Immunogenicity and protection of the *Theileria parva* CTL antigen Tp1, with or without a leader sequence, using HAd5/MVA prime-boost vaccination

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Summary: 4 groups of 5 BoLA-typed animals were immunized with the *T. parva* Tp1 antigen with or without leader sequence in the HAd5 viral vector and boosted with the same antigens in the MVA vector. Most animals generated CTL to the known epitope measured using tetramer staining, ELISpot and Cr-51-release assay. The CTL expressed perforin and lysed peptide pulsed PBMC. CD4 cells were shown to proliferate to the antigen. Challenge of the animals resulted in about 30% protection.

FIG 1. Immunization groups: 5 cattle in each group, primed with HAd5-Tp1 and boosted with MVA Tp1 with and without leader sequence:

GROUP 1. Tp1 + tPA* leader sequence (HAd5/MVA)
GROUP 2. Tp1 without leader sequence (HAd5/MVA)
GROUP 3. Tp1 + native leader sequence (HAd5/MVA)
GROUP 4. GFP + tPA* leader sequence (HAd5/MVA)

FIG 2. Regimen and sample points:

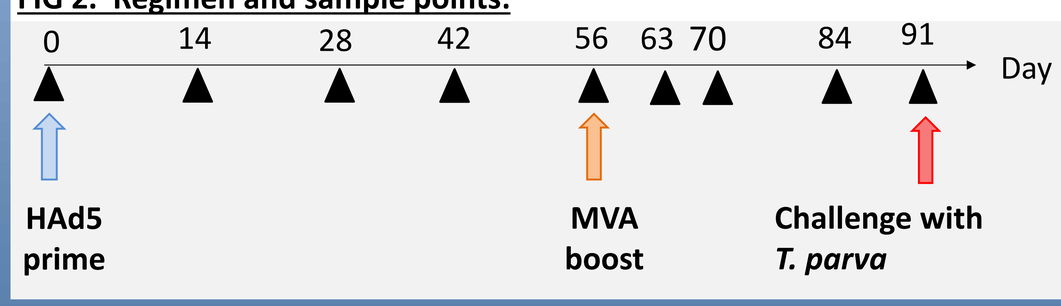


FIG 3. CTL are Tp1 specific (tetramer staining): Example of *ex vivo* tetramer staining of 2 animals, 2 weeks post MVA boost.

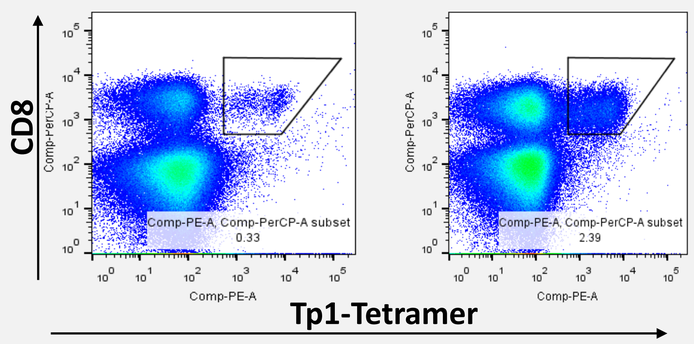


FIG 4. CD8 cells respond to the Tp1 epitope (IFN-γ ELISpot): SFU (per million CD8 cells) 7 days after MVA boost. First diagram shows the group average, the second shows the individual cattle.

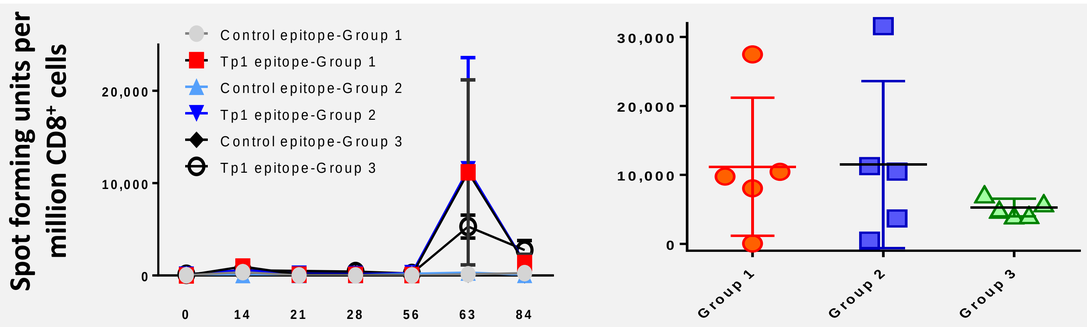


FIG 5. CD4 cells proliferate to the Tp1 antigen. $2x10^5$ CD4 cells were cultured with Tp1 full-length antigen, Tp1 pool (overlapping peptides), ovalbumin (control) and Tp2 pool (control peptides), 3H-thymidine was added, cells were harvested.

