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

Hanako Fukano
National Institute of Infectious Diseases
Tsukasa Terazono
Terazono Animal Hospital Hiroshima, JP
Aki Hirabayashi
National Institute of Infectious Diseases
Mitsunori Yoshida
National Institute of Infectious Diseases
Masato Suzuki
National Institute of Infectious Diseases
Shinpei Wada
Nippon Veterinary and Life Science University, Faculty of Veterinary Science Musashino, Tokyo, JP
Norihisa Ishii
National Institute of Infectious Diseases
Yoshihiko Hoshino (yhoshino@nih.go.jp)
National Institute of Infectious Diseases https://orcid.org/0000-0002-3132-0715

Case Report

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Abstract

Mycobacterium kansasii is one of the most prevalent and pathogenic nontuberculous mycobacteria in the world. Herein, we report the first case of *M. kansasii* infection in an indoor domestic cat in Japan. Complete genome sequence analysis of the isolate showed this pathogen is genetically identical to human pathogenic *M. kansasii*.

Introduction

Nontuberculous mycobacterial (NTM) infections in humans have increased in prevalence in recent decades(1). Most NTM species that are pathogenic to humans are also pathogenic to cats, including *Mycobacterium avium*, *Mycobacterium abscessus,* and *Mycobacterium xenopi*(2). However, little evidence exists of the zoonotic potential of NTM infections.

Mycobacterium kansasii is one of the most prevalent human pathogenic NTM species worldwide and is phylogenetically closest to *Mycobacterium tuberculosis*(3). Initially, PCR-restriction pattern analysis identified seven subtypes of *M. kansasii* (subtypes I–VII)(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 case of *M. kansasii* infection in an indoor 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 4.0 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 cat was treated with broad-spectrum antibiotics and steroids, but eyelid swelling worsened after 1 to 2 weeks. The swelling site was surgically excised, and the pus drained (Figure 1A).

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

We used a mycobacterial isolation technique to isolate the strain, referred to as Kuro-I, from the nodular tissue lesion. For long-read and short-read sequencing, we extracted genomic DNA using phenol-chloroform technique and a NucleoSpin plant kit (Macherey-Nagel), respectively. 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). Preparation for the sequencing library and genome assembly using long/short sequenced data is described in the Appendix. The circular genome size of Kuro-I is 6,649,596 bp, with a G+C content of 66.1%, containing 8,110 putative coding sequences, 3 rRNAs, 54 tRNAs, and 5 CRISPR region.

To compare the Kuro-I strain genome to other MKC species, we obtained next-generation sequencing data for the MKC species from the National Center for Biotechnology Information (NCBI) Assembly and Sequence Read Archive (SRA) databases, and obtained source information from the NCBI BioSample database (Appendix Table). We annotated the genomes with Prokka (Galaxy ver. 1.14.5) and used Roary (Galaxy ver. 3.13.0) for pan-genomic analysis and core-gene alignment, which we used to build a maximum-likelihood phylogenetic tree using RAxML (Galaxy ver.1.0.0), Interactive Tree of Life (iTOL) and Phandango. We calculated the genome-based average nucleotide identity (ANI) with FastANI (Galaxy ver.1.3). We performed pan-genomic analysis for 17 *M. kansasii* strains by Roary (Galaxy ver. 3.13.0).

Phylogenetic analysis based on core-gene alignments discriminated the MKC species. 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 2). The 17 *M. kansasii* pan-genome has a total 15037 gene; 3100 are shared between the core (3100) and soft-core (0) genes; while 3,767 and 8,170 genes from shell and cloud genes, respectively (Appendix Figure. 1). Coding sequence of *espACD* operon was present in Kuro-I strain (Appendix Figure. 2).

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 (Appendix Figure. 3).

The cat received treatment 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 in both eyes. 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(10), the infection sources for all of the MKC species remain poorly understood.

M. kansasii has been isolated from a wide variety of animals such as dogs (*Canis familiaris*), Rhesus Macaque (*Macaca mulatta*) and cats (*Felis catus*). However, the respective *M. kansasii* subtypes and zoonotic potential are unknown(11–13). In this study, we isolated the Kuro-I strain from a diseased indoor domestic cat. Using complete genome sequence analysis, we identified the isolate as *M. kansasii* (former subtype□). The Kuro-I strain was genetically similar to *M. kansasii* isolated from patients with pulmonary diseases and Rhesus macaques. 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(14). 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.

In this study, 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*(14).

Although *M. kansasii* is a drug-sensitive NTM species, inappropriate use of clarithromycin can lead to a high prevalence of macrolide-resistant strains (15). Our data suggest that the treatment of companion animals requires proper antibiotic management by veterinarians to avoid resistance.

In conclusion, we report the first case of *M. kansasii* infection in an 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.

Declarations

Biographical Sketch

Dr. Fukano is a senior researcher in the Department of Mycobacteriology, Leprosy Research Centre, National Institute of Infectious Diseases, Tokyo, Japan. Her research interests are antimicrobial resistance and transmission of mycobacteria.

