Evaluation of multiple tegument proteins and FhTLM as vaccines against *Fasciola hepatica* in cattle

T W Spithill La Trobe University, Australia; H Toet La Trobe University, Australia; V Rathinasamy La Trobe University, Australia; G Zerna La Trobe University, Australia; G Anderson Virbac (Australia) Pty Ltd, Australia; R Dempster Virbac (Australia) Pty Ltd, Australia; T Beddoe La Trobe University, Australia

Australian Government Australian Research Council

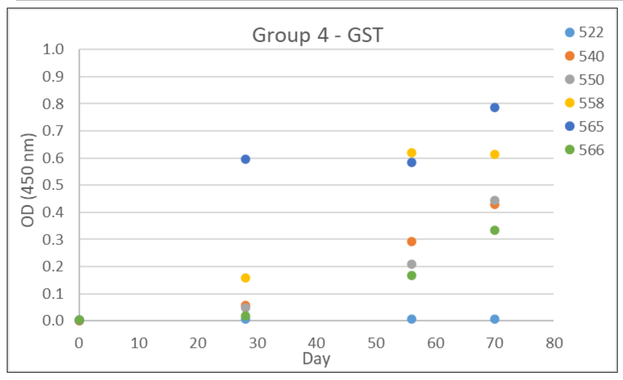
Background and Summary:

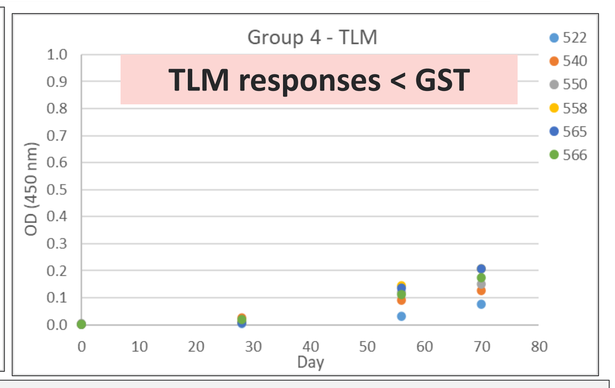
- Despite being a logical vaccine target, surface tegument proteins of *Fasciola hepatica* have not yet been tested (Spithill et al 2022).
- Nine surface-exposed (data not shown) recombinant tegument proteins from *F. hepatica* were evaluated as vaccines in cattle (Tetraspanins (TSP2, 3); Annexins (Anx2, 3, 8); novel Tegument proteins expressed in juvenile/immature flukes (Teg1, 5, 22, 25).
- Transforming growth factor beta (TGF-β)- like homologue protein (FhTLM), an immunomodulator (Sulaiman et al 2016), also tested.
- Tetraspanin 2 was evaluated intranasally fused to *E. coli* heat-labile entero-toxin B subunit LTB adjuvant (LTB-TSP2)(Zerna et al 2021).
- Native Glutathione *S*-transferases (GST) were tested in combination with TSP2, TSP3, Anx2, Anx3/Anx8 and FhTLM.
- No significant efficacy was observed with either TSP2, LTB-TSP2, TSP3 or Anx2 alone; nor with the Teg1/5, Teg22/25 combinations.
- Significant reductions in mean fluke numbers/group (38-48%) were observed in 4 vaccine groups with combinations of Anx2/TSP3, Anx3/Anx8 as well as with FhTLM/GST using 2 adjuvants.
- Three doses of FhTLM in Freund's adjuvant was superior (48% efficacy) to 2 doses (11% efficacy).
- However, there was variation between trials in vaccine efficacy with certain combination vaccines.