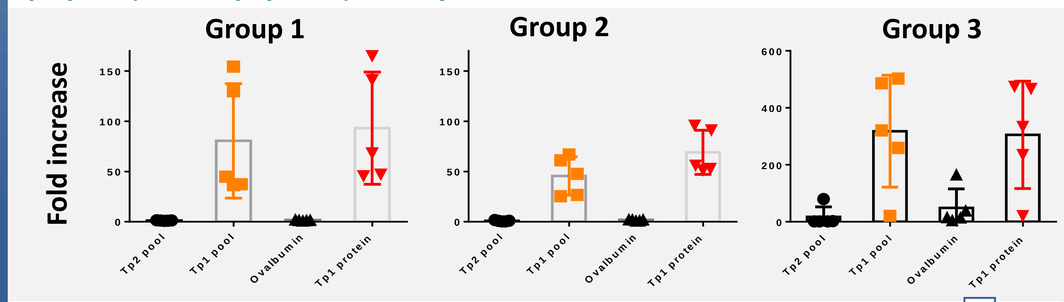


FIG 6. Tp1 specific CTL are cytotoxic and they express perforin: Upper panel: PBMC were restimulated 3 times using infected autologous cells, CD8 cells were purified and cytotoxicity were measured by pulsing autologous PBMC with the peptide or infected cell line (Cr-51 release). Lower panel: CD8 cells were costained with Tp1 tetramer and a perforin mAb.

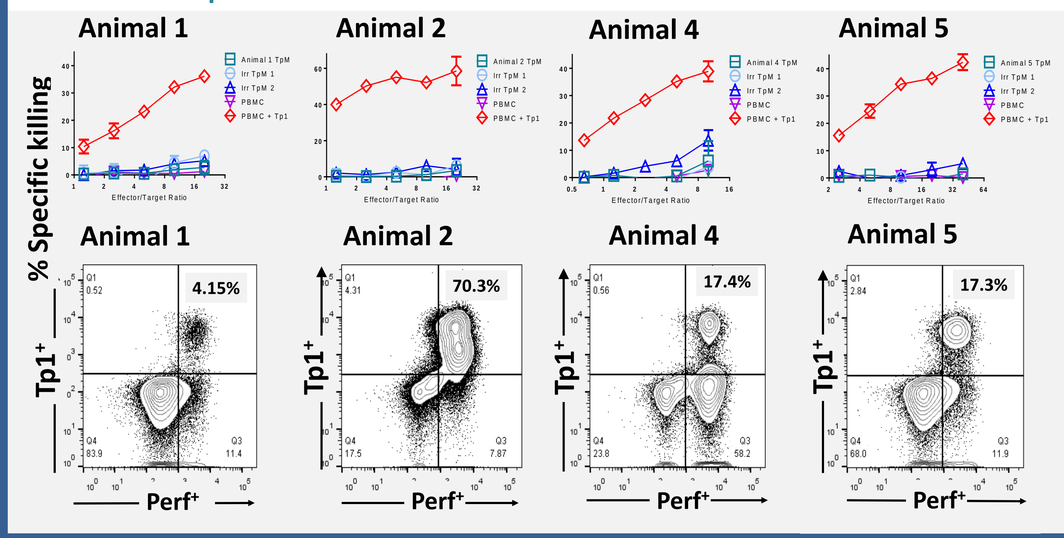
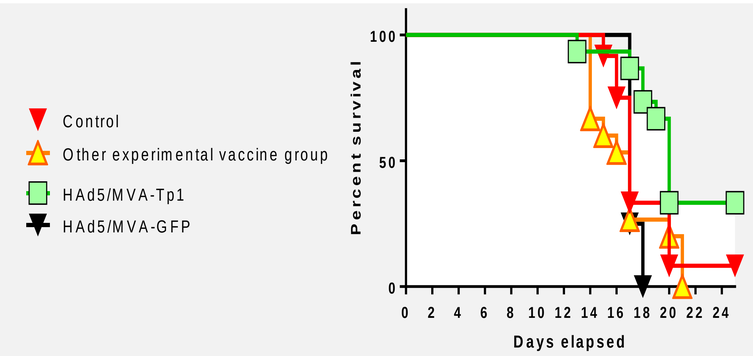


FIG 7. Protection by prime-boost regimen with Tp1 in HAd5/MVA vectors. Groups of cattle (Fig. 2) were challenged with a lethal dose of *T. parva* sporozoites. Kaplan-Meier survival plot is shown.



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