

The Occurrence of *Mycoplasma haemofelis* and *Candidatus Mycoplasma Haemominutum* in Cats in China Confirmed by Sequence-Based Analysis of Ribosomal DNA

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Abstract: The present study aimed to examine whether *Mycoplasma Haemofelis* (MHF) and *Candidatus Mycoplasma Haemominutum* (CMH) occur in cats in Mainland China. Genomic DNA was extracted from 87 cat blood samples collected from Guangzhou, China and they were examined by conventional Polymerase Chain Reaction (PCR) assay to detect and distinguish infection of MHF and CMH. The total infection rate of cats with MHF and CMH was 42.5% (37/87), with one female cat being infected with MHF, while 41.4% (36/87) of the infections were due to CMH and none of the cats was positive for concurrent infection with both MHF and CMH. Sequencing of representative amplicons confirmed the results of PCR amplifications. This result, for the first time, demonstrated the existence of MHF and CMH in cats in mainland China.

Key words: Cat, China, '*Candidatus Mycoplasma Haemominutum*, *Mycoplasma haemofelis*, occurrence, PCR

INTRODUCTION

The hemoplasmas are a group of hemotropic bacterial species, which parasitize animal red blood cells and hemoplasma organisms have been reclassified within the genus *Mycoplasma*, based on phylogenetic analysis of 16S rRNA gene sequences (Foley and Pedersen, 2001; Neimark *et al.*, 2001, 2002; Messick *et al.*, 2002). By sequencing of the 16S rRNA genes, two feline haemotropic mycoplasmas were recognized (Foley *et al.*, 1998) as follows: *Mycoplasma Haemofelis* (MHF) (Neimark *et al.*, 2002) and *Candidatus Mycoplasma Haemominutum* (CMH) (Foley and Pedersen, 2001). Recently, a third species of feline hemoplasma named "*Candidatus Mycoplasma turicensis* (Willi *et al.*, 2005, 2006) has been identified.

Feline hemoplasmas have been shown to exhibit worldwide geographical distribution and several molecular studies have investigated the prevalence of infections in different countries or regions including South Africa (Lobetti and Tasker, 2004), the USA (Eberhardt *et al.*, 2006; Hackett *et al.*, 2006; Ishak *et al.*, 2007), Australia

(Tasker *et al.*, 2004), Switzerland (Willi *et al.*, 2006), the United Kingdom (Tasker *et al.*, 2003), Germany (Bauer *et al.*, 2008), Brazil (Macieira *et al.*, 2008), Italy (Gentilini *et al.*, 2009), Japan (Watanabe *et al.*, 2003), Canada (Kewish *et al.*, 2004) and Southern Europe (Criado-Fornelio *et al.*, 2003). However, it is yet to know whether hemoplasmas occur in cats in mainland China. A single conventional PCR assay for the concurrent detection of both feline hemoplasmas MHF and CMH was developed (Jensen *et al.*, 2001), which was widely used to assess the prevalence of that two feline hemoplasmas (Messick, 2004).

The objective of the present study was to determine whether MHF and CMH exist in cats in mainland China, by using that conventional PCR assay.

MATERIALS AND METHODS

Specimen preparation and DNA extraction: Eighty-seven EDTA anti-coagulated blood samples were obtained from cats admitted to the Animal Hospital of South China Agricultural University, Guangzhou, China. Data on sex

was obtained for all animals, but clinical information was incomplete. Total DNA was obtained from 200 μ L blood using the Genome DNA Extraction Kit (Sangon Ltd, Shanghai, China) and it was eluted into 40 μ L TE buffer following the manufacturer's instructions. Amplification of target DNA and denaturing polyacrylamide gel electrophoresis of the amplicons Primers that target the 16S rRNA gene, producing a 170 bp product for MHF and a 193 bp amplicon for CMH (forward primer, 5-ACG AAA GTC TGA TGG AGC AAT A-3 and reverse primer 5-ACG CCC AAT AAA TCC GRA TAA T-3), described previously (Jensen *et al.*, 2001) were used in the present study. PCR reactions comprised Premix-Taq 25 μ L (TaKaRa Ex Taq 1.25 U, 2 \times Ex Taq Buffer, 4 mmol L⁻¹ Mg²⁺), 1 μ L of each primer with the concentration of 50 pM and 5 μ L of template DNA, made up to a final volume of 50 μ L with sterile water. PCR was performed with an initial incubation at 50°C for 3 min and then 95°C for 5 min, followed by 45 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 30 sec, then finally incubated at 72°C for 7 min. CMH DNA (a gift from Dr. Jane Sykes, University of California) was used as a positive control. A DNA sample from a non-infected cat and water were subjected to PCR as negative controls.

