Modification of a novel Whipworm vaccine candidate with a highly immunogenic Tetanus epitope

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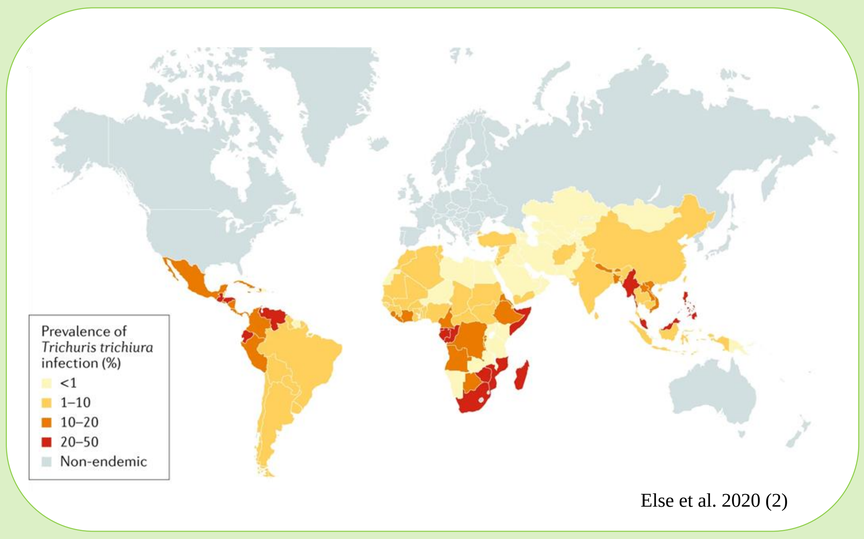
Background

- *Trichuriasis* affects **477 million people worldwide** resulting from infection with *T. trichiura*, (whipworm).
- **The low effectiveness of drugs** currently used to control *T. trichiura* has driven the search for alternative treatments including **anti-*Trichuris* vaccines**.
- Here we modified Virus-Like particle (VLP) vaccine candidates consisting of the Hepatitus B core antigen (HBCAg) genetically fused to *Trichuris spp.* MHC-II T cell epitopes developed by **Zawawi et al. 2020 (1)** with a highly immunogenic **Tetanus epitope** in an attempt to improve their efficacy *in vivo*.

