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LETTER

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Human pathogenic *Mycobacterium kansasii* (former subtype I) with zoonotic potential isolated from a diseased indoor pet cat, Japan

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ABSTRACT

Nontuberculous mycobacterial (NTM) infections in humans have increased in prevalence in recent decades. *Mycobacterium kansasii* is one of the most prevalent human pathogenic NTM species worldwide. Herein, we report the first isolation of *M. kansasii* from an indoor domestic cat in Japan. Comparative genome sequence analysis of the feline isolate showed this pathogen is genetically identical to human pathogenic *M. kansasii*.

This finding suggests that *M. kansasii* has a potential risk of zoonoses and requires the "One Health" approach to control NTM infection.

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Nontuberculous mycobacterial (NTM) infections in humans have increased in prevalence in recent decades. Most NTM species that are pathogenic to humans are also pathogenic to cats, including *Mycobacterium avium*, *Mycobacterium abscessus,* and *Mycobacterium xenopi* [1]. However, little evidence exists of the zoonotic potential of NTM infections.

Mycobacterium kansasii is one of the most prevalent human pathogenic NTM species worldwide [2]. Initially, PCR-restriction pattern analysis identified seven subtypes of *M. kansasii* (subtypes I–VII) [3,4]. However, comparative genomic analysis reclassified these subtypes as *M. kansasii* (former subtype I), *Mycobacterium persicum* (former subtype II), *Mycobacterium pseudokansasii* (former subtype III), *Mycobacterium innocens* (former subtype V), and *Mycobacterium attenuatum* (former subtype VI) [5]. Currently, the *M. kansasii* complex (MKC) comprises the *M. kansasii*, *M. persicum*, *M. pseudokansasii*, *M. innocens*, *M. attenuatum* and *M. gastri* species [5,6]. These species names will be used throughout this manuscript.

A recent study showed that, compared to other MKC species, *M. kansasii* has ESX-1 type VII secretion system and *espACD* operon associated with its pathogenicity [6,7]. Of the MKC species, *M. kansasii* is the most frequently isolated from patients with pulmonary diseases, while *M. persicum* is associated with immunodeficient HIV-infected patients [8,9]. The remaining MKC species are considered non-pathogenic colonizing agents and are typically isolated from tap water samples or animals [8,9]. To date, the risk of *M. kansasii* infection and its major environmental reservoir remain poorly understood.

In this study, we report the first isolation of *M. kansasii* from infected domestic immunocompetent cat in Japan. Complete genome sequence analysis identified the isolate as human pathogenic *M. kansasii*.

The Study

The case is a 13-year-old, neutered, female domestic cat weighing 4 kg. The cat lived outdoors for the first 2–3 years but has remained indoors only for nearly 10 years. Tests for Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) were both negative.

Initially, the cat exhibited lid swelling and eye mucus of the left eye. The swelling site was surgically excised, and the pus drained (Supplementary Figure 1A).

Giemsa staining of cell smear demonstrated a large number of intracellular non-staining long rod bacilli in macrophages (Supplementary Figure 1B). Histopathological examination revealed pyogranulomatous lesions with neutrophil aggregation and multinucleated giant cell infiltration (Supplementary Figure 1C). We observed a large amount of acid-fast bacilli (Supplementary Figure 1D).

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We isolated strain Kuro-I, from nodular lesion. Long-read and short-read sequencing were performed on the MinION platform (Oxford Nanopore Technologies, Oxford, UK) and NovaSeq 6000 Platform (Illumina., San Diego, CA). The circular genome size of Kuro-I is 6,649,596 bp, with a G+C content of 66.1%, containing 8110 putative coding sequences, 3 rRNAs, 54 tRNAs, and 5 CRISPR regions.

To compare the Kuro-I strain genome to other MKC species, we obtained sequencing data for the MKC species from the NCBI Assembly and SRA databases, and obtained source information from the NCBI BioSample (Supplementary Table).

32 MKC strains listed in Supplementary Table were used for phylogeny. Phylogenetic analysis based on core-gene alignments discriminated the MKC species clearly. The Kuro-I strain isolated from the domestic cat and two strains isolated from Rhesus macaques were positioned in the internal clade of *M. kansasii* consist of isolates from pulmonary diseased human (Figure 1). The 16 *M. kansasii* strains and Kuro-I were used for pan-genomic analysis. The 17 isolates pan-genome has a total 15037 genes; 3100 are shared between the core (3100) and soft-core (0) genes; while 3767 and 8170 genes from shell and cloud genes, respectively (Supplementary Figure. 2). The coding sequence of *espACD* operon was present in Kuro-I strain (Supplementary Figure. 3).

The 17 *M. kansasii* strains and type strain of other MKC species were used for ANI calculation. The ANI values between the Kuro-I strain and the MKC strains *M. kansasii* ATCC12478 T, *M. attenuateum* MK41 T, *M. pseudokansasii* MK142 T, *M. gastri* DSM43505 T, *M. innocens* MK31 T, and *M. persicum* AFPC-000227 T were 99.37%, 90.38%, 92.71%, 91.59%, 93.43%, and 93.27%, respectively (Supplementary Figure. 4). The Kuro-I strain showed the highest similarity to the *M. kansasii* ATCC12478 T strain isolated from diseased human (99.37%). 17 strains of *M. kansasii* showed a mean ANI value of 99.23% (range 98.87%–99.97%).