Methods: Five Proof of Concept trials: POC1-5

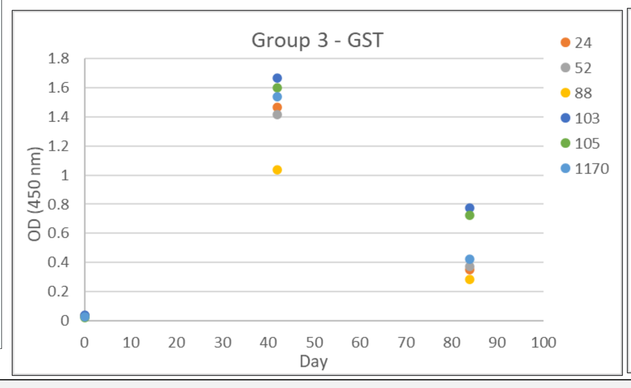
Cattle: female Angus/Angus cross cattle (n=6-7/group; age 6-18 months); Recombinant proteins: expressed in *Pichia*; purified by Ni-NTA chromatog.; Native GSTs: purified from flukes by Glutathione-agarose chromatography; Adjuvants: Freund's Complete/Incomplete adjuvant (FCA/FIA) (POC1-5); *E. coli* heat-labile entero-toxin B subunit LTB adjuvant (termed LTB) (POC3); novel Nanoparticle adjuvant (POC5); Doses: 2 doses of protein (0.1-0.4 mg/dose) POC1-5; 3 doses in POC4; Route: subcutaneous in neck (POC1-5); or intranasal with LTB in POC3; Challenge: 350-535 metacercariae; control mean fluke counts 72-111; Postmortem days 120-150: fluke burdens in liver/gall bladder/small intestine; ELISAs: Total IgG/IgG1 responses assessed by indirect ELISA

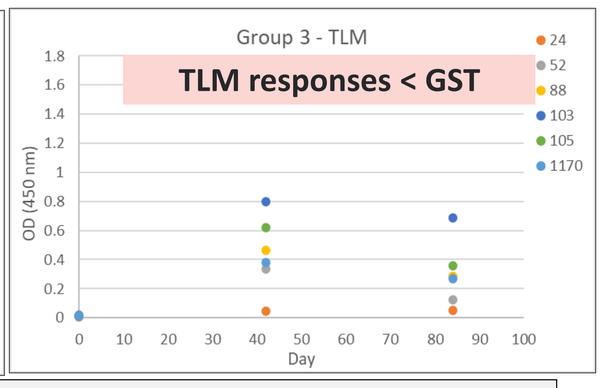
Total IgG responses POC4: GST/TLM/FCA/IFA; at 1/100