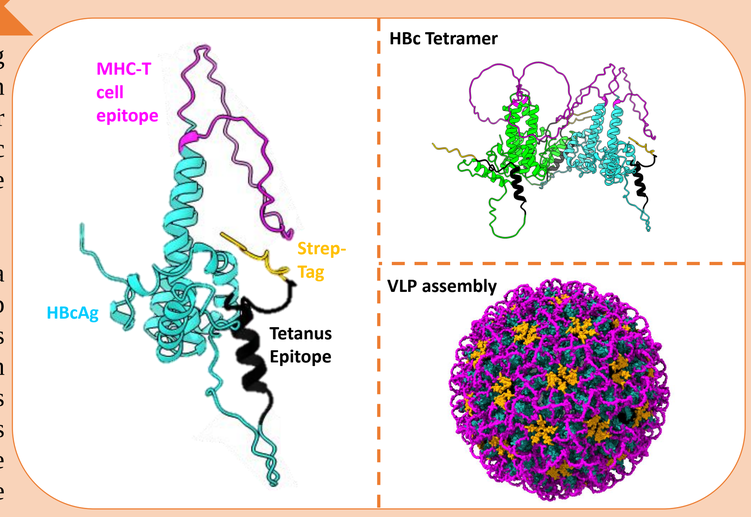


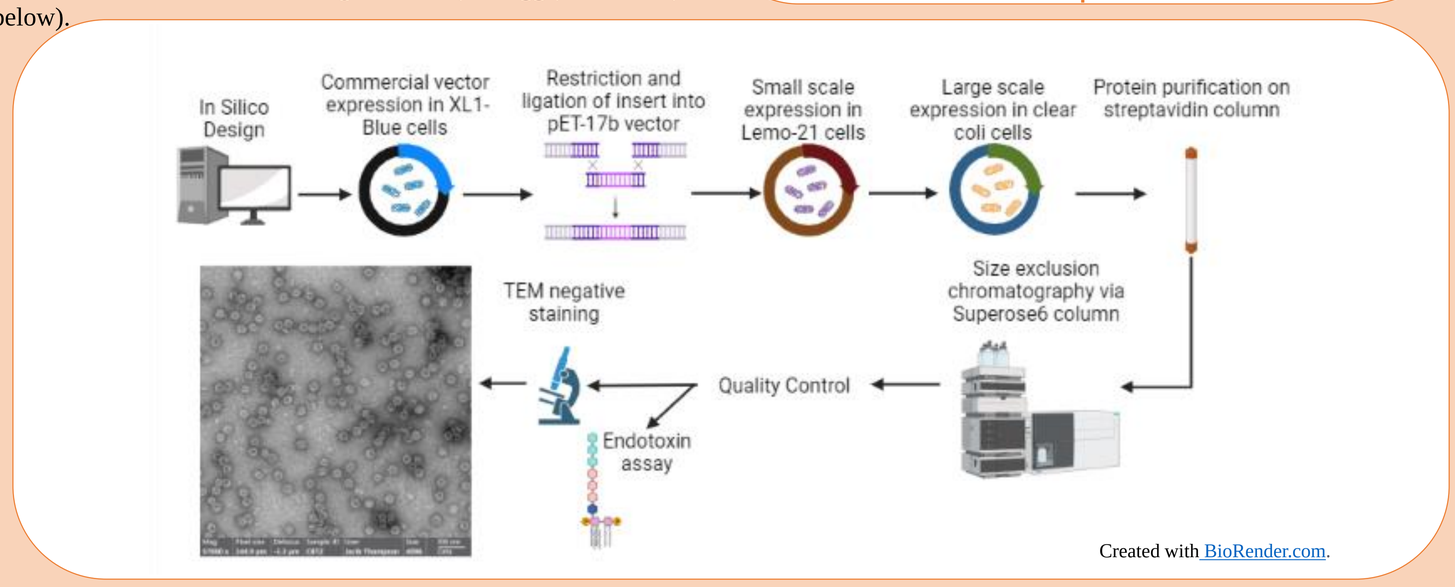
Else et al. 2020 (2)

VLP expression and purification

VLPs genetically fused to a *Trichuris spp.* T cell epitope pertaining to either a whipworm chitin-binding domain-containing protein (CBD) or chymotrypsin-like serine protease (CLSP) at the major immunodominant region (MIR) and the universally immunogenic P2 (QYIKANSKFIGITEL) Tetanus epitope at the C terminus were designed *in silico* (right).

These constructs were cloned and expressed in Clear Coli bacteria that exhibited a non-toxic truncated LPS. Subsequent two step protein purification produced six high quality VLPs, including VLPs containing a CBD or CLSP epitope, and the native HBcAg, all with and without the addition of a Tetanus epitope (below). Constructs containing CBD and CLSP epitopes were used togehter in cocktails with and without an additional Tetanus epitope to immunize **C57BL/6 mice** infected with a high dose of **(150 eggs) *T. muris*** (see below).





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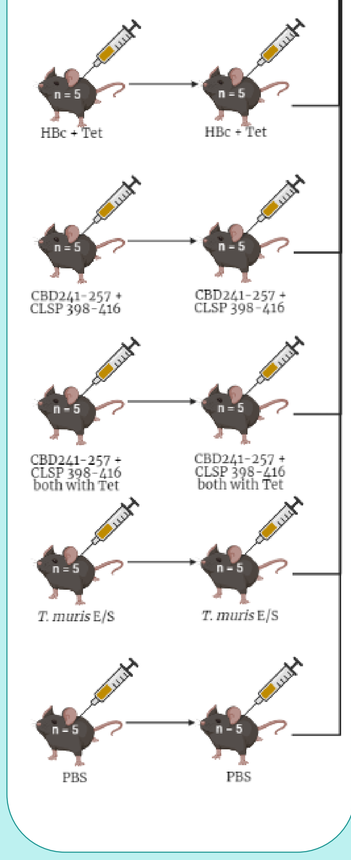
In Vivo Experiment

Experimental Design



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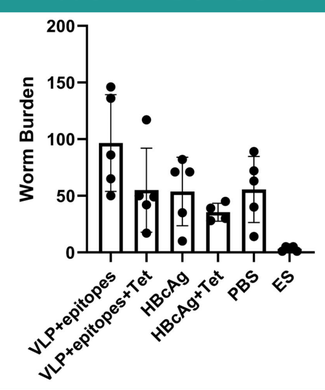
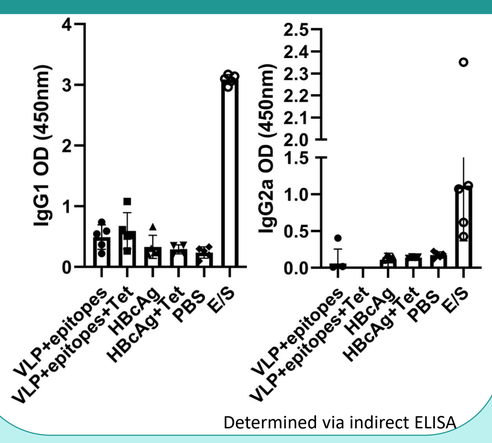
Worm Burden

