



NOTE

Internal Medicine

Clinical findings and treatment of disseminated '*Mycobacterium avium* subspecies *hominissuis*' infection in a domestic cat

Ryoji KANEKI¹⁾, Mayo YASUGI²⁾, Tomoyo NABETANI¹⁾, Toshiyuki TANAKA¹⁾, Yusuke WADA¹⁾, Kotaro HIRAI³⁾, Kikuya SUGIURA⁴⁾ and Shingo HATOYA^{4)*}

¹⁾Veterinary Medical Center, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano, Osaka 598-8531, Japan

²⁾Department of Bioenvironmental Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano, Osaka 598-8531, Japan

³⁾Higashi Kishiwada Animal Hospital, 2-30-15 Habucho, Kishiwada, Osaka 596-0825, Japan

⁴⁾Department of Advanced Pathobiology, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58 Rinku-oraikita, Izumisano, Osaka 598-8531, Japan

ABSTRACT. A cat was referred because of diffuse parenchymal lung disease. Close examinations revealed a swollen abdominal lymph node and multiple nodules of the liver. *Mycobacterium avium* subspecies *hominissuis* infection was confirmed by culture and single nucleotide polymorphism analysis of samples recovered from the liver and bronchoalveolar lavage. After administration of combination antibiotics for 6 months, culture results were negative. Though atonic seizures were observed during the treatment, it disappeared after isoniazid discontinuation and pyridoxal phosphate administration. On day 771 of illness, no clinical signs, lung diseases, or obvious swelling of lymph nodes was observed. This is the first report to confirm *Mycobacterium avium* subspecies *hominissuis* infection in cats through gene analysis and to completely cure it with combination antibiotics.

KEY WORDS: antibiotic, feline, isoniazid, lung disease, mycobacteria

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Mycobacterium avium subspecies *hominissuis* (MAH), a species of nontuberculous *Mycobacterium* (NTM), is a slow-growing bacteria that is widely detected in the environment. In human medicine, NTM produce pulmonary infiltrates and disseminated diseases, mainly in immunocompromised hosts [5, 7]. Incidence of NTM infections is reportedly increasing worldwide, and the prevalence of NTM species varies between countries. In Japan, MAH is the most common pathogen of NTM diseases [14, 24, 25]. A previous study has revealed that combination antibiotics following guidelines for the treatment of pulmonary NTM disease achieves positive clinical results [16].

In dogs and cats, MAH infection occurs via ingestion of infected meat or water, or contact with polluted soils [6]. Despite the widespread distribution of MAH, infection in dogs and cats is uncommon due to their innate immunity [13]. Little is therefore known about the clinical implications, treatment, and prognosis of MAH infection in cats. We report here a case of disseminated MAH infection in a young male Somali cat who recovered due to combination antibiotics and describe a side effect observed during treatment.

A 4-year-old neutered male Somali cat was kept completely indoors, and received a medical check-up every year. Diffuse parenchymal lung disease had been detected without any symptoms or blood test abnormalities in a medical check-up 3 months previously. Antibiotics and prednisolone (0.55–1.1 mg/kg every 24 hr) had been administered for the previous 2 months due to symptoms such as a poor appetite, moderate tachypnea, and a mild fever. The antibiotics clindamycin (5.5 mg/kg every 12 hr), doxycycline (11 mg/kg every 12 hr), and enrofloxacin (5.6 mg/kg every 24 hr) were administered separately. Despite the drug administration, his symptoms and lung disease were not improved. He was therefore referred to the Veterinary Medical Center of Osaka Prefecture University.

