

TITLE: INVESTIGATION OF THE SPREAD OF BOVINE TUBERCULOSIS IN SOUTHERN BRAZIL BY WHOLE-GENOME SEQUENCING

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ABSTRACT:

Mycobacterium bovis is the causal agent of bovine tuberculosis, one of the most important diseases currently facing the cattle industry worldwide. Tracing the source of *M. bovis* infections that result from movement of livestock is an important tool to understand the epidemiology of bovine tuberculosis (bTB) and defining control/eradication strategies. Whole genome sequencing (WGS) provides a higher resolution than other established typing methods and greatly improves the definition of the regional localization of *M. bovis* types. Cultures of *M. bovis* were isolated from 58 bovine granulomatous tissue using conventional methods (Stonebrink medium) from eight dairy farms of the State of Rio Grande do Sul, Southern Brazil. The isolates were sequenced using both Illumina technologies NextSeq 500 System and HiSeqX System. Raw genomic data were trimmed using the TRIMMOMATIC-v0.32 and the quality control of the samples was performed using kraken/bracken. Sequence reads were mapped to the *Mycobacterium bovis* AF2122/97 reference genome (NC0002945) using Burrows-Wheeler Aligner (BWA) (minimum and maximum insert sizes of 50 and 1000 bases, respectively). Single nucleotide polymorphisms (SNPs) were processed with SAMtools mpileup and BCFtools (minimum base call quality of 50 and minimum root squared mapping quality of 30). Variant sites in the alignment were extracted using snp-sites and a maximum likelihood phylogenetic tree was constructed using FastTree2 (nucleotide general time-reversible tree). The resulting tree was annotated and rooted using iTOL. Applying WGS, the role of cattle movements (in two farms) or spatial proximity in transmission (in six farms) was suggested. Nevertheless, further molecular epidemiological analysis such as Bayesian phylogeographic approaches will be necessary to quantify the spread of *M. bovis* across the landscape.

Keywords: *Mycobacterium bovis*, whole genome sequencing, bovine

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