Preclinical development of a trivalent typhoid/non-typhoidal *Salmonella* glycoconjugate vaccine for sub-Saharan Africa

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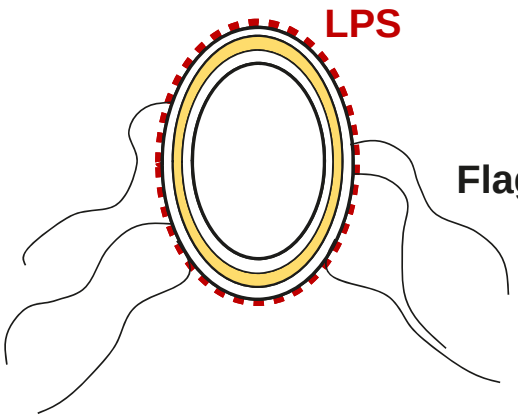
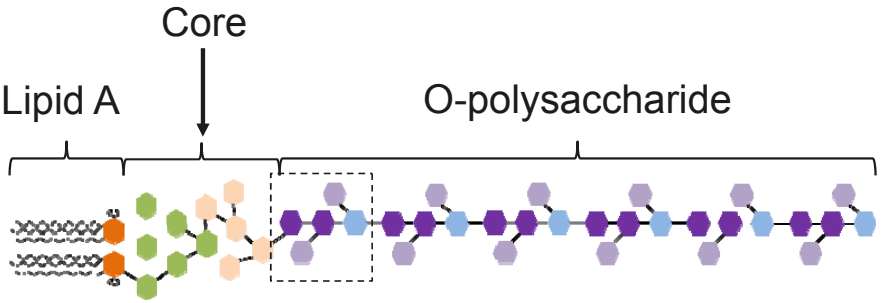
A comprehensive typhoid/iNTS vaccine for use in sub-Saharan Africa

- *Salmonella* Typhi and invasive non-typhoidal *Salmonella* (Enteritidis, Typhimurium) are major problems in sub-Saharan Africa (Marks, *Lancet Glob Health*, 2017).
  - ≥33% of all bacteremias, and multidrug resistance (*S.* Typhi = 47%; iNTS = 48%)
- Regional variability in typhoid fever/iNTS incidence, and heterogeneity in iNTS burden over time.
  - Kenya: Lwak vs. Kibera (Verani, *Clin Infect Dis*, 2015).
- Antibodies recognizing surface polysaccharides correlate with protection in children.
  (Klugman, 1987; MacLennan, 2008; Nyirenda, 2014; de Alwis, 2018).
- **An effective vaccine against invasive *Salmonella* disease should target all three.**

Trivalent typhoid-iNTS glycoconjugate formulation (*S.* Enteritidis COPS:FliC + *S.* Typhimurium COPS:FliC + Typbar-TCV™)

S. Typhimurium (B), *S.* Enteritidis (D)



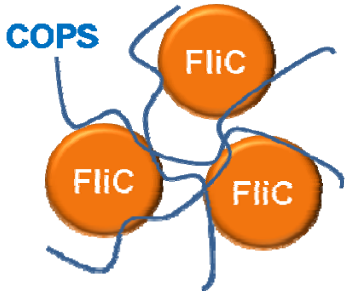


conjugate vaccines:

STm COPS:FliC

Baliban, *PloS NTD*, 2017

SE COPS:FliC



Simon, *Infect Immun*, 2011

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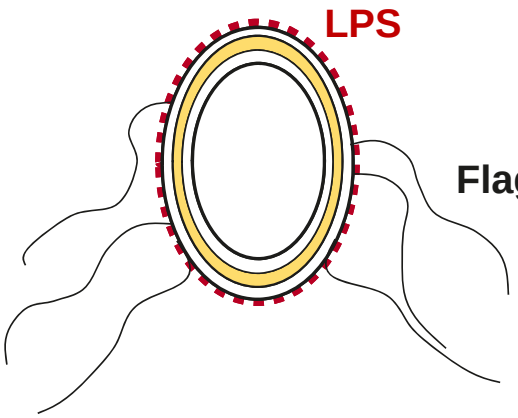
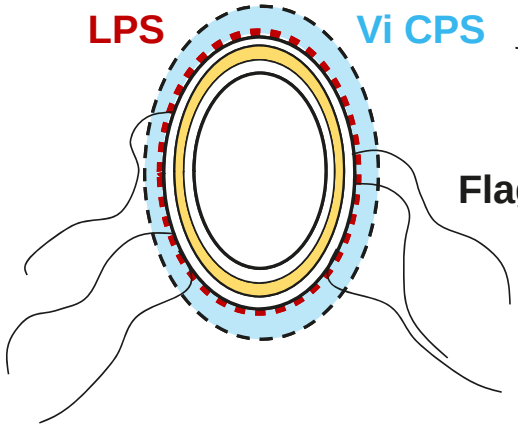
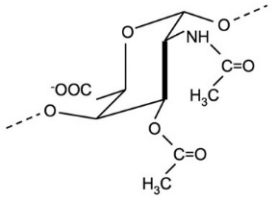


