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Implementing the MALDI-TOF library to predict taxonomy of non-tuberculous mycobacteria: a comparison between spectrometry and sequencing on animal isolates

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Introduction
Non-tuberculous mycobacteria (NTM) are a group of bacteria that belong to the genus Mycobacterium and encompass more than 170 species other than those belonging to the *Mycobacterium tuberculosis complex* and *Mycobacterium leprae*. They can be found in a wide range of environments and can cause, among others, lymphnode granulomatous lesions in various animals and human, which can interfere with the diagnosis of tuberculosis.
NTM are difficult to identify by biochemical methods, due to their microbiological properties and only genetic probe assays and sequencing can provide a reliable taxonomic identification. Recently, Lorente-Leal et al. [1] suggested that MALDI-TOF MS can be used as a promising tool to overcome the expensiveness and the long-timescales of molecular methods but, unfortunately, to date, the available databases are still limited and are not able to predict the taxonomy of all NTM.
It is therefore advisable the implementation and the refinement of the MALDI-TOF MS analysis for the screening of NTM. The aim of this study is to build a database for MALDI-TOF analysis for a reliable and a fast identification of NTM in veterinary laboratories.

Materials and methods
Tonsils, head, chest, abdominal lymphnodes and nasal swabs from 731 wild boars and 112 free-roaming pigs were analysed for the presence of mycobacteria. They were collected from various Italian regions from 2016 to 2021, within the routine diagnostic surveillance on hunted/wild animals.
NTM were isolated from 78 wild boars, and 24 black-pigs and they were classified into rapid growing (RGM) (< 7 days to form colonies) and slow growing mycobacteria (SGM) (? 7 days) [2].
All NTM isolates were submitted to 16s rDNA sequencing using Microseq 500 method (Applied Biosystems) [3]. Sequencing of *rpoB* and *hsp65* genes was performed on RGM and SGM, respectively. All DNA sequences were manually curated and compared against the NCBI nucleotide database, using BLAST for taxonomy identification. All isolates were submitted to MALDI-TOF MS analysis using the Vitek MS instrument (Biomerieux).

Results
Out of 102 NTM, 98 were classified as SGM and 4 as RGM. By sequencing, we identified 32 *M. colombiense*, 15 *M. nonchromogenicum*, 13 *M. avium subsp. hominissuis*, 9 *M. timonense*, 7 *M. intracellulare*, 4 *M. fortuitum* and *M. terrae*, 2 *M. engbaekii*, *M. lentiflavum*, and *M. triplex* and 12 other different mycobacteria species present in only one isolate.
MALDI-TOF analysis based on the existing database was able to identify 21 samples: 14 *M. avium* (confidence of 85-99.9%), 2 *M. arupense/non-chromogenicum* (78.2%), 2 *M. furtuitum complex* (92.4-99.9%) and 2 *M. chimaera*. For 81 isolates, spectrometry analysis was unable to determine the species and their spectra were acquired and added to the existing database. The samples were reanalyzed by MALDI-TOF and the results obtained showed a good correlations with those obtained by sequencing.

Conclusions
Our study expands the utility of MALDI-TOF MS in the identification of NTMs in animals by providing a reliable database for discriminating NTM species in routine veterinary practice.