 

# DNA prime/protein boost regime and CTLA-4 mediated targeting as strategies to improve the potency of DNA vaccine encoding the phosphoglycerate kinase of *Fasciola hepatica* in sheep

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The use of genetic vaccines has a great potential. However, despite the promise shown in laboratory animals, DNA vaccination in large animals has been often associated with poor immunogenicity. To overcome the problem few approaches have been evaluated to enhance the DNA vaccine efficacy in target species. Previously, we have tested the efficacy of a FhPGK delivered as a DNA vaccine in Merino sheep. We have observed neither reduction in worm burden nor any differences in the course of immune responses following vaccination and liver fluke infection between vaccinated and control animals. Here, we tested a cDNA encoding a phosphoglycerate kinase from *Fasciola hepatica* (cDNA-FhPGK/pCMV) as a vaccine against ovine fasciolosis and investigated whether DNA prime/protein boost regime or CTLA-4 (cytotoxic lymphocyte antigen 4) mediated targeting can improve DNA vaccine efficacy.

We did not observe significant reductions in worm burdens and the level of liver damage was comparable between groups. Cellular responses induced by DNA primed/protein boosted or cDNA-FhPGK-CTLA-4/pCMV vaccinated sheep were not significantly different from those generated by respective control groups. However, an analysis of specific antibody responses in DNA primed/protein boosted sheep revealed significantly increased IgG1 titers when compared with the control group, while CTLA-4 targeting failed to enhance cellular responses in vaccinated animals. Moreover, humoral responses in sheep vaccinated according to the prime/boost regime were also different from those generated previously by cDNA-FhPGK/pCMV non-targeted vaccination. Nevertheless, increased titers of specific IgG1 did not contribute to the protection against the infection since no differences in liver fluke recoveries were reported.

Further research aimed at designing new vaccination regimens and identification of effective vaccine antigens is needed to provide enhancement for DNA vaccination in large animal models. If DNA vaccines against fasciolosis in target species are to reach the market one day, reasons underlying the failure of genetic vaccination to promote adequate immune responses in large animals have to be elucidated.