On physical examination, a body weight of 4.4 kg, body temperature of 39.0°C, heart rate of 180/min, and rhonchi lung sounds (respiratory rate: 30/min) were observed. A complete blood cell count revealed neutropenia (Table 1). The general chemical profile

*Correspondence to: Hatoya, S.: hatoya@vet.osakafu-u.ac.jp

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Table 1. The results of blood tests on the first day of illness

Analyte	Value	Reference range
White blood cell count (/μl)	7,540	(6,300–19,600)
Neutrophils (/μl)	1,659	(2,500–12,500)
Lymphocyte (/μl)	5,881	(1,500–7,500)
Monocyte (/μl)	0	(0–850)
Eosinophils (/μl)	0	(0–1,500)
Red blood cell count (×10 ⁶ /μl)	8.49	(6.00–10.10)
Hemoglobin (g/dl)	10.3	(8.1–14.2)
Hematocrit (%)	30.9	(27.7–46.8)
Mean corpuscular volume (fl)	36.5	(41.3–52.6)
Mean corpuscular hemoglobin (pg)	12.1	(12.0–16.0)
Mean corpuscular hemoglobin concentration (%)	33.2	(27.0–32.8)
Platelet count (×10 ³ /μl)	392	(300–800)
Total protein (g/dl)	7.6	(5.8–8.9)
Albumin (g/dl)	3.1	(2.4–3.8)
Blood Urea Nitrogen (mg/dl)	21.3	(16.0–36.0)
Creatinine (mg/dl)	0.86	(0.8–2.1)
Calcium (mg/dl)	10.9	(8.8–11.1)
Asparate aminotransferase (U/l)	68	(8–52)
Alanine aminotransferase (U/l)	38	(18–108)
Total Bilirubin (mg/dl)	0.08	(0.00–0.10)
Total cholestrol (mg/dl)	115	(67–232)
Glucose (mg/dl)	114	(63–132)
Sodium (mEq/l)	155.4	(147.0–158.0)
Potassium (mEq/l)	3.64	(3.10–5.00)
Chloride (mEq/l)	118.7	(113.0–127.0)
Prothrombin time (sec)	9.2	(9.3–11.3)
Activated partial thromboplastin time (sec)	32.9	(20.0–42.0)
Fibrinogen (mg/dl)	231	(120–240)
Feline coronavirus titer	<1:100	Not defined
Alpha 1-acid glycoprotein (μg/ml)	1,060	Not defined
Feline serum amyloid A (μg/ml)	26.2	(0.0–2.5)
Anti-filaria antibody	Negative	Negative

of serum showed no abnormalities (Table 1). The chest X-ray revealed an interstitial lung pattern (Fig. 1A). Ultrasound imaging indicated a swollen lymph node in the right abdomen (Fig. 1B). When fine needle aspiration (FNA) cytology of the swollen lymph node was performed, small lymphocytes and large macrophages were observed without neutrophils and eosinophils, which indicated granulomatous inflammation of the lymph node (Fig. 2). According to additional examinations, such as feline coronavirus titer, alpha 1-acid glycoprotein, serum amyloid A, and anti-filarial antibody, feline infectious peritonitis and filariasis were ruled out and aggressive inflammation was suspected (Table 1).

Due to worsening of lung disease and lymph node swelling, dynamic computed tomography (CT) and bronchoscopy were performed on day 38 of illness. The dynamic CT was performed in the arterial phase (20 sec after the injection of contrast medium), in the portal vein phase (60 sec) and in the equilibrium phase (180 sec) using 2 ml/kg nonionic contrast medium (300 mg/ml iohexol, Daiichi Sankyo Co., Tokyo, Japan). CT scan revealed bilateral peribronchial consolidation, swollen jejunum lymph node with uniform distribution of contrast medium, and multiple prominent nodules of the liver (Fig. 3A). These nodules exhibited lower CT values than that of liver parenchyma in plain image and were not enhanced with dynamic CT (Fig. 3B). Contrast enhancement of peripheral areas of the liver nodules was observed in the arterial phase; however, it disappeared in the portal vein phase. During bronchoscopy, intratracheal foreign bodies, increased mucus production, and redness of bronchial mucosa were not found. Cytology of bronchoalveolar lavage (BAL) showed a small number of neutrophils and macrophages without any bacteria. When FNA cytology of the liver was performed, neutrophils, small lymphocytes, and large macrophages were observed. The specimens of bronchoscopy and liver FNA were submitted to research laboratory (Japan Clinical Laboratories, Inc., Kyoto, Japan) for culture of general bacteria, fungus, and *Mycobacterium* species. These examinations revealed that general bacteria and fungus were culture-negative, and *Mycobacterium* species were smear-negative with Ziehl-Neelsen staining but culture-positive in Mycobacteria Growth Indicator Tube systems (Becton, Dickinson and Co., Franklin Lakes, NJ, U.S.A.). The pathogen was confirmed as *M. avium* through DNA-DNA hybridization techniques with DDH Mycobacteria “Kyokuto” (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) [18]. Antitubercular drug susceptibility testing determined by the proportion test method on egg-based ogawa media (Vite Spectrum SR, Kyokuto Pharmaceutical Industrial Co., Ltd.) revealed that the bacterial isolate was resistant to isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, enviomycin, ethionamide, para-aminosalicylic acid, and

