EVALUATION OF PROTECTION INDUCED BY DIFFERENT NOVEL *Brucella* SPECIES SHOWING DIFFERENT ELIMINATION PATTERNS AGAINST *B. melitensis* IN A MURINE MODEL OF INFECTION

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Brucella spp are a diverse group of gram negative pathogens causing disease in many hosts including humans. Vaccination against animal brucellosis is an important control strategy to prevent the disease. Research for novel vaccines has focused upon the development of live vaccine strains[1]. Based on their different clearance pattern in mice, some of the new Brucella species described lately[2,3], were selected for being potential candidate vaccines against brucellosis. THE AIM OF THIS WORK is to evaluate the efficacy induced by *B. microti*, *B. ceti* and *B. pinnipedialis*, against *B. melitensis*, in a murine model of infection.

MATERIALS AND METHODS

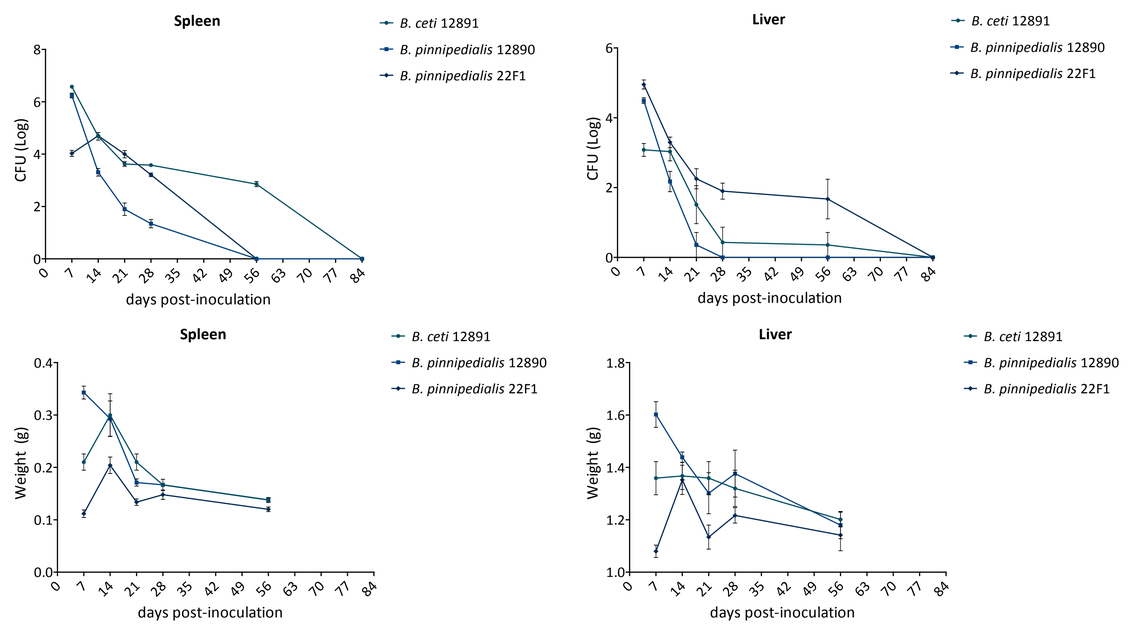
Kinetics of infection. Female balb/c mice were intraperitonelly (ip) inoculated with 10^4 colony forming units (cfu) of *B. microti* or 10^5 cfu of the following strains: *B. ceti* 12891, *B. pinnipedialis* 12890, and *B. pinnipedialis* 22F1. At selected times post inoculation animals were euthanized and their spleen and liver removed to determine cfu/organ (Figure 1).

***In vivo protection* studies**. In parallel, mice were vaccinated with the same strains and doses using the standard vaccine Rev 1 inoculated ip or subcutaneously (sc) as vaccine control. A lot remained unvaccinated. Half of the vaccinated animals were ip challenged 4 weeks later with 10^5 cfu of *B. melitensis* 16M ~~wild type~~ and the other half were similarly challenged 22 weeks later. One week after the challenge, spleens were aseptically removed and weighed to determine the number of cfu/organ (Figure 3).

***In vitro* studies**. Correlates of protective immunity were evaluated via splenocyte cytokine memory responses at 4 and 22 weeks. Splenocytes isolated from vaccinated mice were stimulated with Heat Killed *B. melitensis* for 72 h and IFN-γ and TNF-α production were measured (Figure 2).

Data are represented as Mean±SEM. * p<0.05, **p<0.01, ***p<0.001.

Figure 1. Number of *Brucella* and weight of **spleen** (left) and **liver** (right) of mice inoculated ip with 10^5 cfu of *B. ceti* 12891, *B. pinnipedialis* 12890 and *B. pinnipedialis* 22F1 or 10^4 cfu *B. microti* and analysed at 7, 14, 21, 28, 56 and 84 days post-infection. (Mean±SD). **p<0.01, ***p<0.001.



