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First Cases of *Mycobacterium elephantis* in Zimbabwe Revealed by 16S Ribosequencing

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Abstract

Mycobacterium elephantis is a Nontuberculous *Mycobacterium* (NTM) and was originally discovered in an elephant that had died of a respiratory disease. This *Mycobacterium* species was later found in human clinical specimens in a number of countries. In Zimbabwe, *M. elephantis* was not previously reported. This report is the first one. Using 16S ribosequencing, we discovered one case of *M. elephantis* in cow dung and two cases in human sputum samples collected in Zimbabwe. In all three cases, the 16S ribosomal RNA gene sequences were 100% identical to the *M. elephantis* sequences found in the Genbank. The sources of human infection by *M. elephantis* in Zimbabwe were not clear. Further studies are therefore necessary to ascertain the prevalence and clinical relevance of *M. elephantis* in humans in Zimbabwe.

Keywords: *Mycobacterium elephantis*, cases, ribosequencing, Zimbabwe.

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Introduction

Mycobacterium elephantis is a NTM that was originally isolated from an elephant which had died of chronic respiratory infection in Sri Lanka in 2000 [1]. Since then, several reports of *M. elephantis* infection in humans have been published. *M. elephantis* was isolated in humans in Asia, Belgium, Italy, Canada and Turkey [2-6]. In Africa, *M. elephantis* in humans was reported recently in Zambia [7]. In most reported cases, the infected humans were sick and had respiratory disease symptoms. Recently, a draft genome for *M. elephantis* was successfully sequenced [8]. In Zimbabwe, no previous studies have been reported on the identification of *M. elephantis* in animals or humans. The objective of this study was to report the isolation of *M. elephantis* from cow dung and human sputum samples in Zimbabwe.

Methods

In 2014, we began isolating Mycobacteria from cow dung samples collected throughout Zimbabwe [9]. During the same time, there was also a national Tuberculosis (TB) survey in Zimbabwe in which 961 nontuberculous *Mycobacterium* isolates were made. DNA mycobacterial isolates was isolated as previously described [9]. The 5'-end hypervariable region of the 16S ribosomal RNA gene of *Mycobacterium* was amplified using polymerase chain reaction from the extracted DNA as previously described [9]. The *Mycobacterium*-specific forward primer, 5'-CCT GCA CTT CGG GAT AAG CCT G -3', and reverse primer, 5'-CAA CGC GAC AAA CCA CCT ACG A -5' were used in the polymerase chain reaction. The following cycling program was used for amplification: initial denaturation of 5 minutes at 95°C followed by 35 amplification cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 45 seconds at 72°C, and ending with a final extension step of 7 minutes at 72°C. The amplicons were analyzed by 2% agarose gel electrophoresis. DNA Sequencing of 26 amplicons from cow dung isolates and 81 amplicons from human sputum isolates was performed at Inqaba Biotech (South Africa) using standard protocols. Sequence data were analyzed using Geneious Basic program (Biomatters, USA) and Basic Local Alignment Search Tool (BLAST) programs from the internet. *Mycobacterium* species were identified using these software programs by comparison of the alignment with known sequences in the Genbanks.

Results

From cow dung samples, 26 isolates of Mycobacteria were identified. Out of these, 1 sample was identified as *Mycobacterium elephantis*. From the 81 *Mycobacterium* isolates from human sputum, 2 were identified as *Mycobacterium elephantis.* The ribosomal DNA sequence **(Figure 1)** of the three isolates was 100% identical. The sequence was also 100% identical to 6 *M. elephantis* sequences from Genbanks (Accession numbers

FJ497247, AF385898, GQ924944, GU142921, NR_025296, AJ536100) **(Figure 2)**. Sequence with Accession number FJ497247 is from the original *M. elephantis* isolate from the infected Sri Lanka elephant. AF385898 isolate was from human sputum sample in Canada, GQ924944 isolate was from human sample in Greece and GU142921 from the first human isolate in Asia. AJ536100 was isolated from human sample in Italy and the sequence differed by others by only one nucleotide base. This sequence was identical with two sequences of *M. pulveris*, a vey close relative *M. elephantis* **(Figure 2)**.