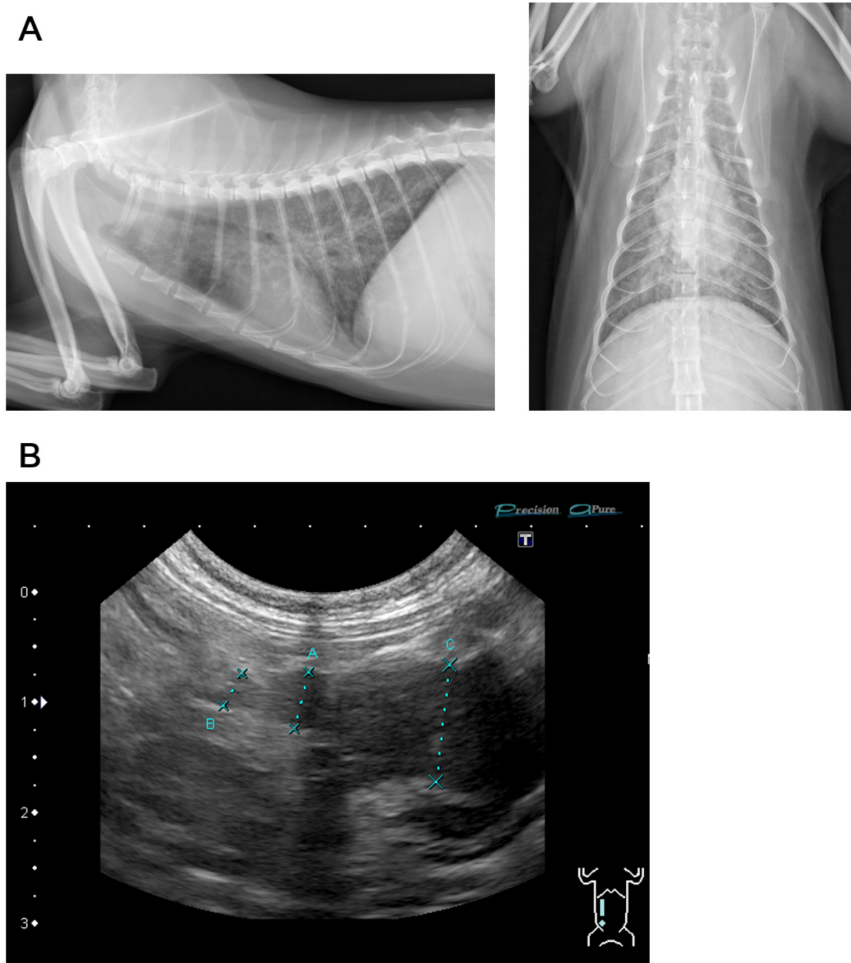


Fig. 1. Medical imaging. (A) Right lateral and ventral view of the chest radiograph. Diffuse parenchymal lung disease was observed. (B) Ultrasound imaging of the swollen abdominal lymph node. The maximum diameter of the lymph node was 10.7 mm.

