P115

Non-tuberculous mycobacteria isolated at the end of the world

X Ferrara Muñiz[1], C Tortone[2], S Oriani[2], C Garro[3], S Garbaccio[3], P Farace[1], M Encinas[1], ME Eirin[1], MJ Zumárraga[1]

[1]Instituto de Agrobiotecnología y Biología Molecular (IABIMO, INTA-CONICET), Hurlingham, Buenos Aires, Argentina.
[2]Facultad de Veterinaria, Universidad Nacional de La Pampa, La Pampa, Argentina.
[3]Instituto de Patobiología Veterinaria (IPVeT, INTA-CONICET), Hurlingham, Buenos Aires, Argentina.

Introduction

Non-tuberculous mycobacteria (NTM) are a group of mycobacteria widely distributed in the environment (e.g. soil and water). NTM can also cause infections in different mammals, mostly in immunocompromised individuals. Moreover, they may interfere with the ante-mortem diagnosis of bovine tuberculosis (BTB) by the tuberculin skin test. In this work, two isolates of NTM species were obtained from soil of Tierra del Fuego, the only province free of BTB and brucellosis in Argentina.

Objective

Isolate and identify non-tuberculous mycobacteria from soil from Tierra del Fuego province.

Materials and Methods

Samples: Two soil samples were taken from a dairy farm in Río Grande, Tierra del Fuego, with in the framework of a bovine blood sampling to perform the Interferon Gamma Release Assay (IGRA).

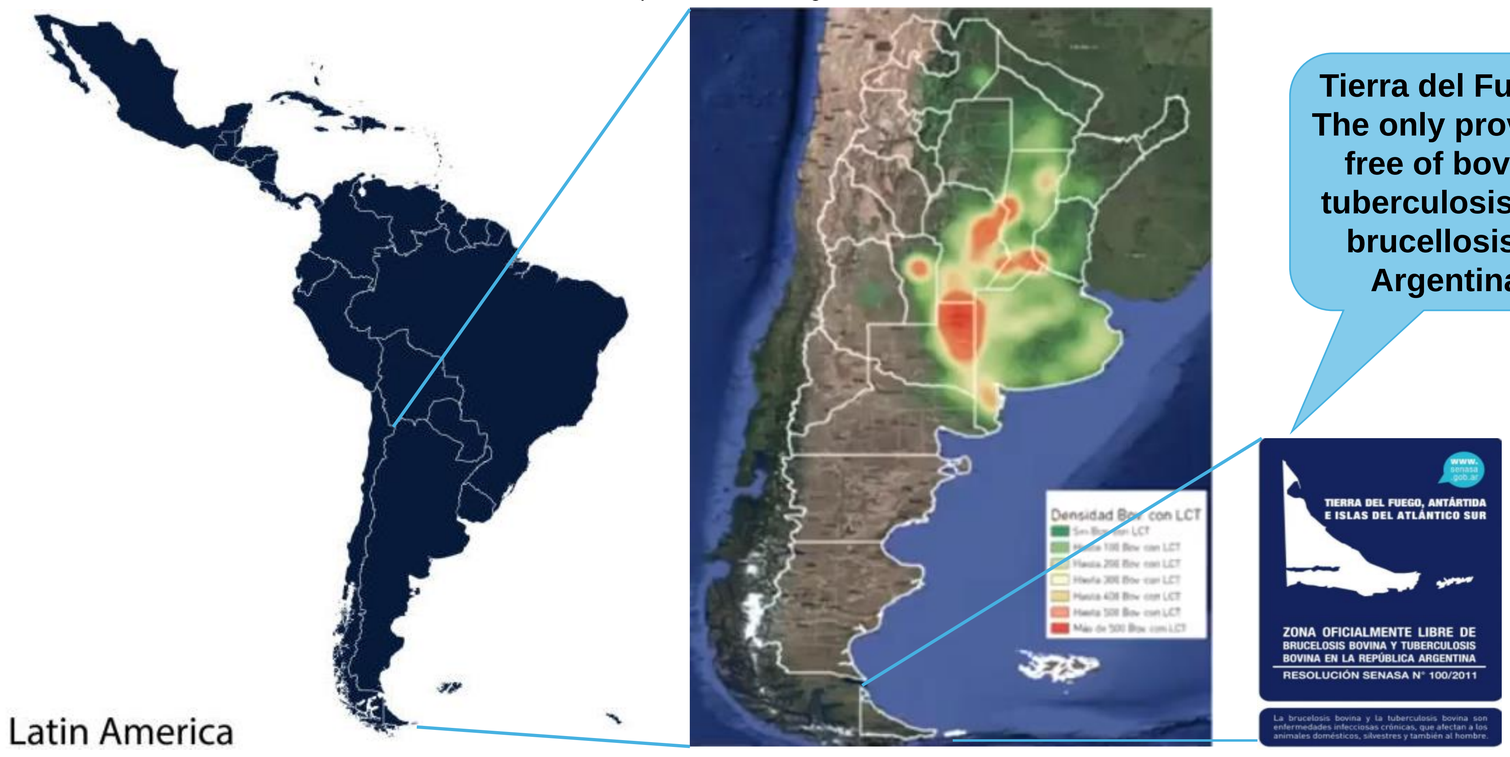
Culture: Soil samples were processed using the protocol described by Livanainen *et al*., (1997), and then inoculated in Löwenstein–Jensen and Stonebrink media.