The cat treated for 6 months with Clarithromycin (20 mg/kg; BID) and Rifampicin (7 mg/kg; BID), which improved clinical signs, including eyelid swelling and eye mucus. There was no recurrence six months after chemotherapy, and the owner was not infected.

Conclusions

Next-generation sequencing has reclassified the MKC species and revealed pathogenic characteristics [7]. Although studies show that the major reservoir of *M. kansasii* infection is tap water instead of environmental water sources or soil, the infection sources for all of the MKC species remain poorly understood [10].

M. kansasii has been isolated from a wide variety of animals; dogs (*Canis familiaris*), Rhesus Macaque (*Macaca mulatta*) and cats (*Felis catus*) [11–14]. To our knowledge, three cases of MKC infetion in cat were reported previously [12,14]. All the three cats were presented with cutaneous and systemic lesion. In one case, the cat was treated with trimethoprim-sulphamethoxazole and doxycycline, but it was unsuccessful [12]. Other two cats were sibling, indoor-only cats and treated successfully with rifampicin, azithromycin and pradofloxacin [14]. In these cases, commercial identification kit or PCR sequence analysis using partial house keeping genes were performed for bacterial identification. Thus, their exact species among reclassified MKC are unknown.

In this study, we isolated the Kuro-I strain from a diseased domestic cat. Using complete genome

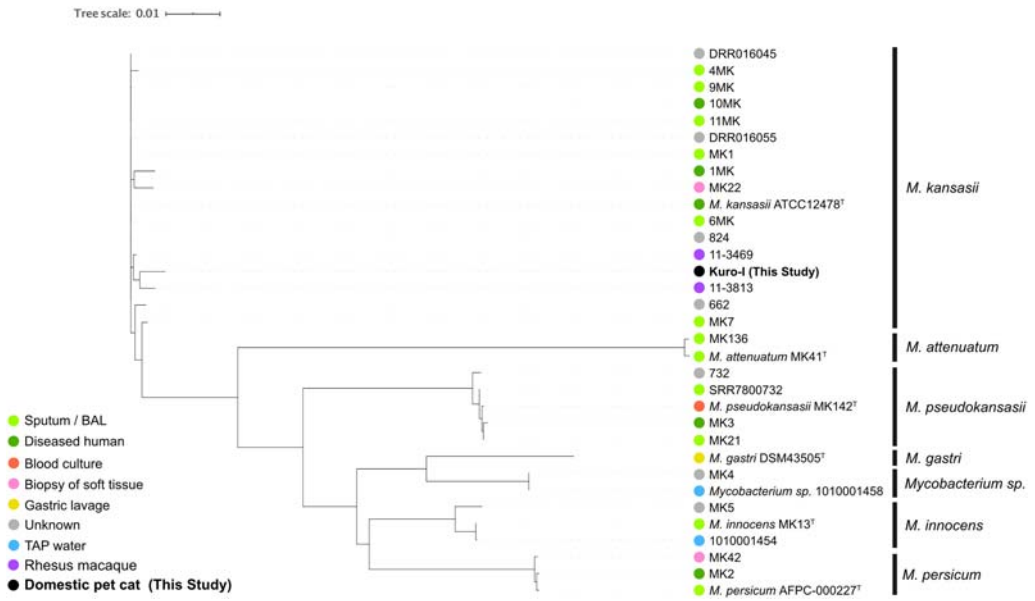

Figure 1. A phylogenetic tree based on core gene alignments of *M. kansasii* complex (MKC) species. Coloured circles indicate the isolate determined by deposited information from the archived NCBI BioSample database.

sequence analysis, we identified the isolate as *M. kansasii* (former subtype I) and genetically similar to *M. kansasii* isolated from patients with pulmonary diseases. We report the first comparative genomic analysis of *M. kansasii* isolated from animals and humans based on core-gene phylogeny and genome-to-genome distance.

A recent study identified an incidence of cat-to-human transmission of *M. bovis* infection in England, where two people who had close contact with an infected pet cat developed active *M. bovis* diseases [15]. Whole-genome sequencing analysis confirmed cat-to-human transmission for the first time and demonstrated a risk of companion animal associated mycobacterial infection in humans. In this present case, we isolated *M. kansasii* from an immunocompetent domestic cat who has remained indoors for the past decade. This demonstrates that domestic cats, which are among the most common pets, are a susceptible host for *M. kansasii* infection under immunocompetent conditions and could be a reservoir for this emerging pathogen.

Histopathological analysis revealed pyogranulomatous lesions filled with pus containing large amounts of mycobacterial cells. This suggests aerosol transmission capability, which is the primary transmission route for human pulmonary NTM infections, including *M. kansasii* [15].

In conclusion, we report the first isolation of *M. kansasii* from an infected indoor pet cat in Japan. This finding suggests that *M. kansasii* has a potential risk of zoonoses and requires the "One Health" approach to control NTM infection. Further studies exploring the environmental sources of *M. kansasii* are necessary to understand the transmission modes or infectious risk of this emerging worldwide disease.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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