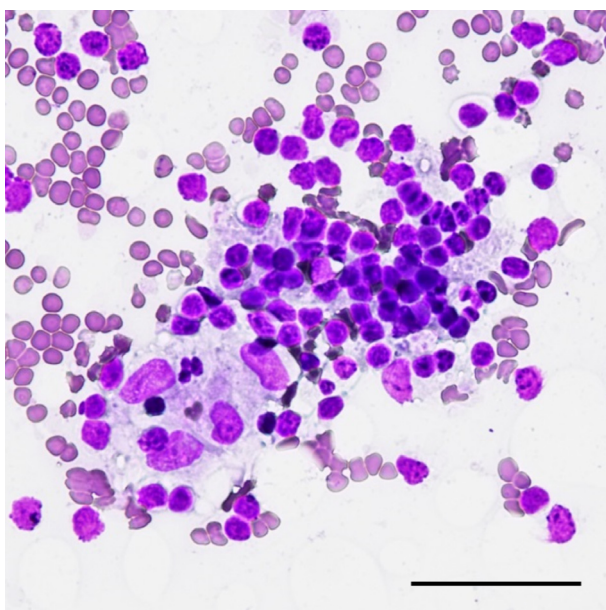


Fig. 2. Cytology of the swollen abdominal lymph node. The fine needle aspiration sample was Giemsa-stained. Small lymphocytes and large macrophages were observed without neutrophils and eosinophils, which indicated granulomatous inflammation of the lymph node. Scale bars=50 μ m.

levofloxacin; however, it was sensitive to cycloserine (Table 2) [26].

In order to identify the subspecies of *M. avium* isolate recovered from the clinical sample, we determined the nucleotide sequence of *hsp65* (GenBank accession number LC497502) and compared single nucleotide polymorphisms (SNPs) among the

