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▣ CASE REPORT

Two Clinical Cases of Feline Hemoplasmosis in Korea

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Abstract: Feline hemotropic mycoplasmosis (hemoplasmosis) is an infection of the red blood cells caused by the *Mycoplasma haemofelis* (*Mhf*), *Candidatus Mycoplasma haemominutum* (*CMhm*), and *Candidatus Mycoplasma turicensis* (*CMt*). The existence of *Mhf*, *CMhm*, and *CMt* has been demonstrated in feral cats in Korea using molecular methods, but no clinical cases have yet been reported. This study reports 2 clinical cases of hemotropic mycoplasmosis caused by *CMhm* and *CMt* in 2 anemic cats. The first case was a client-owned intact female domestic shorthair cat that presented with fever, pale mucous membranes, and normocytic normochromic non-regenerative anemia. Prior to referral, an immunosuppressive prednisolone dose was administered at the local veterinary clinic for 1 month. The cat was diagnosed with high-grade alimentary lymphoma. Organisms were found on the surface of the red blood cells on blood smear examination. The second case was of a rescued cat that presented with dehydration and fever. The cat had normocytic normochromic non-regenerative anemia. Necropsy revealed concurrent feline infectious peritonitis. Polymerase chain reaction assay targeting 16S rRNA revealed *CMhm* infection in case 1 and dual infection of *CMhm* and *CMt* in case 2. Normocytic normochromic non-regenerative anemia was observed in both cats before and during the management of the systemic inflammation. This is the first clinical case report in Korea to demonstrate *CMhm* and *CMt* infections in symptomatic cats.

Key words: Feline hemoplasmosis, *Candidatus Mycoplasma haemominutum*, *Candidatus Mycoplasma turicensis*, hemolytic anemia, PCR

INTRODUCTION

Feline hemotropic mycoplasmosis (hemoplasmosis) is primarily associated with 3 hemotropic *Mycoplasma* species that attach themselves to the surface of red blood cells (RBCs) in cats: *Mycoplasma haemofelis* (*Mhf*), *Candidatus Mycoplasma* haemominutum (*CMhm*), and *Candidatus Mycoplasma* turicensis (*CMt*) [1-6]. Molecular studies have demonstrated the existence of *Mhf* and *CMhm* species since 2007 and *CMt* species since 2017 in feral cats in Korea [7-9]. These species are mainly transmitted through arthropod vectors, such as fleas and ticks, and cat bites, or via blood transfusions [1,2,10-12]. The pathogenicity varies among species, among which *Mhf* is the most pathogenic [1,2,13,14]. Although the other 2 feline hemoplasma species are not the primary cause of hemolytic anemia, concurrent disease or immune suppression may predispose a cat to life-threatening anemia in these cases [2,15]. One previous case report described a clinical case of *Mhf* in a cat in Korea; however, clinical reports of other species of *Mycoplasma* associated with clinical signs have not been reported to date [16]. Here we report clinical cases of *CMhm* and *CMt* infections in 2 cats with anemia.

CASE DESCRIPTION

Case 1

A 6-year-old, client-owned, intact female domestic shorthair cat was admitted to our veterinary medical teaching hospital for an abdominal mass accompanied by peritoneal effusion. The cat was a rescued feral cat, but clinical signs were absent prior to the present illness. Local veterinarians suspected the abdominal mass to be a tumor, and an immunosuppressive dose of prednisolone (2 mg/kg per oral, twice in a day) was administered for 4 weeks. On presentation, the cat had a fever (40.8℃), dehydration, and pale mucous membranes. Manual packed cell measured by microhematocrit tube centrifugation was 25% with a total solid volume of 7.3 mg/dl. A complete blood cell count revealed a mild normocytic normochromic

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non-regenerative anemia with a red blood cell (RBC) count of 5.36×10^6/μl ($6.54\text{-}12.2 \times 10^6$/μl); hematocrit, 23.7% (30.3-52.3%); hemoglobin concentration, 7.9 g/dl (9.8-16.2 g/dl); reticulocyte count, 43.4×10^3/μl, with leukocytosis 22.71×10^3/μl ($2.87\text{-}17.02 \times 10^3$/μl), and a platelet count, 882×10^3/μl ($151\text{-}600 \times 10^3$/μl). A blood smear examination stained with Diff-Quik revealed intracellular organisms on the surface of the RBCs (Fig. 1). Molecular analysis to detect feline hemotropic parasites was performed using EDTA anti-coagulated whole blood. Multiplex polymerase chain reaction (PCR) analysis (feline anemia panel, IDEXX laboratory, Westbrook, Maine, USA) showed that the cat was negative for *Anaplasma* spp., *Bartonella* spp., *Cytauxzoon felis*, and *Ehrlichia* spp., except for feline hemotropic *Mycoplasma*. Feline leukemia virus and feline immunodeficiency virus infections were excluded using the rapid diagnostic heartworm-feline leukemia virus antigen-feline immunodeficiency virus antibody test kit (IDEXX Laboratories). Abdominal computed tomography revealed asymmetrical wall thickening of the proximal jejunum with loss of the wall layer and heterogeneous contrast enhancement. A round, peripheral enhancing mass was also identified in the abdominal wall adjacent to the 13th chondrocostal junction. Enlarged pancreaticoduodenal and hepatic lymph nodes were revealed. A mass from the abdominal wall revealed a homogenous population of large lymphocytes, with features typical of feline high-grade alimentary lymphoma based on the results of fine-needle aspiration and intestinal mass cytology (Supplementary Fig. S1). A septic exudate due to intestinal perforation was also observed, and the owner elected humane euthanasia. No necropsy was performed per the owner's preference.