S. Typhi (D)





conjugate vaccines:

STm COPS:FliC

Baliban, *PloS NTD*, 2017

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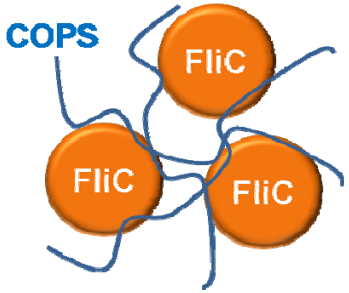
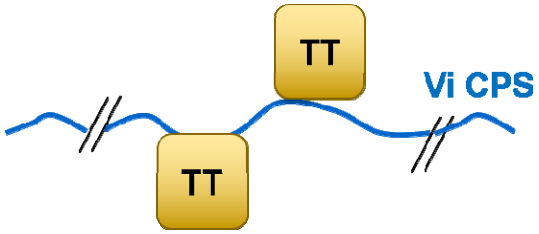


Simon, *Infect Immun*, 2011

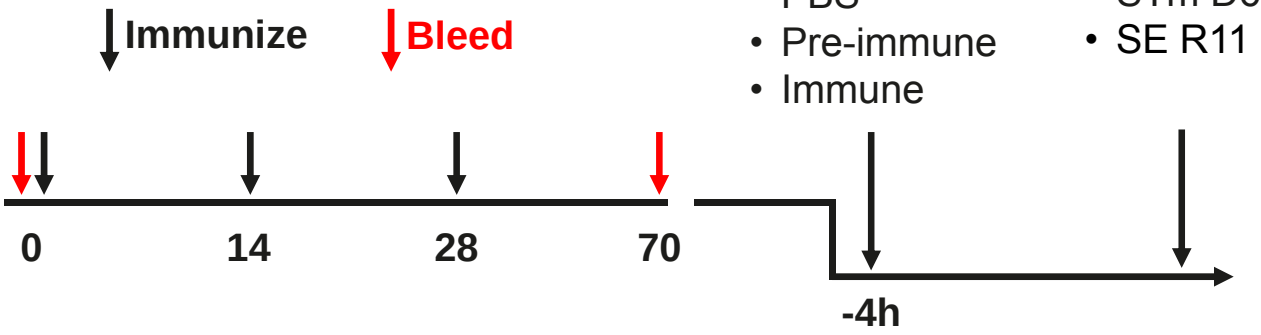
Typbar-TCV



Mohan, *Clin Infect Dis*, 2015

Overview of study

NZW
n = 5 m/f



IP Transfer
- PBS
- Pre-immune
- Immune

Challenge
- STm D65
- SE R11

6-8 wk CD-1
n = 18 f/group

Immunogen
SE COPS:FliC (5 µg) +
STm COPS:FliC (5 µg) +
Typbar-TCV™ (5 µg)

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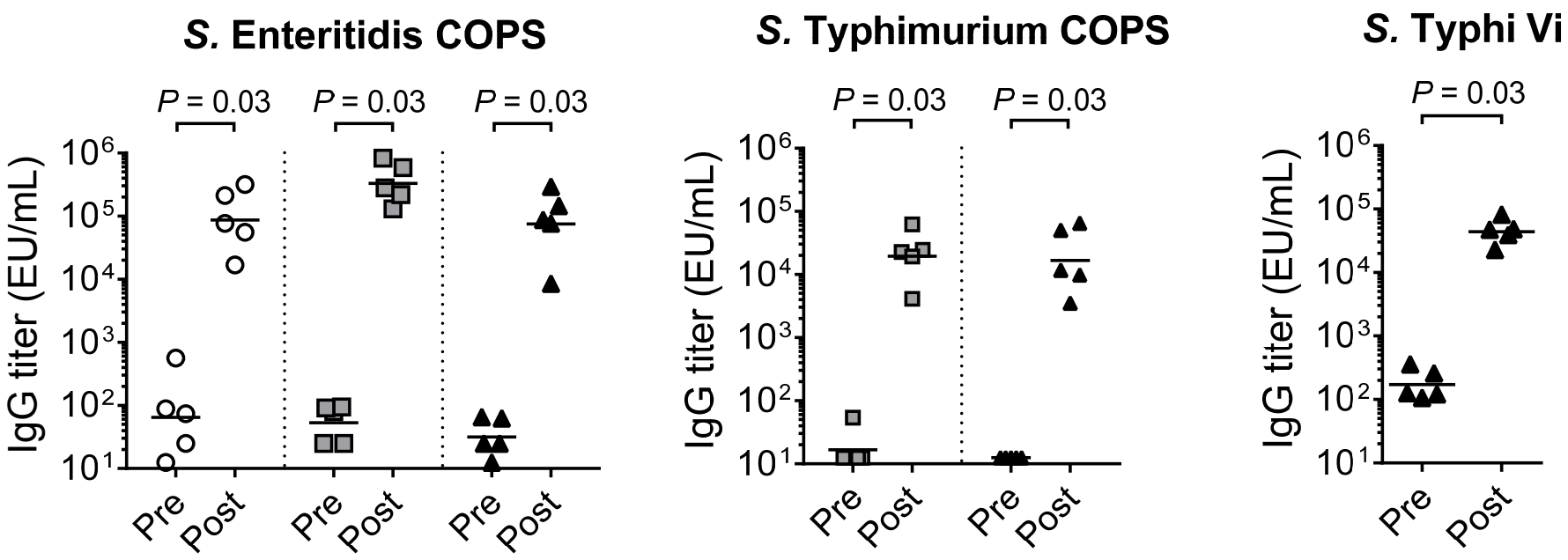
Serum anti-polysaccharide IgG: Impact of conjugate valency in rabbits



