P050

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Comparative study of two deleted *Mycobacterium bovis* strains in experimental animal models

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Introduction

Bovine tuberculosis (bTB) constitutes a problem for livestock and Public Health due to its negative impact on farms and its zoonotic nature. In Argentina, the application of the National Control and Eradication Program reduced bTB prevalence. Due to its complexity, the disease continues to be a challenge. Vaccination, as a complementary control tool, could reduce its impact on cattle.

Currently, there is no commercial vaccine approved for its use in bovines. Two attenuated strains, *M. bovis Δmce2* and *M. bovis Δmce2-phoP*[1,2] induced protection against a virulent *Mycobacterium bovis* (*M. bovis*) strain, during a challenge in cattle[3] and the murine model[4], respectively.

The objective of this work was to evaluate the virulence and safety of *M. bovis Δmce2* and *M. bovis Δmce2-phoP* in mice and guinea pigs, to compare how these two strains behave in both animal models .

Ethical approval

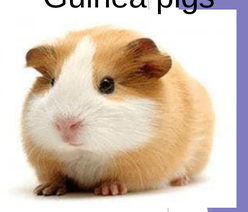
The trials were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL), Faculty of Veterinary Sciences, University of Buenos Aires (protocols N º 2018/36-2021/33).

Materials and Methods

Attenuated strains: *M. bovis* Δ*mce2*, *M. bovis* Δ*mce2-phoP* — 1 x 10^5 CFU

Controls: *M. bovis* NCTC10772 (1 x 10^5 CFU), *M. bovis* 04-303. PBS (1 x 10^4 CFU)

Animal models: Mice, Guinea pigs



Necropsy: 60 and 90 dpi* ; 178 dpi*

*dpi: days post inoculation

Parameters evaluated:

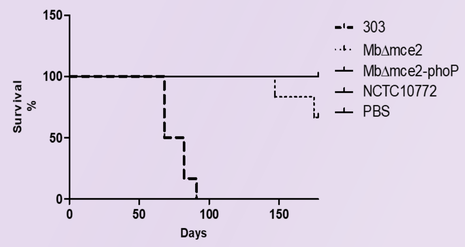
- Survival (days).
- Pathology (granulomas).
- Bacteriology.
- Tissue-IS*6110*-PCR (lung, liver, lymph nodes and spleen).

Results

Survival

100 % survival of all the animals of the different groups at 60 and 90 dpi was observed.

Figure 1. Kaplan-Meier survival plot.



Kaplan-Meier survival plot. After 90 dpi, total animals inoculated with the *M. bovis* 04-303 strain died (bold line). In the case of the group inoculated with the *M. bovis Δmce2* strain, 33% of the guinea pigs died before 176 dpi (light line). In the rest of the groups, 100% survival was observed until the end of the trial (black line).

Pathology

Absence of macroscopic lesions in all of the animals of the different groups at 60 and 90 dpi was recorded.

Figure 2. Enlarged lymph node (LN) of a guinea pig inoculated with *M. bovis Δmce2*.

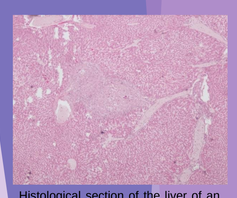
Sagittal section of the lymph node. Note the caseous content inside.

Figure 3. Liver of a guinea pig inoculated with *M. bovis Δmce2-phoP*.

Enlarged liver without lesions compatible with tuberculosis.

Figure 4. Number of guinea pigs with LCT (A) and positive histopathology (B) in the different groups.

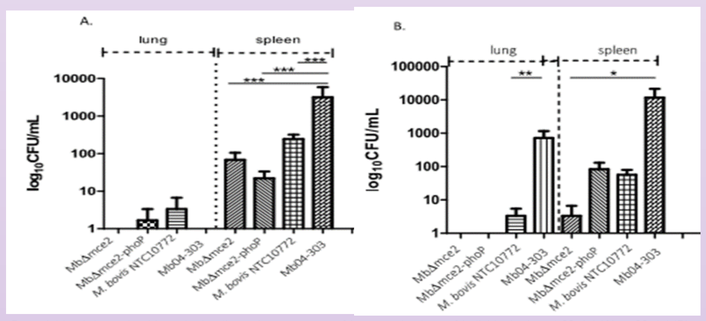
The groups inoculated with the *M. bovis Δmce2-phoP* strain and with PBS, did not present macro and microscopic lesions.