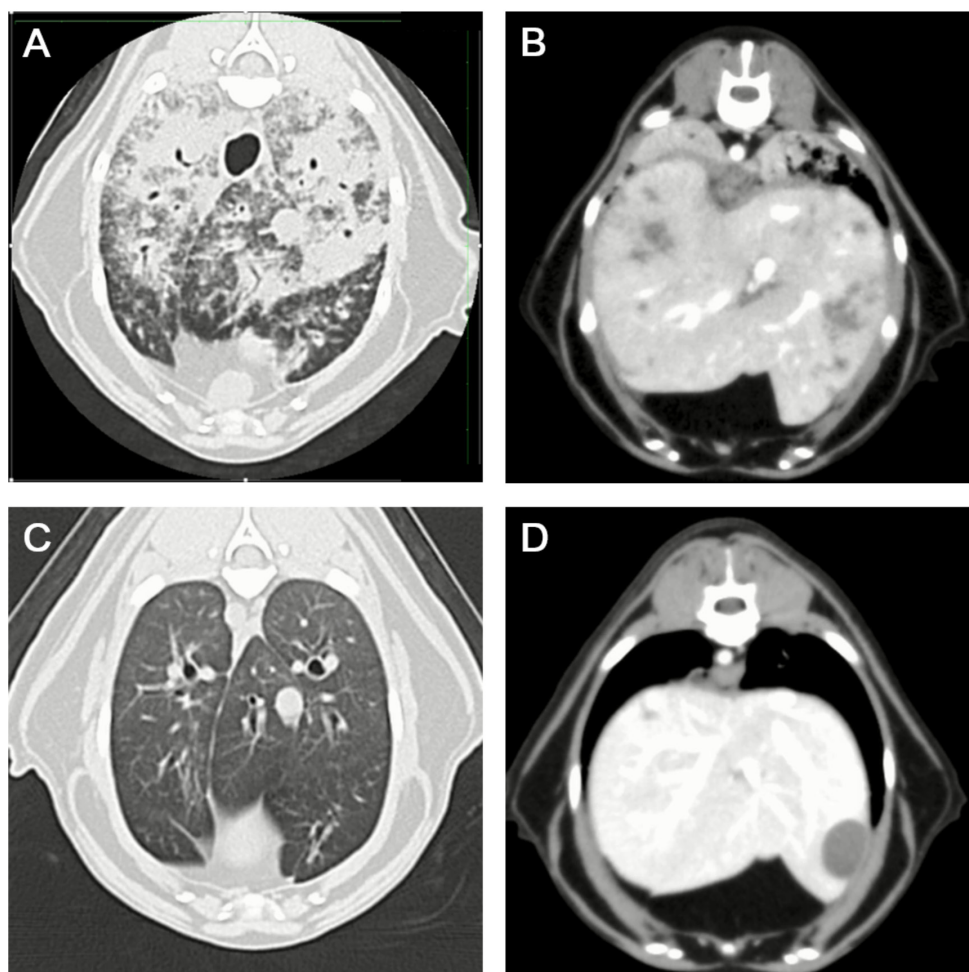


Fig. 3. Computed tomography scan. (A) Image of the lung at the 8th thoracic vertebral level without contrast medium on day 38 of the illness. Bilateral peribronchial consolidation was detected. (B) Image of the liver at the 11th thoracic vertebral level at the portal vein phase on day 38 of the illness. Multiple nodules of the liver without distribution of contrast medium were found. (C) Image of the lung at the 8th thoracic vertebral level without contrast medium on day 246 of the illness. Bilateral peribronchial consolidation was not detected. (D) Image of the liver at the 10th thoracic vertebral level at the portal vein phase on day 246 of the illness. No nodules were observed.

Table 2. The results of antitubercular drug susceptibility testing for *Mycobacterium avium* subspecies *hominissuis* recovered from bronchoalveolar lavage and liver

Antitubercular drug	Concentration ($\mu\text{g/ml}$)	Evaluation
Isoniazid	1.0	R
Rifampicin	40	R
Streptomycin	10	R
Ethambutol	2.5	R
Kanamycin	20	R
Enviomycin	20	R
Para-aminosalicylic acid	0.5	R
Levofloxacin	1.0	R
Cycloserine	30	S

R=resistant, S=susceptible.

M. avium strains, which were categorized into “codes” 1 to 9, and 15 to 17 depending on their sequence (Table 3) as described previously [27]. The sequence from our sample, however, was not a complete match with any *hsp65* sequences from code 1 to 9, and 15 to 17. We then created the phylogenetic tree of *hsp65* using the sequences deposited in GenBank belonging to codes 1 to 9, and 15 to 17 [1, 12] (Fig. 4). Codes 1 to 3, 7 to 9, and 15 to 17 are found in MAH. Code 4 is from *M. avium* subsp. *avium*,

Table 3. Comparison of *hsp65* sequences with *Mycobacterium avium* strains

Representative strain code	Nucleoide at indicated base pair position																				n				
	240	276	324	588	612	633	642	645	742	861	928	1092	1128	1136	1218	1269	1272	1277	1296	1350		1435	1468	1488	1536
Thel isolated strain (MY332)	C	G	T	T	C	C	A	C	A	G	C	G	C	C	A	G	C	C	C	G	A	G	G	G	-
Code 1 (DQ284765)	-	-	C	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	3
Code 2 (DQ284766)	-	-	-	-	-	-	G	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	2
Code 3 (DQ284767)	-	-	C	-	-	-	G	-	-	-	-	-	G	-	G	C	G	G	-	-	-	-	-	-	6
Code 4 (DQ284768)	-	-	C	-	-	-	G	T	-	-	-	-	G	-	G	C	G	G	-	-	-	-	-	-	7
Code 5 (DQ284769)	-	-	C	-	G	G	G	-	-	T	-	-	G	-	G	T	G	-	A	-	-	A	C	-	12
Code 6 (DQ284770)	T	-	C	-	G	G	G	-	-	T	-	-	G	-	G	C	G	-	-	-	G	A	C	-	13
Code 7 (DQ284771)	-	C	C	-	-	-	G	-	-	-	-	A	G	-	-	C	G	-	-	A	-	-	-	-	8
Code 8 (DQ284772)	-	-	-	C	-	-	G	-	-	-	-	-	-	-	T	G	-	-	-	-	-	-	-	-	4
Code 9 (DQ284773)	-	-	C	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Code 15 (EU085419)	N.A.	N.A.	N.A.	-	-	-	G	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	A	3
Code 16 (AB453830)	N.A.	N.A.	N.A.	-	-	-	G	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Code 17 (AB453831)	N.A.	N.A.	N.A.	-	-	-	G	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	A	3