S. Enteritidis COPS:FliC

S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC

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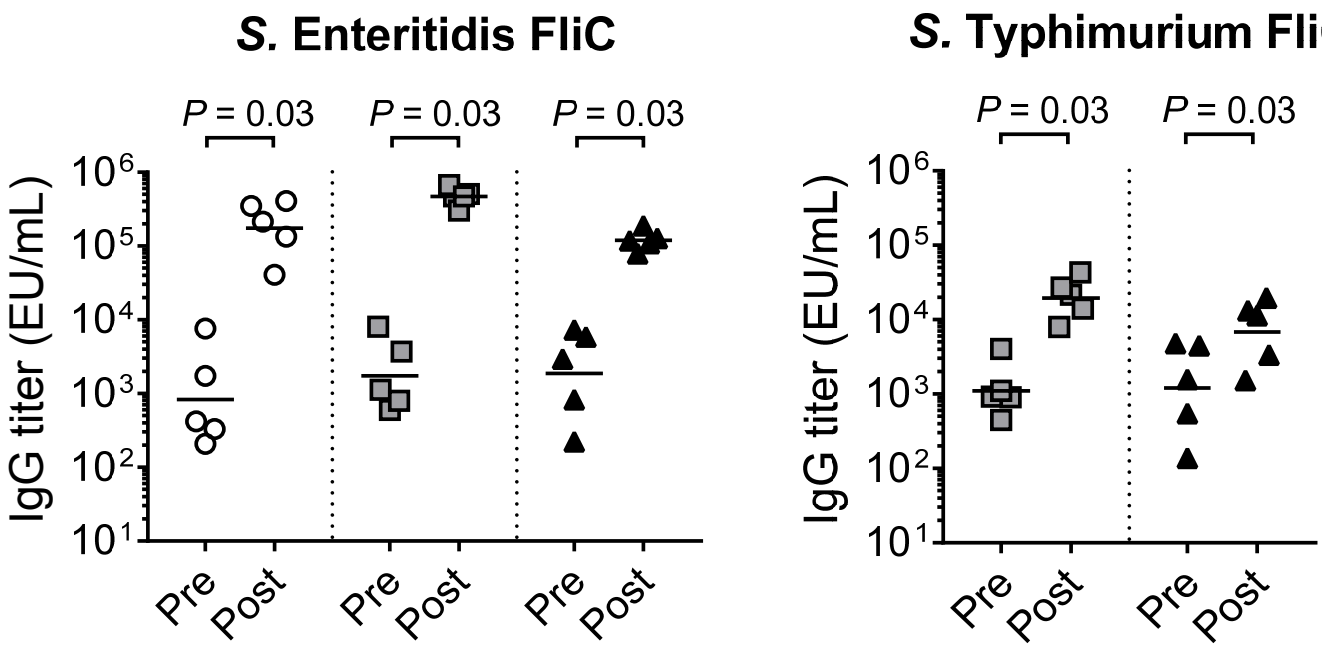
Baliban, 2018

