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"*Candidatus* Mycoplasma Haemominutum" Infection in a cat in Antalya

Kerem URAL [1]
Cenk SÜER [2]
Zati VATANSEVER [3]
Sırrı KAR [3]
Kemal EKER [2]
Cenker Çağrı CINGI [4]

[1] Republic of Turkey, The Ministry of Agriculture and Rural Affairs, Board of High Stewards, Ankara-TURKEY

[2] Akdeniz Animal Hospital, B. Onat st. 74/1, Antalya, TURKEY

[3] University of Ankara, Faculty of Veterinary Medicine, Department of Parasitology, Ankara – TURKEY

[4] University of Afyon Kocatepe, Faculty of Veterinary Medicine, Department of Internal Medicine, Afyon–TURKEY

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Yazışma Adresi
Correspondence

Kerem URAL
[1]Tarım ve Köy İşleri Bakanlığı
Yüksek Komiserler Kurumu
Ankara-TÜRKİYE

uralkerem@gmail.com

Recently, a total of three feline hemotropic *Mycoplasma* sp., (*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

Bugüne kadar kedilerde anemiyle seyreden üç hemotrofik Mycoplasma türü (*Mycoplasma haemofelis*, "*Candidatus* Mycoplasma haemominutum" ve "*Candidatus* Mycoplasma turicensis") tanımlanmıştır. Bu olgu sunumunda da bahsedildiği gibi "Candidatus Mycoplasma *haemominutum*" enfeksiyonu bulunan sağlıklı kedilerde hayatı tehdit eden anemi ile birlikte diğer hastalıklara veya immun supresyona eğilim artmaktadır. Bu makalede, Antalya'da Siyam melezi bir kedide (polimeraz zincir reaksiyonu ile saptanan) ölümle sonuçlanan "*Candidatus* Mycoplasma haemominutum" enfeksiyonunun bildirilmesi amaçlanmıştır.

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 rRNAgene 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 clinicans in this area.

Case Presentatıon

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 anaemia (Packed Cell Volume 20,4%, Haemoglobin 6,4 g/dl, red blood cell 3.08 x 10^6/uL, 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 targetting the rRNA gene, producing a 170 base pair (bp) product from *M. haemofelis* and a 193 bp amplicon from "Candidatus M. *haemominutum*" with 5'- ACG AAA GTC TGA TGG AGC AAT A–3' forward primer and 5'- ACG CCC AAT AAA TCC GRA 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 $MgCl_2$, 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. *M. haemofelis* and "*Candidatus* M. haemominutum" DNAs were used as positive controls. A reagent negative control (sterile pure water) was included in each PCR run for monitorizing 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 *M. haemofelis* and "*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 "*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 enrofloxacine at a subcutaneously dosage of 5mg/kg once a day for 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 aproach 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 İstanbul (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).

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 "*Candidatus* M. haemominutum" is less pathogenic (19). A life-threatening anemia associated with "*Candidatus* M. haemominutum" in a cat with lymphoma was reported previously (20). In cats experimentally infected both with "*Candidatus* M. haemominutum" and FeLV more severe anaemia occured in contrast to cats only infected with "*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 "*Candidatus* M. haemominutum", detected as single pathogen.

It can be concluded from the present study that "*Candidatus* M. haemominutum" may be prevalent among pet cat population in Antalya. To the present authors knowledge "*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.

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