“*Candidatus Mycoplasma Haemominutum*” Infection in a cat in Antalya

Recently, a total of three feline hemotropic *Mycoplasma* spp., (*Mycoplasma haemofelis*, “*Candidatus Mycoplasma haemominutum*” and “*Candidatus Mycoplasma turicensis*”) in cats with anemia have been recognized. Healthy cats with “*Candidatus Mycoplasma haemominutum*” infection may prone to a life-threatening anemia with accompanying concurrent disease or immune suppression, such as in the case reported here. The present article describes the presence of “*Candidatus Mycoplasma haemominutum*” infection (diagnosed by polymerase chain reaction) resulted in fatality in a Siamese crossbred cat in Antalya.

**Key Words:** “*Candidatus M. haemominutum*”, cat, Antalya.

**Antalya’da bir Kedide “*Candidatus Mycoplasma haemominutum*” Enfeksiyonu**


**Anahtar Kelimeler:** “*Candidatus M. haemominutum*”, kedi, Antalya.

**Introduction**

*Hemobartonella felis*, the causative agent of haemobartonellosis or feline infectious anemia, has recently been reclassified as a hemotropic mycoplasma (hemoplasma) based on phylogenetic 16S rRNA gene sequences. Thus two different species, *Mycoplasma haemofelis* (*M. haemofelis*) (1, 2) and “*Candidatus Mycoplasma haemominutum*” (“*Candidatus M. haemominutum*”) (1-5) have been recognized worldwide. In addition recently, a third novel feline hemoplasma isolate, was designated and recognized as “*Candidatus Mycoplasma turicensis*” (“*Candidatus M. turicensis*”) (6).

In the present article the authors describe an eight month old cat with a diagnosis of “*Candidatus M. haemominutum*” in Antalya. To the present authors’ knowledge the latter infection has not previously been reported in this region (by PCR assay). Thus the aim was to indicate that this infection is prevalent in Antalya region. A further aim also was to inform that this infection should be considered in cats with anaemia and taken into account by the veterinary clinicians in this area.

**Case Presentation**

An 8-month-old female cat was presented with a history of muscle weakness, anorexia and lethargy. At physical examination the cat was anaemic, thin and depressed. According to the owner, the onset of the disease coincided with previous flea infestation. The cat was pyrexic (temperature of 39.6°C, reference range 38–39°C). Routine hematology revealed macrocytic-normochromic anemia (Packed Cell Volume 20.4%, Haemoglobin 6.4 g/dL, red blood cell 3.08 x 10^6/μL, Mean corpuscular volume 66.2 fl, mean corpuscular haemoglobin concentration 31.3 g/dL). Cytological examination of the Romanowsky stained smear on referral demonstrated microscopical evidence of hemoplasmosis.

PCR assay was performed for the present case on referral day. DNA extraction was performed from whole blood by use of a DNA extraction protocol (EZ DNA isolation kit, Dr. Zeydanli, Ankara, Turkey) with regard to the manufacturer’s instructions.
PCR assay was performed as previously described (7-9), by use of primers targeting the rRNA gene, producing a 170 base pair (bp) product from \textit{M. haemofelis} and a 193 bp amplicon from \textit{Candidatus M. haemominutum} with 5'-ACG AAA GTC TGA TGG AGC AAT A–3' forward primer and 5'-ACG CCC AAT AAA TGG TAA T–3' reverse primer, Dr. Zeydanli, Ankara, Turkey.

The PCR amplification was carried out in 25 µl reaction mixtures containing 31 pmol of each primer, 200 µM (Amresco) deoxynucleotide triphosphates (dNTPs), 0.025 units Taq DNA polymerase (Gene Mark), 3.5 mM MgCl₂, 1x PZR Buffer (Gene Mark) and 2 µl sample DNA.