Serum anti-FliC IgG: Impact of conjugate valency in rabbits



S. Enteritidis COPS:FliC

S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC

S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC + Typbar-TCV™

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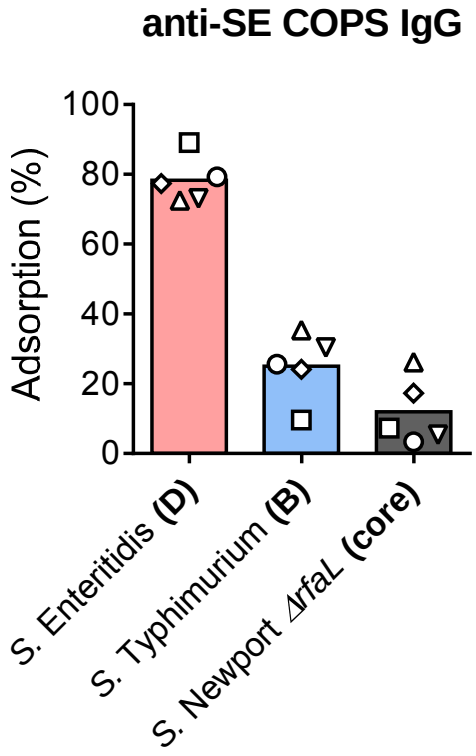
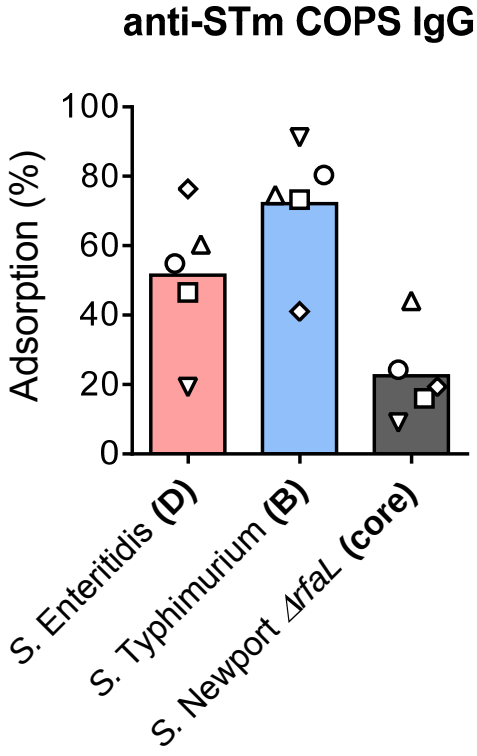
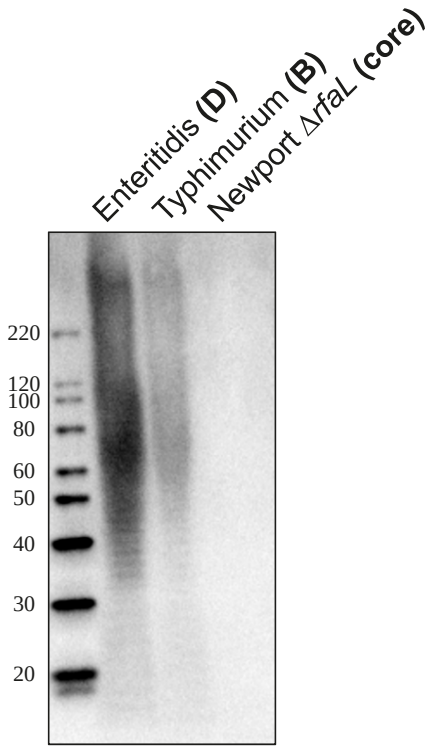
Trivalent-induced anti-OPS IgG is primarily serotype (serogroup)-specific







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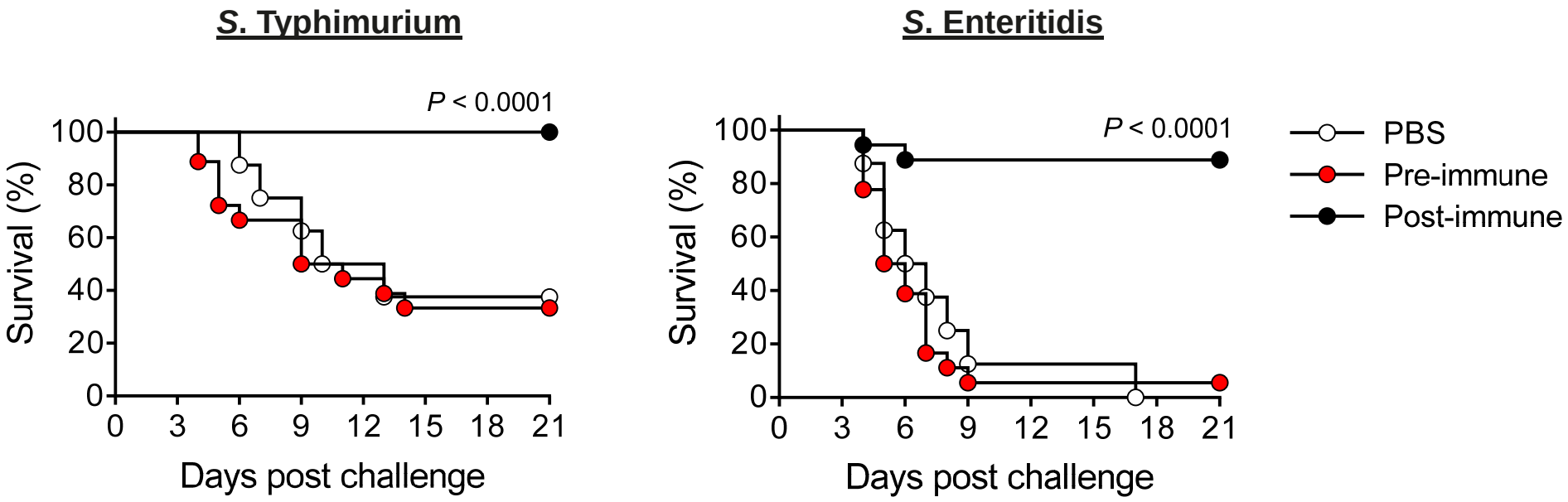
Passive transfer of trivalent vaccine-induced sera protects mice against fatal iNTS challenge