Numbers mean base pair positions on the complete *hsp65* gene. n=number of SNPs from MY332, N.A.=Not available on GenBank. *hsp65* gene sequences from 12 nontuberculous *Mycobacterium* species belonging to codes 1–9 and 15–17 (accession numbers DQ284765–DQ284773, EU085419, AB453830, and AB453831, respectively) were retrieved from the GenBank and compared with that of the present clinical isolate (MY332) [1, 12, 27]. Codes 1–3, 7–9, and 15–17 belong to *M. avium* subsp. *hominissuis*. Code 4 is from *M. avium* subsp. *avium*. Codes 5 and 6 are from *M. avium* subsp. *paratuberculosis*.

and codes 5 and 6 are from *M. avium* subsp. *paratuberculosis*. The *hsp65* sequence from our specimen, shown as strain MY332, was closest to that in code 9 and located in the group composed of codes 1, 2, 8, 9, 15, 16, and 17. These results indicated that the isolate from the clinical sample was MAH.

Due to the positive culture results for the *Mycobacterium* species, we started orally combined administration of antibiotics (isoniazid 10 mg/kg every 24 hr, rifampicin 10 mg/kg every 24 hr, and enrofloxacin 5 mg/kg every 24 hr) on day 66 of illness. However, atonic seizures occurred in increasing frequency from day 93 of illness. The chest X-ray and ultrasound imaging revealed the improvement of lung disease and jejunum lymph node swelling, which indicated the therapeutic response of MAH infection. We administered pyridoxal phosphate (1 mg/kg every 24 hr) and clarithromycin (10 mg/kg every 12 hr) instead of isoniazid. Atonic seizures disappeared within a week. On day 107 of illness, lung disease was in remission and the jejunum lymph node had begun to shrink. On day 246 of illness, CT scan revealed that the peribronchial consolidation and the multiple nodules of the liver had disappeared (Fig. 3C and 3D). Furthermore, bronchus and liver FNA specimens were culture-negative in Mycobacteria Growth Indicator Tube systems. We stopped antibiotic administration on day 429 of illness, and there were no clinical signs, evidence of lung disease in chest X-Ray, or obvious lymph node swelling in ultrasound images on day 771 of illness.

Here, we diagnosed MAH infection in a domestic cat who was referred because of lung disease of an unknown origin. During clinical investigation, we detected an interstitial lung pattern, a swollen abdominal lymph node, and multiple nodules of the liver. Cytology of the lymph node and the liver indicated signs of granulomatous inflammation without any bacteria. However, cultures of bronchus and liver FNA specimens were positive for NTM, which was confirmed as MAH through SNP analysis and analysis of the phylogenetic tree of *hsp65*. After the administration of combination antibiotics for 6 months, NTM culture of bronchial and liver FNA specimens results turned negative. We stopped administration of antibiotics after an additional 6 months, and there were no signs of relapse on day 771 of illness.

Infections of *Mycobacterium* species in domestic cats have been reported sporadically. In Great Britain, previous reports have revealed that 1.16% of 11,782 feline tissue samples submitted to diagnostic laboratories were confirmed to have *Mycobacterium* infections [10], and 15% of cultured *Mycobacterium* species were *M. avium*. In Japan, *Mycobacterium* infections in domestic cats have been historically uncommon, with only two cases reported: unclassified *Mycobacterium* species (MFM001 strain) [13] and MAH in the Kanto region [20]. This is the third reported case of *Mycobacterium* species infection in a domestic cat in Japan. In Japan, pulmonary NTM diseases are commonly diagnosed in human medicine with an incidence rate of 14.7 cases per 100,000 person-years in 2016, which is 2.6 times higher than the incidence rate reported in 2007 [25]. The most common pathogens of pulmonary NTM diseases were *M. avium* in the northern and eastern parts of Japan and *M. intracellulare* in the southern and western parts of Japan. *Mycobacterium* infection should also be considered a potential disease for domestic cats. In a previous study in Australia, it was observed that certain lines of Abyssinian and Somali cats likely suffer from a familial immunodeficiency that predisposes them to infection with slow-growing mycobacteria, including *M. avium* [2]. Whilst immunological predisposition of those breeds has not been well proven, it will be beneficial to pay attention to the breed of cats with suspected *Mycobacterium* infections.

