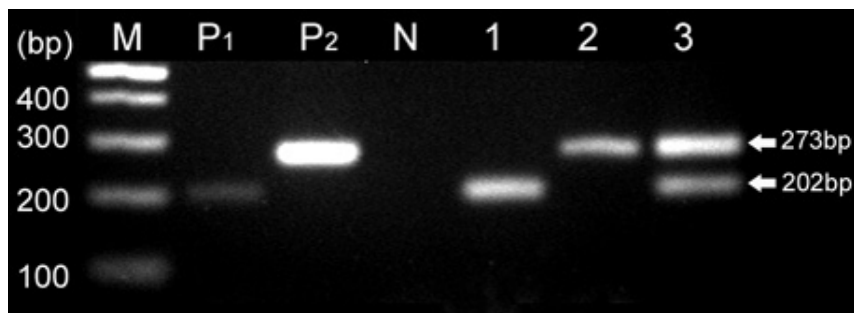


Fig. 1. PCR amplicons of feline hemoplasma 16S rRNA gene. Lanes M, molecular size marker; Lanes P<sub>1</sub>, '*Candidatus M. haemominutum*' positive control; GenBank accession no. U88564; Lanes P<sub>2</sub>, *M. haemofelis* positive control; GenBank accession no. U88563; Lanes N, reagent negative control; Lanes 1, 202-bp DNA fragment; Lanes 2, 273-bp DNA fragment; Lanes 3, both of 202-bp and 273-bp DNA fragment.

Table 1. Names and GenBank accession numbers of the strains submitted to the GenBank

Species	GenBank name	Partial sequence (5'-3')	GenBank Accession numbers
<i>M. haemofelis</i>	H14-Mhf	ATGCCCTCT.....TGGACGAAAG	EF198142
<i>M. haemofelis</i>	H23	ATGCCCTCT.....AGGAACGAAA	EF198143
<i>M. haemofelis</i>	H83-Mhf	ATGCCCTCT.....TAGTCAGCTG	EF198144
' <i>Candidatus M. haemominutum</i> '	H14-Cmh	CTGGGAAACT.....GCAATACCAT	EF198145
' <i>Candidatus M. haemominutum</i> '	H67	CTGGGAAACT.....GCAATACCAT	EF198146
' <i>Candidatus M. haemominutum</i> '	H83-Cmh	CTGGGAAACT.....GCAATACCAT	EF198147
' <i>Candidatus M. haemominutum</i> '	H92	CTGGGAAACT.....GCAATACCAT	EF198148
' <i>Candidatus M. haemominutum</i> '	S3	CTGGGAAACT.....GCAATACCAT	EF198149

of the 16S rRNA gene in this study have been deposited in the GenBank database under the accession numbers as shown in Table 1.

This study reports the prevalence of *M. haemofelis* and '*Candidatus M. haemominutum*' for the first time in Korean feral cats. The successful amplification of feline hemoplasma DNA in this study confirms the existence of both '*Candidatus M. haemominutum*' and *M. haemofelis* in Korea. Indeed, partial sequence of the 16S rRNA gene has confirmed the existence of species with nearly identical 16S rRNA sequences to those previously reported in other countries. However, the overall prevalence of infection with *M. haemofelis* or '*Candidatus M. haemominutum*' in these 331 Korean feral cats was 19.9%, higher than the prevalence of 16.5% detected in 484 American feral cats by PCR assay [4]; Both studies found a similar prevalence of infection with *M. haemofelis* alone (4.3% in U.S.A., 4.2% in Korea), but the prevalence of '*Candidatus M. haemominutum*' alone (10.3%) and both organisms (5.4%) were higher in this study than in the American study (8.3% and 3.9% in U.S.A., respectively). This difference suggests that there may be geographical variations in the prevalence of the different strains. Further study of the extent and significance of this epidemiological survey is needed.

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