- Mice (n = 18/group) transferred PBS, or diluted pre- or post-immune sera
- Challenged 4 hours later with virulent iNTS isolate (STm D65, SE R11)

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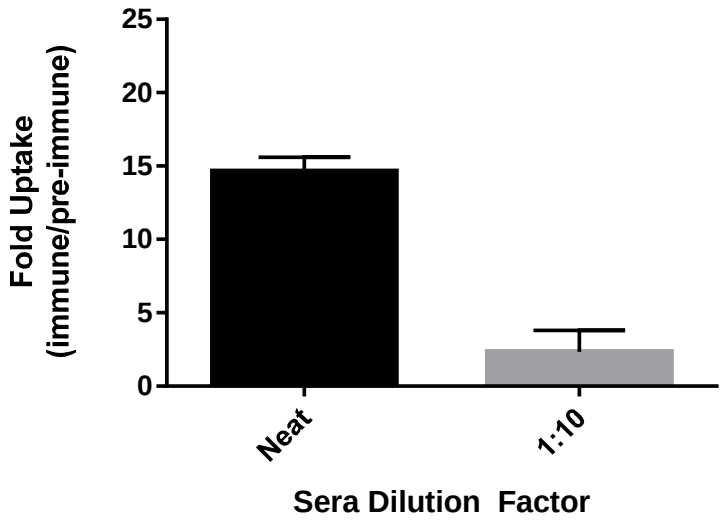
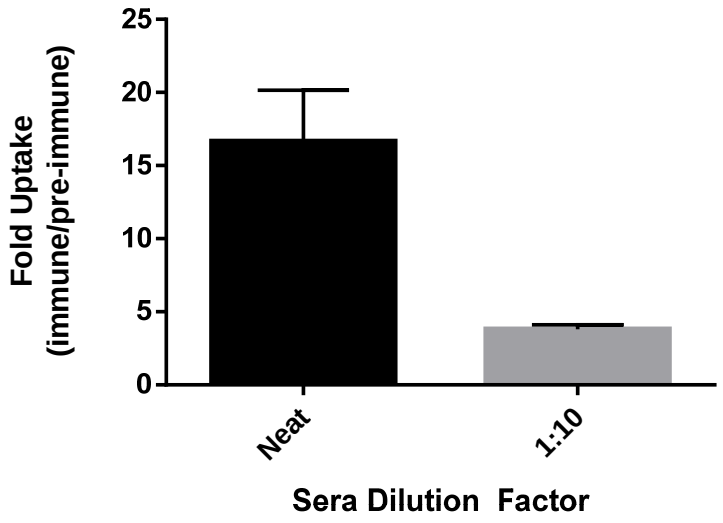
Trivalent post-immune sera mediates robust opsonophagocytosis (OPA) of both *S.* Typhimurium and *S.* Enteritidis

<u>*S.* Typhimurium</u>



<u>*S.* Enteritidis</u>



- Heat-inactivated sera
- Viable CFU measured after incubation with J774 murine macrophages and cell lysis