Species of *M. avium* are divided into four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *hominissuis*. These subspecies are genetically very close. However, their host range and pathogenicity differs. Furthermore, recent studies have suggested that genomic differences affect the virulence and antibiotic resistance of MAH [4, 14]. In domestic cats, there have been a few reports that have identified the pathogen of NTM infection as MAH [3, 15, 20]. In this report, SNP analysis and analysis of the phylogenetic tree of *hsp65* enabled the correct evaluation of the

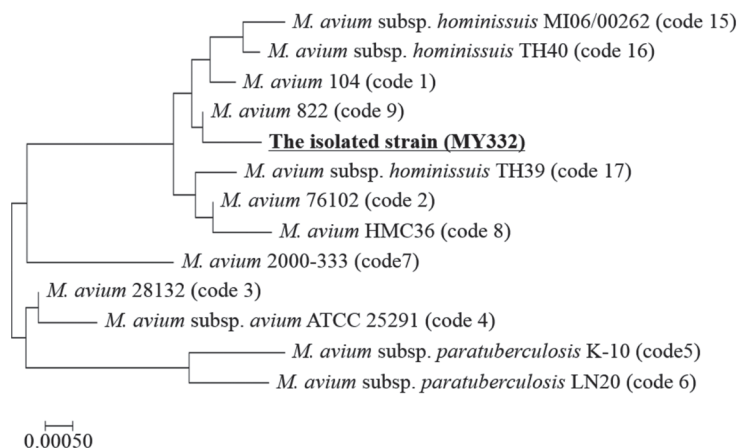


Fig. 4. *hsp65* gene sequences from 12 nontuberculous *Mycobacterium* (NTM) species retrieved from GenBank belonging to codes 1 to 9, and 15 to 17 (accession numbers DQ284765–DQ284773, EU085419, AB453830, and AB453831, respectively), and from the present clinical isolate (strain MY332) were aligned using Mega7 [17]. Phylogenetic tree was constructed using the neighbor-joining method. Codes 1-3, 7-9, and 15-17 belong to *Mycobacterium. avium* subsp. *hominissuis*. Code 4 is from *M. avium* subsp. *avium*. Codes 5 and 6 are *M. avium* subsp. *paratuberculosis*.

clinical findings, pathology and therapeutic response depending on the bacterial species.

M. avium is ubiquitous worldwide in soil and water under certain conditions and remains viable in the environment for at least two years [8]. Despite the widespread distribution and survivability of *M. avium*, infections in human and veterinary medicine are quite rare. Therefore, individual innate immunity is the most important for the prevention of *M. avium* infection, and no evidence has been found for the spread of *M. avium* among humans or animals. However, due to uncommon diseases, it is difficult to conclude that the epidemiology of each *Mycobacterium* species, subspecies, and strain has been established. Thus, we should prevent immunocompromised humans or animals (f.e., resulting from chemotherapy) to come into contact with infected animals.

M. avium infection in cats is usually caused by ingestion of the organism via the environment or contaminated food. Our case was completely kept indoors; therefore, we suspected inapparent infection in youth or infection from polluted water or food. After ingestion, *M. avium* is phagocytosed by intestinal macrophages and eventually causes diseases due to stress or acquired or inborn immunosuppression [8]. In human and veterinary medicine, some cases of *M. avium* infection have been diagnosed with blood culture; therefore, hematogenous dissemination of *M. avium* has been considered [1, 5, 19]. Previously reported clinical findings of MAH infection in cats are lymphadenopathy, skin abscesses, skin granulomas, meningoencephalitis, and lung nodules [3, 15, 20]. In the present study, diffuse parenchymal lung diseases and granulomatous inflammation of the lymph node and liver were observed. We suspected that our case was infected via ingestion of polluted water or food and MAH disseminated to the jejunum lymph nodes, liver, and lungs. MAH infection should therefore be considered a differential diagnosis for lung diseases or granulomatous inflammation of uncertain cause. A previous study identified the same clinical findings and splenomegaly in cats with *M. avium* infection, though potential subspecies were not analyzed [2]. In the present study, no spleen abnormalities were found with ultrasound imaging or CT scan. However, as we did not perform cytology of the spleen, we should consider the possibility that minute spleen lesions were formed. Further studies will be required to elucidate the relationship between clinical findings and *M. avium* subspecies.