Case 2

An unconscious feral cat with severe dehydration and fever (39.6℃) was rescued. Physical examination revealed tachycardia (240/min), tachypnea (96/min), pale mucous membranes, temporary jerk nystagmus, bilateral purulent oculonasal discharge, and gingivitis with severe dental tartar. Fluid was administered intravenously for resuscitation, and enteral feeding via a nasoesophageal tube was performed for 5 days. Despite doxycycline administration (5 mg/kg, bid), the initial manual packed cell volume of 27% decreased to 16% on day 5. A complete blood count on admission revealed a mild normocytic normochromic non-regenerative anemia, with an RBC count, 5.57×10^6/μl ($6.54\text{-}12.2 \times 10^6$/μl); hematocrit, 26.1% (30.3-52.3%); hemoglobin level, 8.3 g/dl (9.8-16.2 g/dl), and reticulocyte count, 12.8×10^3/μl, along with leukocytosis, 23.85×10^3/μl ($2.87\text{-}17.02 \times 10^3$/μl). Feline leukemia virus and feline immunodeficiency virus infections were excluded using the SNAP® Feline Triple® test kit. Molecular analysis was performed to identify the cause of anemia using EDTA anti-coagulated whole blood. Multiplex PCR analysis (feline anemia panel, IDEXX laboratory) revealed that the cat was negative for *Anaplasma* spp., *Bartonella* spp., *Cytauxzoon felis*, and *Ehrlichia* spp., except for feline hemotropic *Mycoplasma*. The neurological signs worsened with seizures and nystagmus; euthanasia was performed. The Rivalta test was positive, and necropsy re-

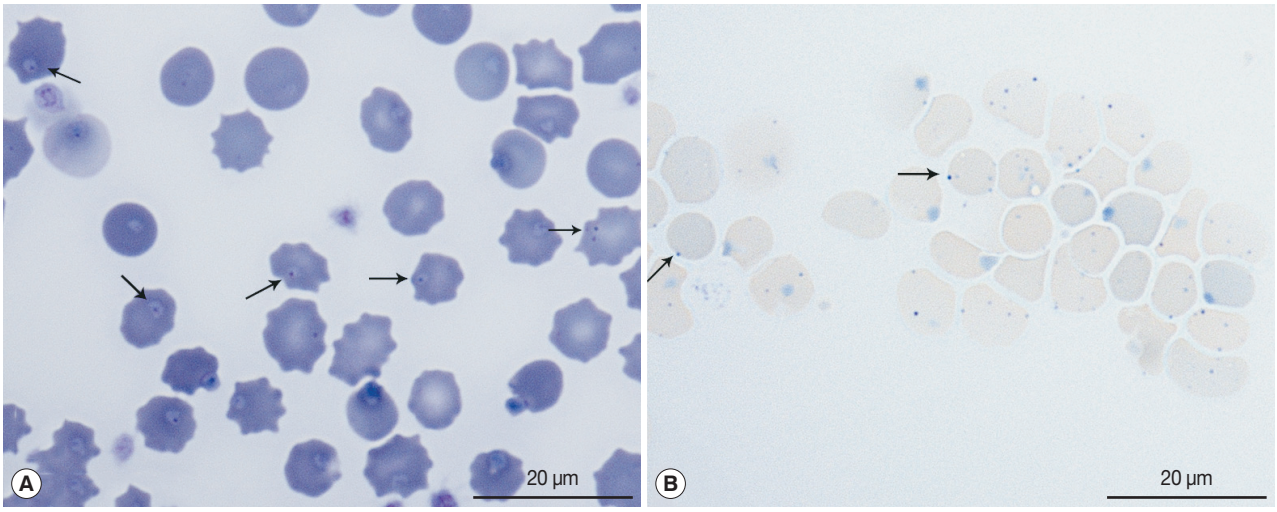

Fig. 1. Blood smear examination of case 1. Organisms are seen on the surface of the erythrocytes (arrow). (A) Organisms are seen as single dark purple and blue dots on the surface of erythrocytes with Diff-Quik staining. (B) Some erythrocytes have many distinct organisms on their surface, visualized with New Methylene Blue staining.

vealed a small amount of viscous and straw-colored pleural/peritoneal effusion. Multifocal granulomatous lesions were observed in the kidneys, mesentery, liver, and brain. Immunohistochemistry with mouse anti-FIP virus monoclonal antibody (Custom Monoclonals International, clone FIPV3-70, Sacramento, California, USA) indicated a feline coronavirus infection in the kidneys and brain, which supported feline infectious peritonitis diagnosis (Supplementary Fig. S2).