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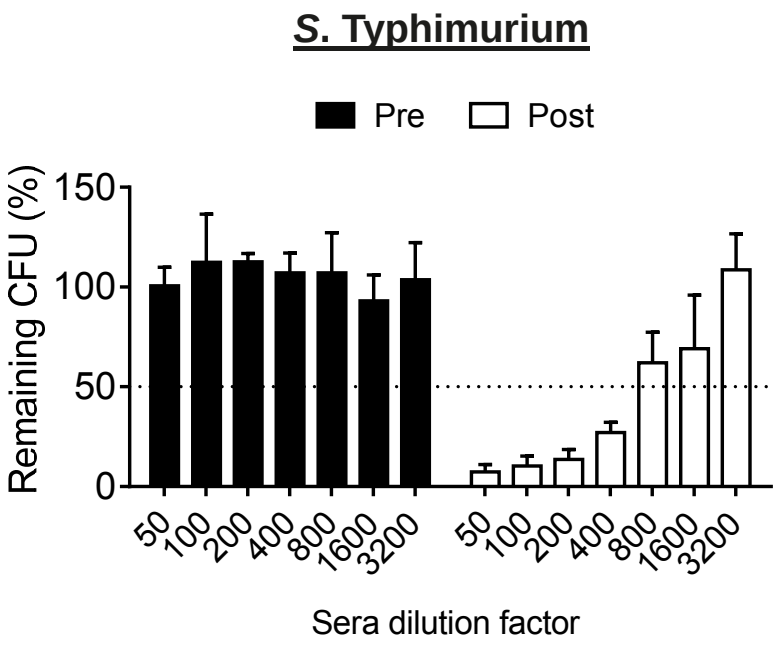
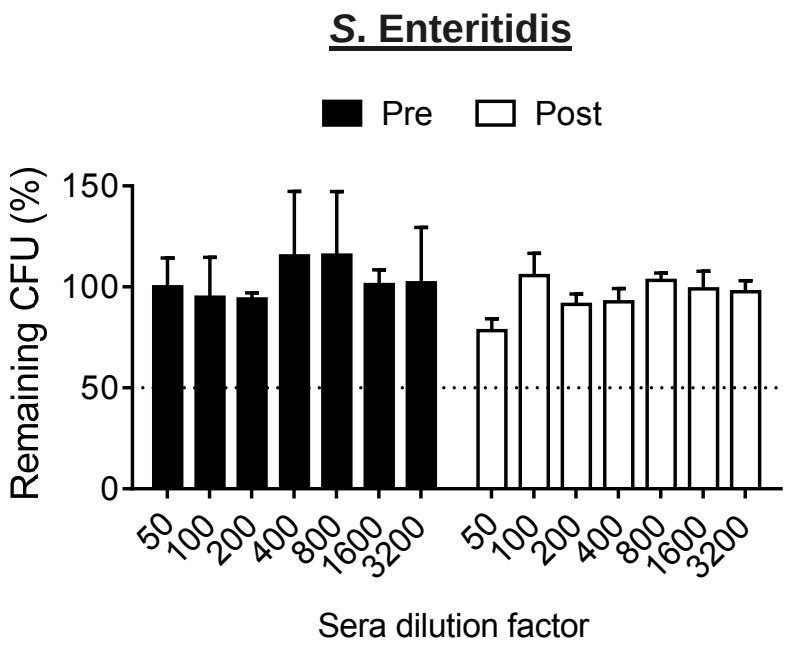
Trivalent post-immune sera mediates strong complement-dependent serum bactericidal activity (SBA) against *S.* Typhimurium





- Heat-inactivated sera + baby rabbit complement
- Viable CFU measured after incubation

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Summary

- Immunization with the trivalent typhoid-iNTS conjugate formulation elicited robust IgG responses to all three polysaccharide antigens.
- Anti-COPS IgG antibodies directed primarily against serogroup-specific OPS epitopes.
- Post-vaccination rabbit sera mediated functional OPA (STm + SE) and SBA (STm) *in vitro*.
- Passive transfer of post-vaccination sera protected against challenge with virulent STm or SE Malian blood isolates (88-100% efficacy).

Partnership with Bharat Biotech

- UK Wellcome Trust Strategic Translational funding to UMSOM-CVD with Bharat Biotech (Hyderabad, India)
- Phase 1 & 2 clinical trials with trivalent *S*. Enteritidis COPS:FliC + *S*. Typhimurium COPS:FliC + Typbar-TCV™ formulation

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wellcome trust

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Team & Funding

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Fina Biosolutions LLC

Andrew Lees
John Van Druff

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NIH/NIAID
UK Wellcome Trust

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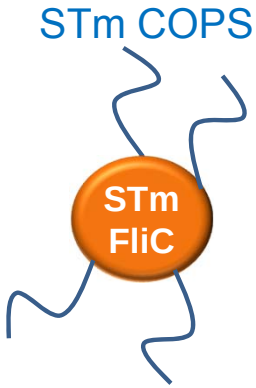
Extra slides

Trivalent glycoconjugate vaccine formulation to prevent invasive *Salmonella* disease in sub-Saharan Africa

S. Typhimurium (STm)

Antigens: STm COPS & FliC
Architecture: Sun-type (end-link)
Chemistry: thioether
Linkers: GMBS (FliC lysines), aminooxy-thiol (COPS-KDO)
Linkage: COPS-KDO -> FliC amines