RESULTS

The used strains showed different multiplication patterns (Figure 1). All the new strains of Brucella tested induced protection against *B. melitensis* 16M at 4 and 22 weeks after vaccination independently on the clearance pattern (Figure 2). It was a correlation between protection induced and cytokine production at both time points (Figure 3).

Figure 2. Concentration of **IFN-γ** and **TNF-α** produced by splenocytes isolated from mice inoculated ip with 10^5 cfu of *B. ceti* 12891, *B. pinnipedialis* 12890 and *B. pinnipedialis* 22F1 or 10^4 cfu *B. microti* for 4 (left) or 22 (right) weeks and stimulated with Heat Killed *B. melitensis* for 72 h. (Mean±SD). **p<0.005, ***p<0.001.

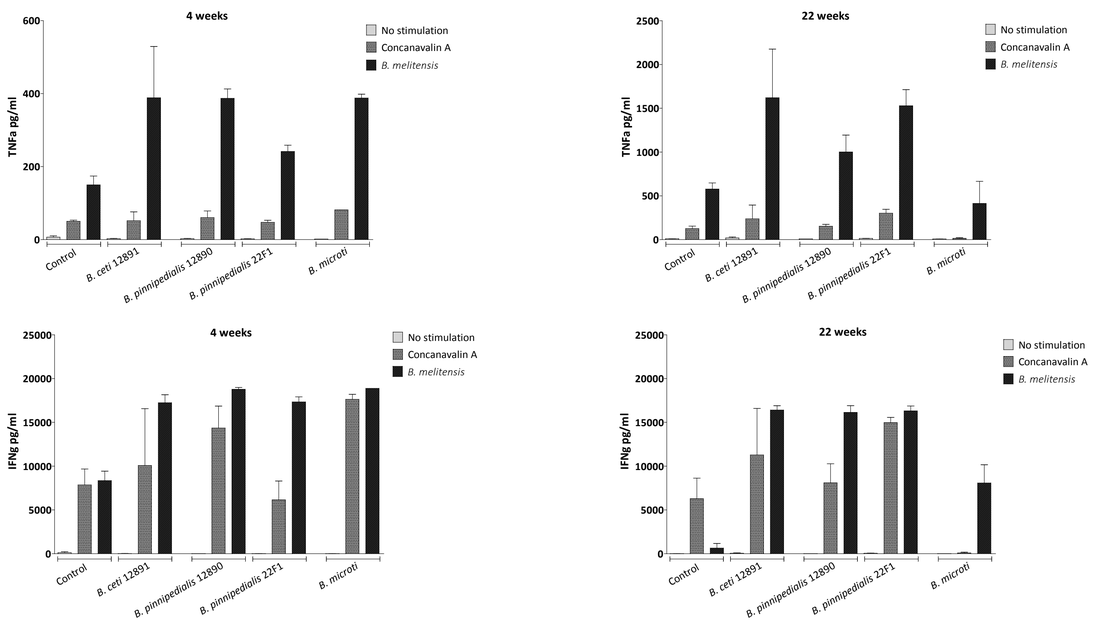
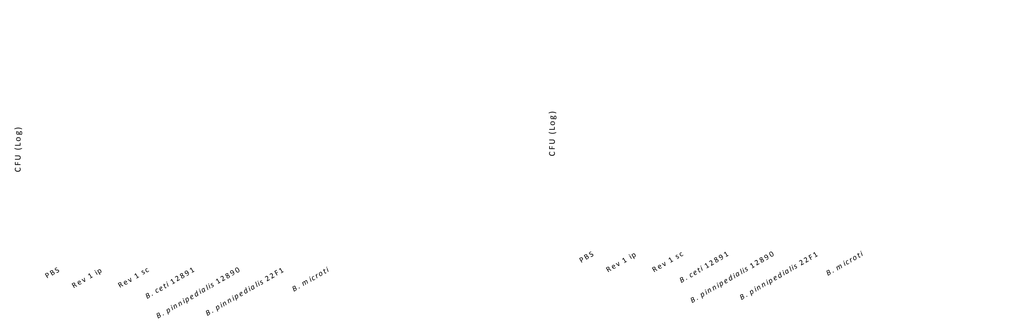


Figure 3. Number of *Brucella* per **spleen** of mice inoculated ip with 10^5 cfu of *B. ceti* 12891, *B. pinnipedialis* 12890 and *B. pinnipedialis* 22F1 or 10^4 cfu *B. microti* for 4 (left) or 22 (right) weeks and challenged ip with 10^5 cfu of *B. melitensis*, analysed at 7 days post-infection. (Mean±SD). **p<0.005, ***p<0.001.



CONCLUSION

The new species of Brucella tested could be considered as potential candidates vaccines against *B. melitensis*.
Moreover, cytokine production reflected a memory response with subsequent protection against challenge.

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