Molecular identification of feline hemotropic organisms

The cat's DNA was extracted from EDTA-anticoagulated whole blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Nested PCR targeting 16S rRNA gene was performed to detect feline hemotropic organisms, as described in a previous study [5]. PCR products were visualized by electrophoresis on a 2% agarose gel with ethidium bromide staining. Case 1 had *CMhm* infection and case 2 had both *CMhm* and *CMt* infections. Amplicons were cloned using the TA-cloning Vector Kit II (iNtRON, Seongnam, Korea) to identify the gene sequences. Plasmid DNA for sequencing was purified using the Plasmid Extraction Mini Kit (Favorgen Biotech Corp., PingTung, Taiwan), according to the manufacturer's instructions. Purified recombinant plasmid DNA was sequenced by 3730 capillary DNA Analyzer (Applied Biosystems, Foster City, California, USA). The DNA sequences were evaluated using Chromas software (Ver 2.6.6), aligned using

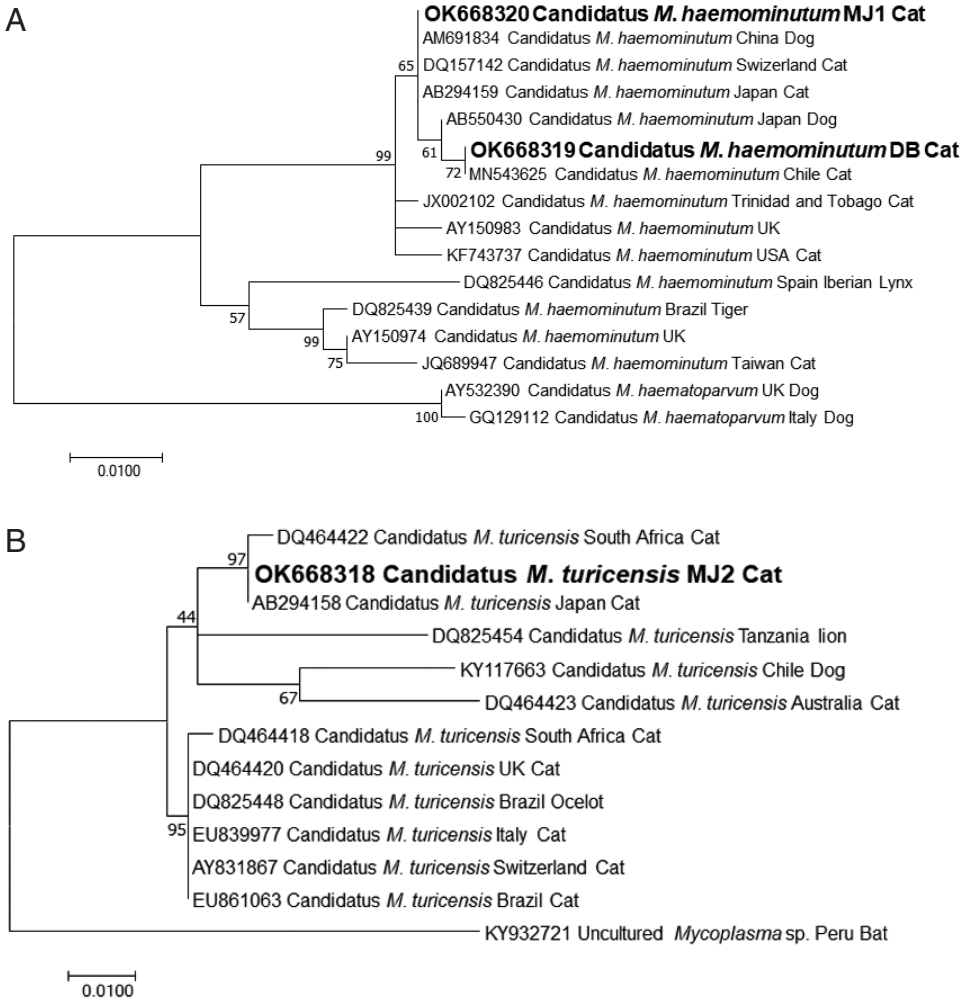

Fig. 2. Molecular phylogenetic analysis by the Maximum Likelihood Estimation Method. Phylogenetic relationships for 402 bp-long *Candidatus Mycoplasma haemominutum* (A) and 271 bp-long *Candidatus Mycoplasma turicensis* (B), based on partial nucleotide sequences of the 16S rRNA gene fragments. The Maximum Likelihood Estimation Method based on the Kimura 2-parameter model was used for constructing the phylogenetic tree. The numbers at the nodes are the proportions of 1,000 bootstrap iterations that support the topology shown. Bolded letters indicate the *Mycoplasma* sequences obtained from the cats in this study.