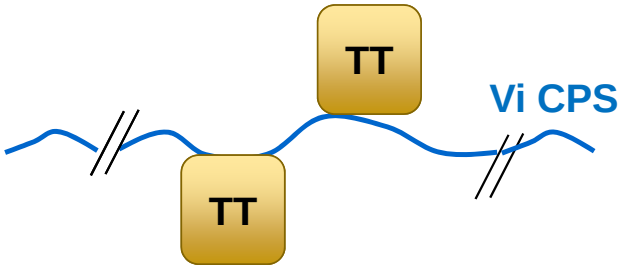


S. Enteritidis (SE)

Antigens: SE COPS & FliC
Architecture: Lattice (multi-point linkage)
Chemistry: CDAP cyanylation
Linkers: Adipic acid dihydrazide (FliC carboxyls)
Linkage: COPS hydroxyls -> FliC amines & carboxyls

S. Typhi (ST, Typbar-TCV™)

Antigens: ST Vi CPS & TT
Architecture: Bead-on-string
Chemistry: carbodiimide
Linkers: Adipic acid dihydrazide (Vi-TT carboxyls)
Linkage: Vi-carboxyls -> TT carboxyls



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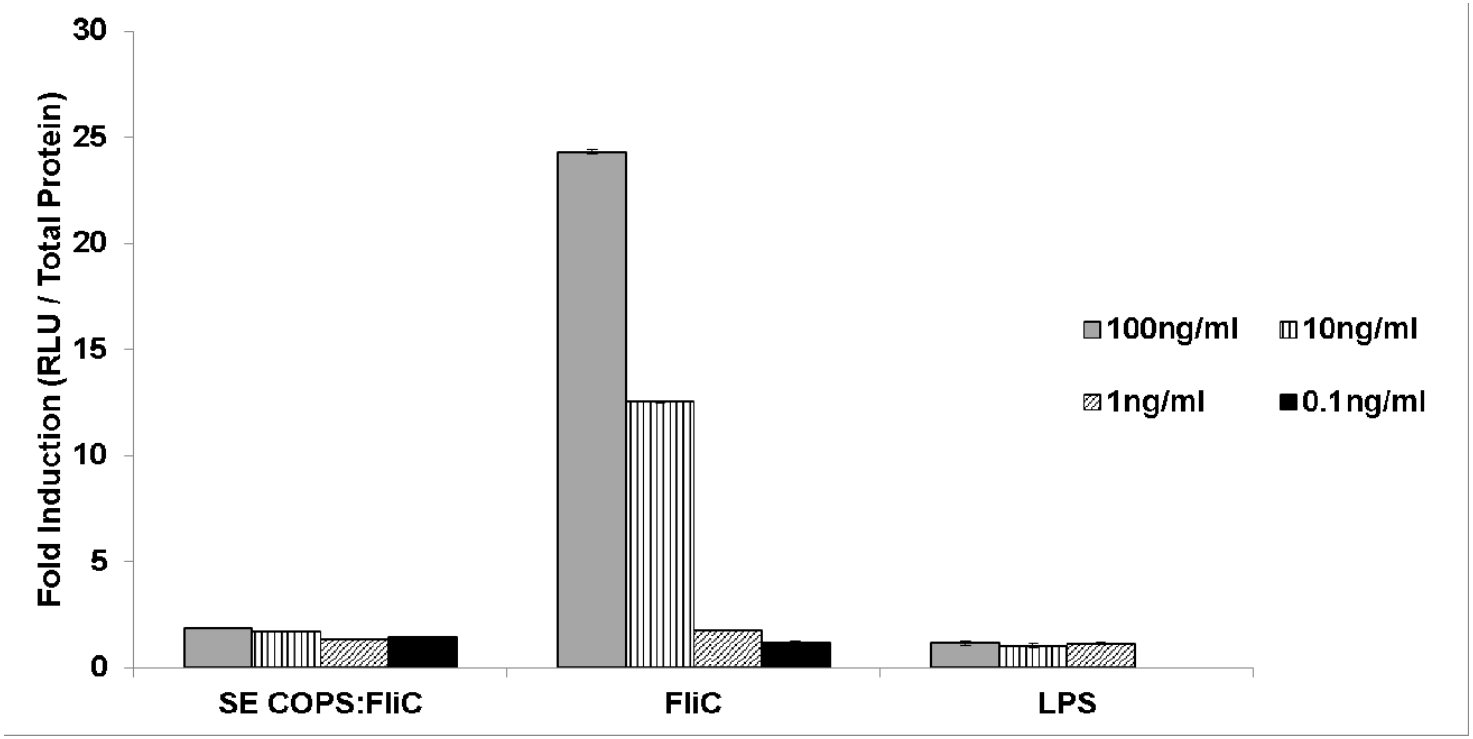
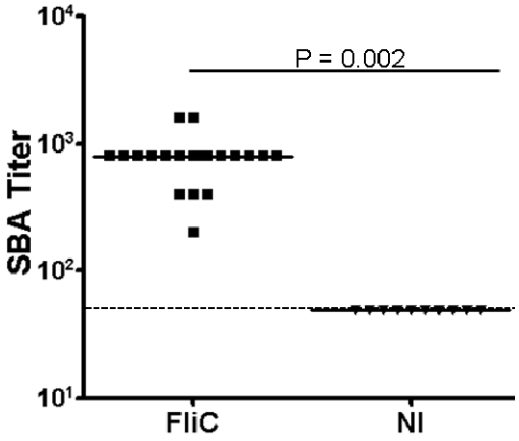
Conjugation ablates TLR5 bioactivity in SE COPS:FliC conjugates