IgG1 responses POC5: GST/TLM/Nanoparticle adj.; at 1/200

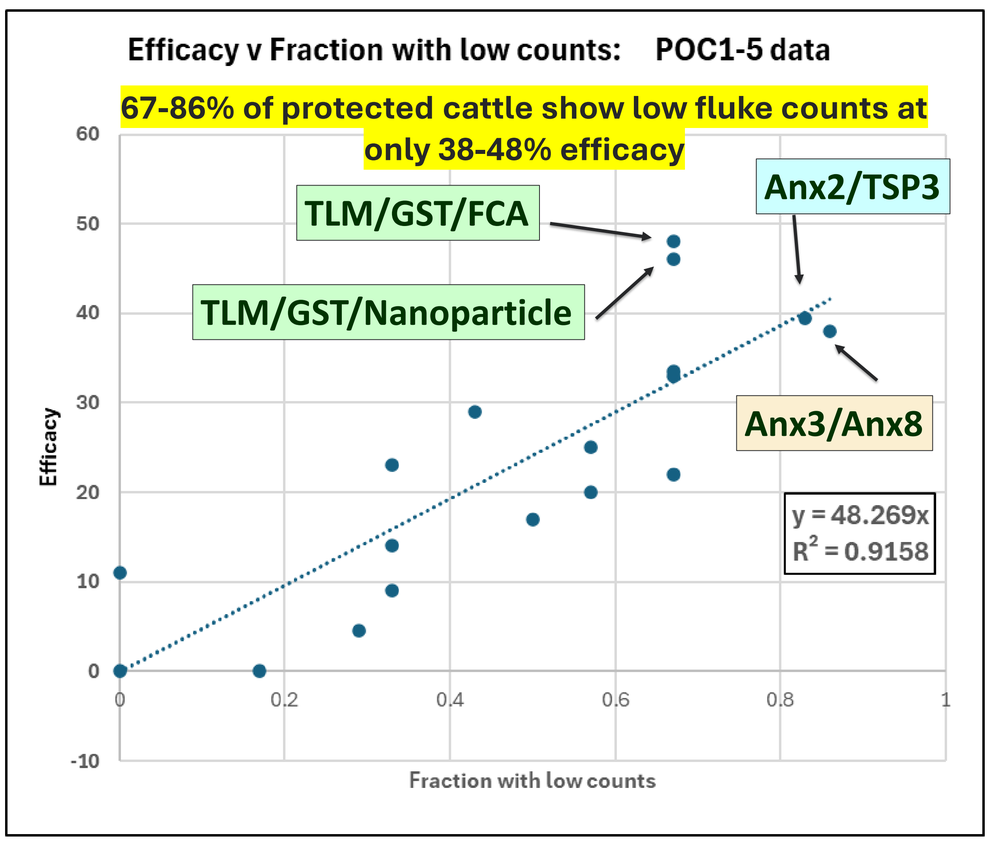




Summary of efficacy data over 5 trials: POC1-5

Antigens (2 doses except POC4)	POC1	POC2	POC3	POC4 3 doses	POC5	Comments
Anx2 + TSP3	39% (p=0.034)	20%				Sig. efficacy in POC1; Anx2 /TSP3 efficacy variable
Anx2 + TSP3 + TSP2		29%				No synergy between Anx2/TSP3 and TSP2
Anx2 (or TSP3) alone		25% (0%)				No efficacy with either antigen alone
Anx3 + Anx8		38% (p=0.026)				Sig. efficacy in POC2
Anx3 + Anx8 + Anx 2 + TSP2			14%			No synergy between these 4 antigens
TSP2		33%	0%			No efficacy
LTB-TSP2			17%			No efficacy with LTB-TSP2 fusion protein delivered intranasally
GST + TSP2			33%			No synergy with GST/TSP2
GST + Anx2				23%		No synergy with GST/Anx2
GST + Anx 3 + Anx 8				22%		No synergy with GST/Anx3/Anx8; low efficacy despite 3 doses of vaccine
GST+ TLM with FCA/IFA				48% (p<0.05) 3 doses	11% 2 doses	Sig. GST/TLM efficacy varies with # of doses (Note: 3 doses POC4 (0.1-0.4mg) cf 2 doses POC5 (0.1-0.2mg)
GST + TLM with Nanoparticle adjuvant					46% (p<0.05) 2 doses	Sig. efficacy with Nanoparticle adjuvant (2 doses, 0.1- 0.2mg); strong IgG1 responses

% of animals showing fluke burdens < lowest fluke count in the control group as an indicator of protection



Conclusions:

- Efficacy was closely associated ($r^2 = 0.915$) with the fraction of animals showing fluke counts < the lowest counts in control animals.
- In the 4 protected groups, 67-86% of protected cattle show relatively low fluke counts with a vaccine efficacy of only 38-48%.
- Although 38- 48% reductions in fluke burdens were observed in cattle with 3 different antigen combinations, interpretation of the data is confounded by variation in efficacy of certain formulations between trials.
- The tegument proteins assessed here are not optimal vaccine candidates.
- TLM/GST data encourage further evaluation of this combination.
- TLM/GST data suggest # of vaccine doses (2 vs 3) and protein concentration (0.1-0.4 mg/dose) are important in inducing protection; repeat trials needed.

Going forward:

- As natural fluke infections of < 50 flukes are common in cattle, future experimental challenges should reflect that level to optimise vaccine evaluation under more realistic conditions (i.e. a challenge of 200-260 metacercariae)(Spithill et al 2022)
- A high % of animals with low fluke counts will result in lower herd production losses which is the key parameter for a commercial vaccine (Turner et al 2016).
- The commercial problem will be how to market a vaccine with partial (50%) efficacy:

- it will be simpler in areas where drug resistance means triclabendazole use is not feasible;
- and simpler for dairy producers where increased milk production is easily assessed following vaccination (Spithill et al 2022).