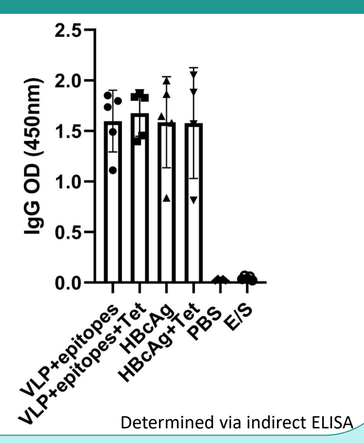


E/S specific serum response day34



Determined via indirect ELISA

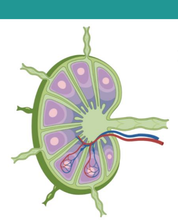
HbcAg specific serum response day34



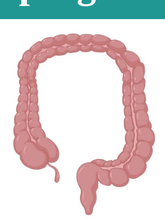
Determined via indirect ELISA

Analysis in progress

1. Cytometric Bead Array (CBA) of E/S re-stimulated mesenteric lymph nodes



2. Histological analysis of goblet cells and crypt lengths in the large intestine



3. Indirect ELISAs to quantify levels of anti-E/S and HBc mucosal IgA in faecal extractions

Key Findings

There is no significant reduction in worm burden between PBS vaccinated mice and mice vaccinated with VLPs containing *Trichuris* specific MHC-II T cell epitopes with and without a universally immunogenic T cell epitope.

- **All mice vaccinated with VLP vaccine candidates based on the HBcAg expressed significantly greater levels of IgG antibodies specific to the native HBcAg compared to non-VLP immunized mice (e.g. *T. muris* E/S and PBS vaccinated mice).**
- **While a small increase in E/S specific IgG1 antibodies can be seen in the serum of mice vaccinated with VLP vaccine candidates containing *Trichuris spp.* specific MHC-II T cell epitopes, this increase was not significant compared to their PBS vaccinated counterparts.**
- **No significant difference was observed in IgG2a serum antibody responses between mice vaccinated with VLP vaccine candidates and PBS vaccinated mice.**

Discussion

- We observed highly variability in worm burden in mice vaccinated with PBS indicative of either **variable infectivity of the *T. muirs* eggs** used to inoculate the mice or **active parasite expulsion from the mice**. Both hypotheses warrant a repeat of the experiment preferably with an earlier end point to ensure mice vaccinated with PBS are not actively expelling the parasite at the time of harvest.
- In line with previous experiments we used a remarkably concentrated **2 nanomole (~50µg) dose** of the VLP vaccine candidates. These high doses may have saturated the immune response of the mice against *T. muris* with anti-HBc responses in a process canonically known as **epitope suppression**.
- **In conclusion**, although no significant reduction in worm burden was observed between PBS vaccinated mice and those vaccinated with VLP candidates containing Trichuris *spp.* specific MHC-II T cell epitopes and a Tetanus epitope, optimization of the vaccine candidate doses and time of sampling may reveal interesting kinetics between the vaccine candidate and the mouse anti-*T. muris* immune response, and elicit improved vaccine efficacy.

Future Work

- In addition to repeating the experiment as detailed above, we also aim to:

1) **Couple the *Trichuris spp.* antigens to the VLP via an alternative process**, which would leave the antigens more exposed to the machinery of the immune system.
2) **Create VLP vaccine candidates containing experimentally screened *Trichuris spp.* antigens** sourced from collaborators at the Manchester Metropolitan University (MMU).
3) Source samples of *T. trichiura* to screen serum from mice vaccinated with VLP vaccine candidates to **ensure cross reactivity between the *T. muris* mouse model and *T. trichiura* in the field**.

Acknowledgements

- **The authors wish to thank the staff in the FBMH EM Core Facility at the University of Manchester (RRID:SCR_021147) for their assistance and the Wellcome Trust for equipment grant support to the EM Facility.**
- **We would also like to thank Iris Mair and Ruth Forman for their help in preforming the animal work in this study.**
- **Finally, we would like to thank Angela Thistlethwaite, Smruti Rashmi, Fidel Ramirez Bencomo, Aljawharah Alabbad, and Laura Burgess Tornaletti for their helpful advice and aid in designing, expressing, and purifying the VLP vaccine candidates.**

References

1.Zawawi A, Forman R, Smith H, Mair I, Jibril M, Albaqshi MH, et al. In silico design of a T-cell epitope vaccine candidate for parasitic helminth infection. PLoS Pathog. 2020;16(3).

2.Else KJ, Keiser J, Holland C V., Grencis RK, Sattelle DB, Fujiwara RT, et al. Whipworm and roundworm infections. Vol. 6, Nature Reviews Disease Primers. 2020.