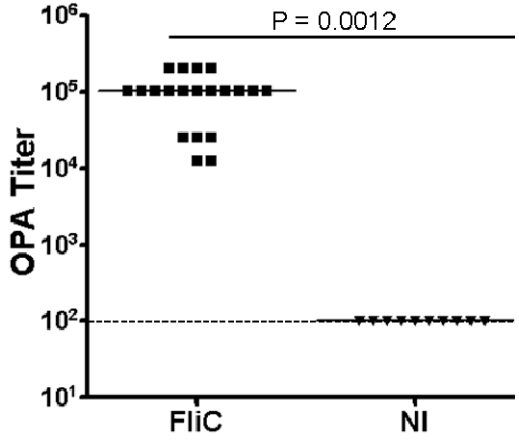


- HEK-293 cells stably expressing luciferase reporter under control of Nf-kB promoter
- Cells treated 4 hours, activation measured by luciferase assay

Functional activity of antibodies directed against *S.* Enteritidis flagellin

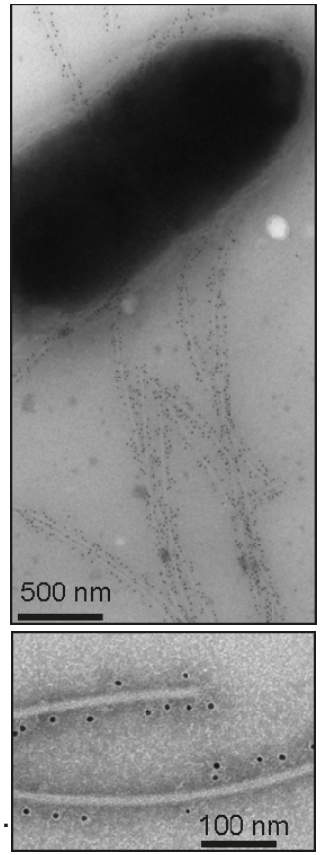


- Complement mediated killing
- Baby rabbit complement
- Polyclonal mouse anti-FliC vs. normal (NI) sera



- Opsonophagocytic uptake and killing
- HL-60 cells
- Polyclonal mouse anti-FliC vs. normal (NI) sera

Immunogold EM with anti-SE flagellin antisera & *S.* Enteritidis



Ramachandran et al. 2016. PLoS One.

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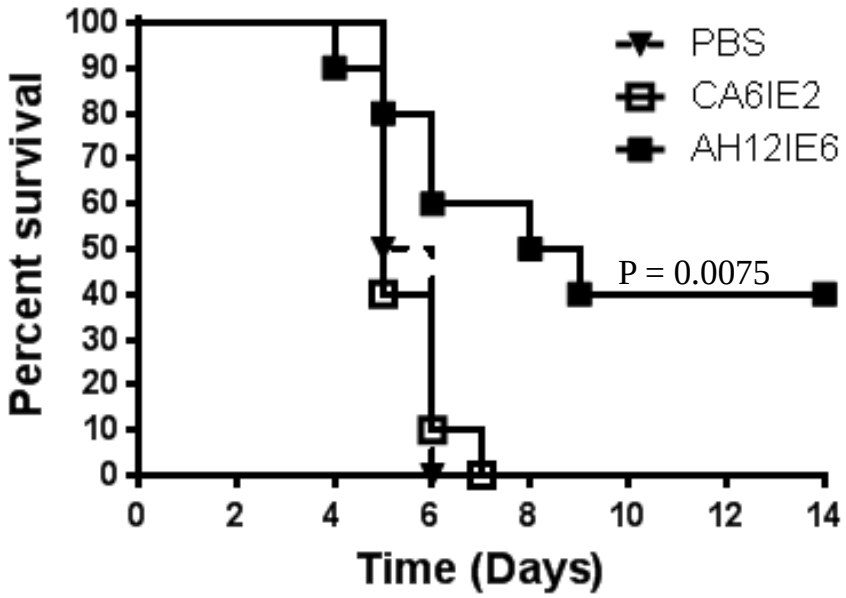
Protective efficacy after passive transfer of monoclonal anti-FliC in mice against *S*. Typhimurim D65 challenge



Mice passively administered anti-SE or anti-STm FliC Mab, challenged IP with STm D65

Ramachandran et al. 2016. PLoS One.