TITLE: GENOTYPING *Mycobacterium* spp. ISOLATES FROM WILD BOARS THROUGH *hsp65* PARTIAL GENE SEQUENCING

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ABSTRACT:

Bovine tuberculosis caused mainly by Mycobacterium bovis, is a zoonosis affecting domestic animals and some species of free living animals. Suidae members are susceptible to mycobacteria belonging to Mycobacterium tuberculosis (MTC) and Mycobacterium avium (MAC) complexes. One of the methods for identifying these bacteria is the genotyping though *hsp65* gene sequencing; this gene encodes a highly conserved constitutive protein, however presenting hypervariable regions. This work aimed to genotyping the hsp65 gene from Mycobacterium spp. isolated from wild boars' tissues from Rio Grande do Sul. Thirty five isolates retrieved from 22 wild boars were evaluated. All the isolates were identified as MTC or MAC through real-time polymerase chain reaction (PCR) or conventional PCR. Of the 35 isolates, 18 were genotyped by hsp65 gene sequencing. After analysis through the Basic Local Aligment Sequence Tool (BLAST), of National Center for Biotechnology Information (NCBI), 7 of the sequences showed similarity to MTC (38.9%) and 11 with MAC (61.1%). Importantly, most of the sequences (n = 14; 77.8%) had not 100% identity with reference sequences. Moreover, sequences were aligned with Multiple Sequence Alignment (MAFFT) method for phylogenetic analysis, which was performed in Geneious software using the HKY85 model (Hasegawa-Kishino-Yano, 85). At this analysis, some samples also clustered separately from the reference ones. These results indicate that there is genetic variability among isolated mycobacteria and suggest that there are different sources of infection for wild boars.

Keywords: Tuberculosis, *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex, *hsp65* genotyping.

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