Discussion

Molecular identification of microorganisms is now becoming an important component in the diagnosis and treatment of infectious diseases such as tuberculosis and non-tuberculosis. Amplification by polymerase chain reaction and sequencing of 16S ribosomal RNA gene is now being used in the rapid identification of bacterial microorganisms [10]. In this study, we employed 16S ribosequencing in identifying *Mycobacterium* species in cattle and humans. From the study, three cases of *M. elephantis* were discovered. This is the first report on the isolation of *M. elephantis* species in cow dung and humans in Zimbabwe. NTM such as *M. elephantis* have clinical importance to low-resource countries such as Zimbabwe. These bacteria which are found in diverse environmental sources such as natural and municipal waters, soil, birds and animals can potentially cause opportunistic infections in humans especially those with underlying medical conditions such as HIV/AIDS [11,6]. The source of human infection by *M. elephantis* in Zimbabwe is still not clear. If the pathogen is present in Zimbabwean elephants, which are many, it can easily move to humans through zoonotic transmission. There is an overlap of elephant, cattle and human ecology in Zimbabwe. Cattle can get infected from elephants and they can potentially transmit the pathogen to humans. In Zimbabwe, humans also live in close proximity with animals and they can easily get infected. The consumption of animal products such as milk and meat can be another potential source of infection. Therefore further studies are necessary to establish the true prevalence and clinical relevance of *M. elephantis* in Zimbabwe.

Conflicts of interest

The authors declare that there is no conflict of interests.

Acknowledgements

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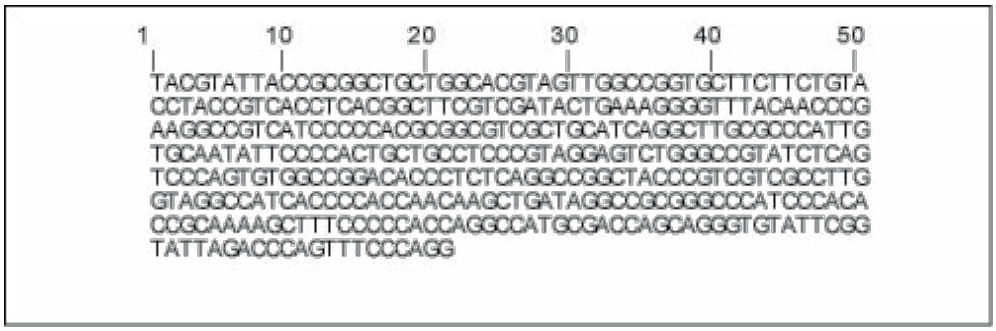

Figure 1 Nucleotide sequence of 16S rRNA gene of 3 *Mycobacterium elephantis* isolates discovered in Zimbabwe. Only the 5'-end of the 16S rRNA gene that is hypervarible across *Mycobacterium* species was amplified, sequenced and analyzed.

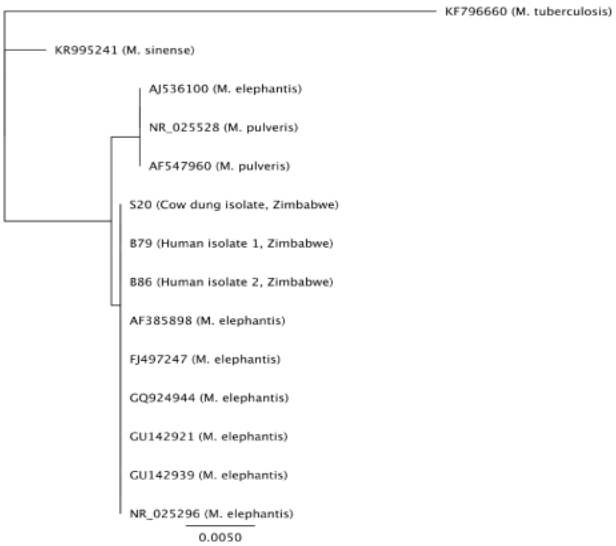

Figure 2 A 16S rRNA gene-based phylogenetic tree of three *Mycobacterium elephantis* isolates from Zimbabwe compared with other isolates found elsewhere around the world. *M. pulveris* and *M. sinense* were used for comparison. The tree was rooted with *M. tuberculosis* sequence (Accession number KF796660).

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