In some reports, NTM phagocytosed by macrophages were detected in cytology of granuloma or BAL, with or without acid-fast staining [15, 21, 22]. However, we did not detect MAH in cytology of the swollen lymph node, liver or BAL with Ziehl-Neelsen staining. Previous studies revealed that *Mycobacterium* species at different stages of infection can sometimes be extremely difficult to find. Thus, it is recommended that *Mycobacterium* infection is not ruled out even in cases of negative results of cytological preparations, and that histological examination or polymerase chain reaction should nevertheless be performed [22, 23]. In the present study, lung disease was detected without any symptoms or blood test abnormalities in a medical check-up three months earlier; therefore, we considered our case to be at the early stage of MAH infection. In such cases, it has been reported that specific culture for NTM species of granuloma, BAL and blood has proved useful in human medicine [28]. In our case, bronchus and liver FNA specimens were submitted for culture of *Mycobacterium* species and shown to be positive. This result indicated that culture for specific NTM species, as well as cytology, should be considered in cases with lung diseases or granulomatous inflammation of uncertain cause.

During combined administration of antibiotics, this case presented atonic seizures. As the possible causes of seizures, we considered MAH infiltration into central nervous systems, side effects of isoniazid, or other intracranial diseases. Chest X-ray and ultrasound imaging revealed the improvement of lung disease and jejunum lymph node swelling, which indicated the therapeutic response of MAH infection. Therefore, a side effect of isoniazid, whose recommended dosage range was 10 – 20 mg/kg every 24 hr [8], was suspected as the cause of seizures. In human medicine, isoniazid administration for patients with concurrent diseases, such as chronic kidney disease and diabetes, sometimes causes atonic seizures, and these side effects can be prevented and treated

with pyridoxal phosphate [7]. In the present case, there were no clinical findings to indicate concurrent diseases. However, we should consider the possibility that the multiple nodules in the liver potentially affected the metabolism of isoniazid even though isoniazid was administered at the minimum recommended dosage.

Three cats were previously reported with MAH infection. Two were diagnosed at necropsy [3, 20]. One, which presented with chronic vomiting, diarrhea, weight loss, anorexia and an abdominal mass, was treated with combined antibiotics, including azithromycin, rifampicin and enrofloxacin [15]. However, after a short period of improvement of clinical symptoms, the cat was euthanized because of severe protein-losing enteropathy. Although treatment and prognosis of *M. avium* infection in domestic cats in Australia has been reported, *Mycobacterium* subspecies were not identified in these cases [2]. This is therefore the first case of MAH infection to be confirmed by gene analysis and to show recovery with combination antibiotic treatment. The poor prognosis in previous cases could be attributed to difficulties in the antemortem diagnosis and delayed start time for treatment of MAH infection after clinical symptoms were observed. This case demonstrates that combined antibiotic administration based on reports from human medicine is effective for MAH infection in cats, and early examinations, diagnosis and treatment will lead to a good result. Susceptibility testing revealed that the MAH isolate was resistant to most antitubercular drugs. However, we administered combined antibiotics including isoniazid and rifampicin, which the MAH isolate was resistant to, and clinical symptoms improved. Previous studies revealed that *in vitro* susceptibility testing for *Mycobacterium* species was of little or no value for predicting clinical efficacy [4, 11]. The present study indicated that it is difficult to predict therapeutic effects of antitubercular drugs for MAH depending on *in vitro* susceptibility testing in domestic cats.

In human medicine, combination antibiotics are administered for 12–24 months or until NTM culture results are negative. However, there is no convincing scientific evidence [4, 9, 16, 28]. In domestic cats, it was recommended that treatment of feline leprosy syndrome, which was caused by *Mycobacterium* species, should ideally be continued for a further 3 months after lesions have regressed in order to reduce the risk of recurrence [23]. However, the optimal length of drug therapy for MAH has not yet been established. Here, we administered combination antibiotics for 6 months until NTM culture yielded negative results, and no symptoms or abnormal findings were detected in X-Ray or ultrasound images. Further studies might be needed to identify the optimal treatment duration of MAH infection.

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