Reaction products (2 μ L) were electrophoresed through 6% denaturing polyacrylamide gel, stained with 0.1% AgNO₃ and photographed. Identification of the cat DNA samples as MHF or CMH was made by comparing the sizes of the PCR products with the known positive control DNA and 20 bp DNA ladder Marker (TakaRa, Japan).

Nucleotide sequencing: In order to confirm the identity of PCR amplicons, one PCR product representing the 170 bp fragment and another one representing the 193 bp fragment were separated by electrophoresis in 1% agarose gel, purified using the Agarose Gel Reclamation Kit (Sangon Ltd, Shanghai). The purified PCR products were cloned into the pGEM-T Easy Vector (Promega) and transformed into JM109 competent cells according to the protocol of the manufacturer (Promega). The transformants were obtained by the blue-white selection and two clones of each sample were sequenced (Sangon Ltd, Shanghai). The obtained sequences were compared with relevant sequences available in GenBank™.

RESULTS AND DISCUSSION

Figure 1 shows the representative denaturing polyacrylamide gel image. Two different amplicons were

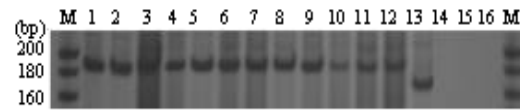


Fig. 1: Representative denaturing polyacrylamide gel (6.0%) demonstrating Polymerase Chain Reaction (PCR) amplified products of haemoplasma 16S rRNA gene from peripheral blood of cats. Lane M represents 20 base pairs (bp) DNA ladder. Lane 1 represents the known genomic DNA of CMH as positive control. Lanes 2-14 represent feline samples (samples 2-12 identified as CMH, sample 13 identified as MHF). Lane 15 represents DNA from a healthy cat. Lane 16 represents No-DNA control

amplified from some of the cat DNA samples (Fig. 1) and their sizes corresponded to that of MHF (the smaller fragment) or CMH (the larger fragment). The two species were able to be differentiated on the basis of their amplicon sizes by denaturing polyacrylamide gel electrophoresis.

The only 170 bp PCR product (the same PCR product presented in lane 13 in Fig. 1 and a representative 193 bp PCR product (the same PCR product presented in lane 11 in Fig. 1) were sequenced.

Similarity comparison with related sequences available in GenBank™ showed that the 170 bp fragment had 99.4% identity with the corresponding region of the 16S rRNA gene of MHF, while the 193 bp fragment was identical to the corresponding region of the 16S rRNA gene of CMH, indicating that they represented MHF and CMH, respectively.

The two sequences were deposited in GenBank™ (accession numbers AM748929 and AM748928). The total infection rate of cats with MHF and CMH was 42.5% (37/87), with one female cat (1.15%, 1/87) being infected with MHF, while 41.4% (36/87) of the infections were due to CMH (18 of 40 male cats were positive for CMH, while 18 of 47 female cats were positive for CMH).

None of the cat blood samples examined in this study was PCR positive for both MHF and CMH. Compared with reported infection rate of feline hemoplasmas in cats in other countries or regions, low infection rate of MHF and high infection rate of CMH in cats in the study show distinct worldwide geographical distributions of feline hemoplasmas in cats.

CONCLUSION

The result of the present study documents, for the first time, the existence of MHF and CMH in cats in mainland China. This result has implications for the effective control of feline infection with hemoplasmas in China.

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