Histological section of the liver of an animal inoculated with the *M. bovis Δmce2* strain stained with Hematoxylin-Eosin.

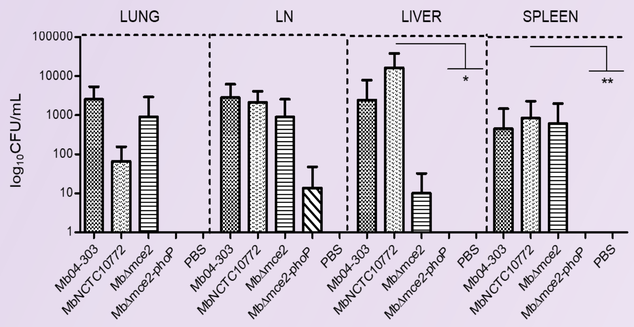
Bacteriology

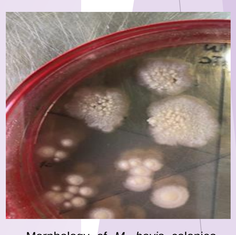
Figure 5. Organ bacillary loads in BALB/c mice inoculated subcutaneously with hypervirulent *Mb*04-303 strain, *Mb* NCTC10772 parental vaccine candidates and the experimental vaccine candidates *MbΔmce2* and *MbΔmce2-phoP.*



Bacterial load determined by culturing tissue homogenates of lungs and spleen of individual mice at 60 dpi (A) or 90 dpi (B). The bars represent the mean CFU value obtained for each group studied. Significant outliers were excluded and then a statistical analysis of CFU was performed using the One-Way Anova and Bonferroni post-test (*p<0.05, **p<0.01 and ***p<0.001). Values considered as outliers (p < 0.05) (Grubbs' test, GraphPad) were excluded from the Oneway ANOVA analysis.

Figure 6, . Bacteriological isolation of lung, lymph node (LN), liver and spleen in guinea pigs inoculated with *M. bovis* 04-303, *M. bovis* NCTC10772, *M. bovis Δmce2*, *M. bovis Δmce2-phoP* and PBS.





Morphology of *M. bovis* colonies growing on 7H10 media supplemented with pyruvate.

Bacteriological isolation of lung, lymph node (LN), liver and spleen in guinea pigs inoculated SC with *M. bovis* 04-303, *M. bovis* NCTC10772, *M. bovis Δmce2*, *M. bovis Δmce2-phoP* and PBS. Bacillary load determined by culture of homogenized tissues of the different organs analyzed. The bars represent the CFU values obtained for each group studied. Kruskal-Wallis non-parametric test, Dunn's post test. *, p < 0.05

Tissue-IS*6110*-PCR

Figure 7. Proportion of animals in the different groups with detection of genomic DNA In lung and spleen

	M. bovis Δmce2		*M. bovis Δmce2-phoP*		*M. bovis* NCTC10772		*M. bovis* 04-303	
	Lung (n/N) *	Spleen (n/N)	Lung (n/N)	Spleen (n/N)	Lung (n/N)	Spleen (n/N)	Lung (n/N)	Spleen (n/N)
60 dpi	4/6	1/6	4/6	0/6	6/6	0/6	2/3	0/3
90 dpi	3/6	3/6	0/6	2/6	2/6	0/6	0/3	2/3

*n/N: proportion of animals with the presence of genomic DNA in lung and spleen at 60 and 90 dpi and the total number of mice included in each group

Figure 8. Number of guinea pigs with positive DNA detection by PCR-IS*6110*.

The group inoculated with the *M. bovis Δmce2-phoP* strain increased the number of positive animals. This technique has allowed us to improve diagnostic sensitivity.

Conclusions

A higher attenuation degree of *M. bovis* Δ*mce2-phoP* compared to *M. bovis* Δ*mce2* was confirmed, especially evident in a highly susceptible model such as guinea pigs, even when these strains were inoculated subcutaneously.

References

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