Molecular typing: One loopful of bacterial growth was suspended in 200 µL of sterile apyrogenic water contained in a 1.5 mL capped tube and then incubated at 95°C in a thermoblock for 40 min. It was then centrifuged 5 min at 12,000 rpm. Ten microliters of the obtained bacterial lysate was used for PCR of the heat shock protein (*hsp*65) (Telenti *et al*., 1993), the 16S subunit of the ribosomal RNA (16S rRNA) (Kirschner *et al*., 1998) and the β-subunit of the RNA polymerase (*rpo*B) (Adekambi *et al*., 2003) genes. PCR products were purified and after that were sent for gene sequencing. The criterion for species identification was in concordance with the identity of at least two of these genes after BLAST comparison. The sequences have not been deposited in GeneBank. In order to assign species, ≥99.7% similarity to type strain sequences was required for 16S rRNA, ≥97% for *rpo*B and ≥99% for *hsp*65 according Monteserín *et al*., (2016) and the consensus of the identity was provided by at least two of the three genes amplified.

The presence of *esx*A (ESAT-6)*, esx*B (CFP-10) and *mb3645c* (EspC) genes was investigated by PCR according to Encinas *et al*., (2018).

Prevalence of bovine tuberculosis in Argentina: Detection of tuberculous lesions in slaughterhouses 0.27 % (2019)

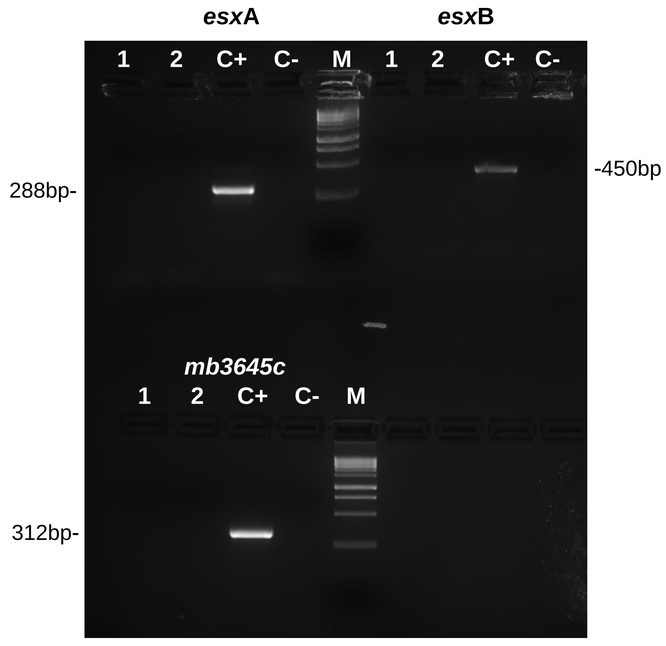
(SENASA, 2020)
https://www.aavld.org.ar/ciclo-micobacterias-de-interes-veterinario/



Tierra del Fuego: The only province free of bovine tuberculosis and brucellosis in Argentina.

Results

- *Mycolicibacterium diernhoferi* and *Mycolicibacterium novocastrense* were identified in the two isolates.
- None of *esx*A, *esx*B and *mb3645c* genes were detected in either *M. diernhoferi* or *M. novocastrense.*



1- *M. diernhoferi*; 2- *M. novocastrense*; C+: *M. bovis* wild type; C-: Negative control; M: 1Kb DNA Ladder Promega.

Discussion and Conclusions

- This work was preliminary. It was done with in the framework of a bovine blood sampling to perform IGRA. An experimental design to carry out a new soil sampling is required.
- Knowledge of the presence and distribution of NTM in the environment and the evaluation of the specificity of the genes used in the diagnosis of BTB can contribute to better control of the disease, especially in areas declared free of the disease.
- This is the first report of *M. diernhoferi* in Argentina.
- Whole genome sequencing of these strains will be performed to compare them with the reference genome of *M. bovis* AF2122/97 to detect antimicrobial resistance, virulence genes and other genes related to BTB diagnosis.

Funding

- PICT 2018-3784 / PICT-2021-1037.
- INTA Project I103.

References

1. Adekambi *et al*., 2003. *rpo*B-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. doi.org/10.1128/jcm.41.12.5699-5708.2003.
2. Encinas *et al.,* 2018. *Mycobacterium bovis* ESAT-6, CFP-10 and EspC antigens show high conservation among field isolates doi: 10.1016/j.tube.2018.06.007.
3. Kirschner *et al*., 1998. Species identification of mycobacteria using rDNA sequencing. doi.org/10.1385/0- 89603-471-2:349.
4. Livanainen *et al*., 1997. Comparison of some decontamination methods and growth media for isolation of mycobacteria from northern brook Waters. doi.org/10.1111/j.1365-2672.1997.tb03306.x
5. Monteserin *et al*., 2016. Combined approach to the identification of clinically infrequent non-tuberculous mycobacteria in Argentina. doi: 10.5588/ijtld.16.0122.
6. Telenti A *et al*., 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. doi.org/10.1128/jcm.31.2.175-178.