For PCR assay optimization PCR machine (Biometra T-gradient) was used, within the following reaction conditions; 30 seconds at 94 ºC, followed by 45 cycles of 30 seconds at 57 ºC and 45 seconds at 72ºC. Prior to the first cycle, initial denaturation for 5 minutes at 94 ºC and after the last cycle 10 minutes extension at 72ºC processes were applied. \textit{M. haemofelis} and \textit{Candidatus M. haemominutum} DNAs were used as positive controls. A reagent negative control (sterile pure water) was included in each PCR run for monitoring contamination. Reaction products including 0.5 µg/ml were electrophoresed within 2.5% agarose gel containing ethidium bromide and visualised by UVP gel documentation system. DNA identification for \textit{M. haemofelis} and \textit{Candidatus M. haemominutum} included comparison of the size of of the PCR product within the size of known positive control DNA and with a 100 bp DNA ladder.

Based on cytology, haematological analysis and PCR assay a diagnosis of \textit{"Candidatus M. haemominutum"} infection was made.

Commercial immunocromatographic rapid test kit (Speed FeLV test kit and Speed FIV test kit, Bioveto, Maya Vet. Ltd. Co., Ankara) was used in an attempt to diagnose FeLV and FIV infections. No sample was negative for FeLV P27 antigen or FIV antibody.

Therapy included enrofloxacin at a subcutaneously dosage of 5mg/kg once for a day 10 days. Fipronil was administered topically at a dosage of 7.5 mg/kg for flea control. At the third day of admission the clinical signs considered to account for haemoplasma worsened and the cat was found dead by the owner. Necropsy was discussed with the owner, who declined further research.

**Discussion**

To the present authors knowledge this is the first article to show that at least one feline hemoplasma specy is prevalent in pet cats in Antalya. The PCR application of the study was performed in line with previously designed and described PCR assay that proved to be highly sensitive and specific and thus accurate for the diagnosis of feline hemoplasma infections.

Prior to recent developments of molecular diagnostic investigations, haemoplasma species were not recognized in detail, only Haemobartonella felis was known, thus the only diagnostic approach relied upon cytological examination (9). Earlier Turkish studies regarding haemoplasma infection in cats had been based on cytological diagnosis (10-12). The recent development of PCR analysis provided more reliable and sensitive diagnosis of hemoplasma infection in cats (13-16). However limited numbers of studies on feline hemoplasma infection in Turkey have been published. There were previous case series reported in Ankara (11) and Istanbul (12). Turkish studies reporting the prevalence of hemoplasma infection had been based on cytological diagnosis with rates reported, as 20 % (11) in Ankara and 14.87 % (10) Van provinces. Apart form those above mentioned studies, in a previous PhD dissertation study regarding haemoplasmosis diagnosis was based mainly on PCR assay and a prevalence of 30.76% was reported in cats in Ankara (17).

\textit{M. haemofelis} infections usually induce severe hemolytic anemia (13, 14, 18), with accompanying signs as apathy, pale mucous membranes, tachycardia, and tachypnea, and may result in fatality. However \textit{"Candidatus M. haemominutum"} is less pathogenic (19). A life-threatening anemia associated with \textit{"Candidatus M. haemominutum"} in a cat with lymphoma was reported previously (20). In cats experimentally infected both with \textit{"Candidatus M. haemominutum"} and FeLV more severe anaemia occurred in contrast to cats only infected with \textit{"Candidatus M. haemominutum"} (21). A previous retrospective study reported approximately 10 per cent of FIV infected cats with haemoplasmosis (22). The present case was free of FeLV antigen and FIV antibody, therefore no dual infection was evident. However life-threatening anemia was in association with \textit{"Candidatus M. haemominutum"}, detected as single pathogen.

It can be concluded from the present study that \textit{"Candidatus M. haemominutum"} may be prevalent among pet cat population in Antalya. To the present authors knowledge \textit{"Candidatus M. haemominutum"} should at least be on the differential diagnosis of cats with anaemia. In addition, further studies based on PCR assay dealing with larger cat populations in this area may corroborate the true nature and real prevalence of this disease. Therefore performing detailed researches will be the subject of our subsequent studies.
References


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