Clustal X (Ver 2.1), and then examined with a similarity matrix. Phylogenetic trees were created using MEGA 7 [17,18].

The partial sequences of *CMhm* from case 1 and *CMhm* and *CMt* from case 2 were deposited in the GenBank database with the access no. OK668319, OK668320, and OK668318, respectively. The phylogenetic tree of *CMhm* 16S rRNA gene is presented in Fig. 2. The partial sequences of the 16S rRNA gene of *CMhm* and *CMt* showed 99-100% identity with those from the published sequences from different geographic origins (Fig 2).

The case 1 was diagnosed with subclinical *CMhm* infection with the clinical signs presenting after the immunosuppressive course of prednisolone, and case 2 was diagnosed with dual infections of *CMhm* and *CMt*, causing feline infectious peritonitis.

DISCUSSION

CMt infection was first reported in Switzerland in 2005 [19]. *CMhm* has the highest prevalence worldwide and the prevalence of *Mhf* and *CMt* is similar [1-3,5,6]; the prevalence of *CMhm*, *Mhf*, and *CMt* is 8.5-46.7%, 0.5-21.3%, and 1-10%, respectively [2,3,6]. Although *CMhm* is known to be more common than *Mhf*, no studies in Korea have compared the prevalence of these 3 species. Overall, the prevalence of *CMhm* and *Mhf* infections is 15.7-19.6% and 4.3-9.6%, respectively [7,8]. It was unknown whether *CMt* existed in Korea, but a recent study reported a 1.7% prevalence of *CMt* in feral cats from urban and rural areas [9]. In this study, the existence of *CMhm* and *CMt* was confirmed in rescued feral cats. Phylogenetic analysis showed that the partial sequences of *CMhm* were similar to those from China and Japan. The partial sequences of *CMt* were 100% identical to those found in Japan. Although no evidence of feline hemoplasmosis among house cats in Korea to date, feline hemoplasmosis should be one of the differential diagnoses if anemic cats are in contact with feral or rescued cats in Korea.

Feline hemoplasma species that are currently recognized show differences in their pathogenicity. A previous Korean article reported *Mhf* in a 6-month-old rescue cat with severe anemia (hematocrit equal to or less than 15.7%) and jaundice (2.7 mg/dl of total bilirubin) [16]. On the other hand, *CMhm* and *CMt* are known to have low pathogenicity, thus do not cause clinically significant signs [14,20]. However, anemia was observed in the present cases, probably due to immunosuppression or preexisting disease [1,2,15,21]. In case 1, an immunosuppressive dose of prednisolone had been administered for 4 weeks before the onset of anemia, and in case 2, necropsy revealed concurrent infection with feline infectious peritonitis. Similar to case 1, a previous study revealed persistent anemia in a cat that worsened after chemotherapy in a cat with lymphoma [21]. Feline infectious peritonitis caused by feline coronaviruses is characterized by fibrinous serositis with fluids accumulation in body cavities, widespread pyogranulomatous lesions, and infection of macrophages and monocytes. Feline infectious peritonitis of viral origin has been associated with severe suppression of natural killer cells and regulatory T cells, leading to cell depletion and decreased cell functionality, whch results in immune suppression [22]. For these reasons, it is believed that both cats in this report were infected with *CMhm* and *CMt*, which are less pathogenic, but exhibited clinically significant anemia. To the best of our knowledge, this is the first clinical case report in Korea to demonstrate *CMhm* and *CMt* infections with clinical signs in a cat. If an anemic cat has immunosuppression or concurrent disease, feline hemoplasmosis should be included in the differential diagnoses, and PCR should be considered.

Cats with outdoor access are more likely to be infected with hemoplasmas [1,2,10,11], supporting the hypothesis that these agents are indirectly transmitted by blood-sucking arthropods, such as ticks and fleas. Both cats in this report were feral, suggesting that they were more likely to be exposed to these vectors or even involved in fights with other cats, increasing their risk of wounds and infections. Therefore, PCR should be performed if a rescued or feral cat shows symptoms of anemia, and prophylactic antibiotic (doxycycline) administration should be considered because feral cats can be asymptomatically infected with mycoplasma bacteria. Events such as immune suppression may precipitate the onset of anemia.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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