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Figures

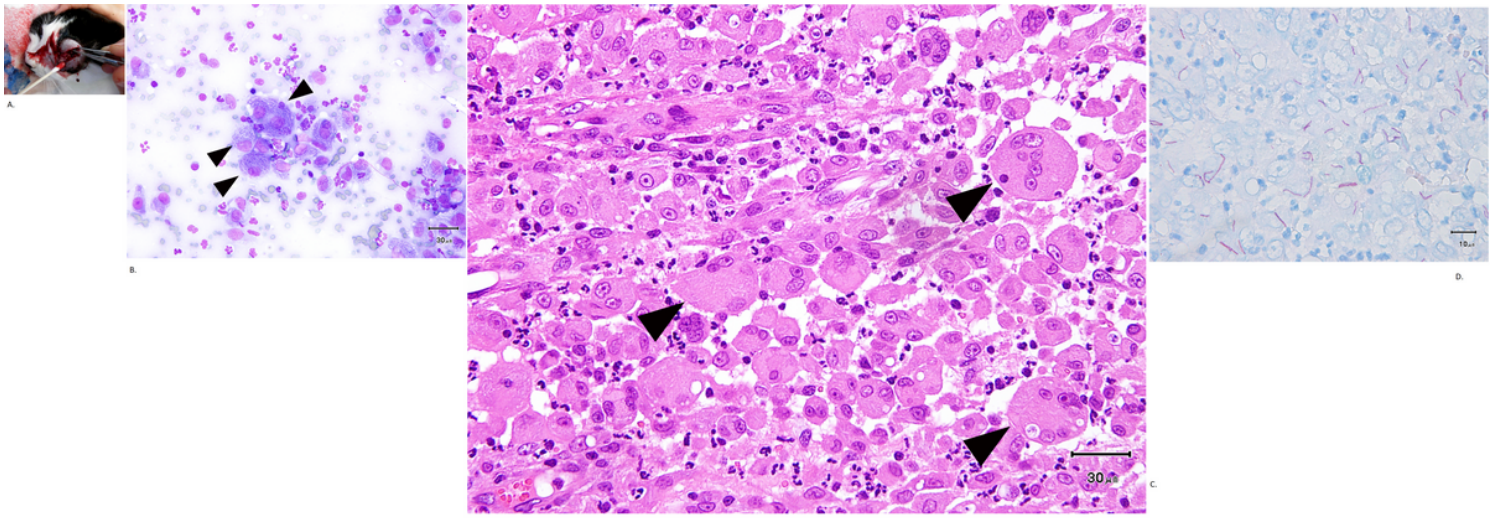

Figure 1

A) A heavily swollen left eyelid caused by M. kansasii infection in an indoor pet cat. B-D) Results of histopathological analyses. B) Giemsa staining of stamp specimens revealed many intracellular non-staining long rod bacilli within macrophages or giant cells (arrowheads). C) Hematoxylin and eosin staining demonstrated neutrophil aggregation and multinucleated giant cell or Langhans giant cell infiltration (arrowheads). D) Ziehl-Neelsen staining showed acid-fast long rod bacilli (arrowheads).

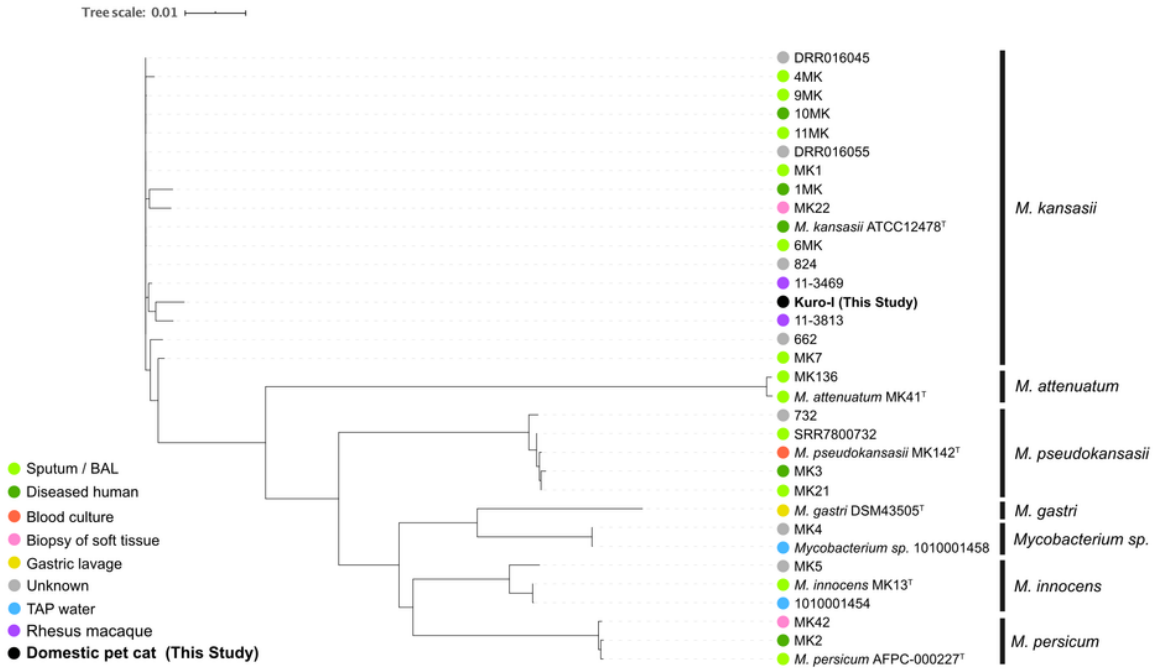

Figure 2

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TechnicalAppendix.docx
- AppendixFig.1Pangenome.png
- AppendixFig.2ACDoperon.png
- AppendixFig.3ANI.png
- AppendixTable.xlsx