Development of a vaccine based on GMMA against invasive nontyphoidal *Salmonella* disease in sub-Saharan Africa

Oliver Koeberling

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Kampala, Uganda

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GSK Vaccines Institute for Global Health (GVGH) profile

Located in **Siena**, Italy, on the same campus as **GSK Vaccines**, **one** of the three GSK Vaccines **R&D centers**, alongside Rixensart (Belgium), and Rockville (USA)

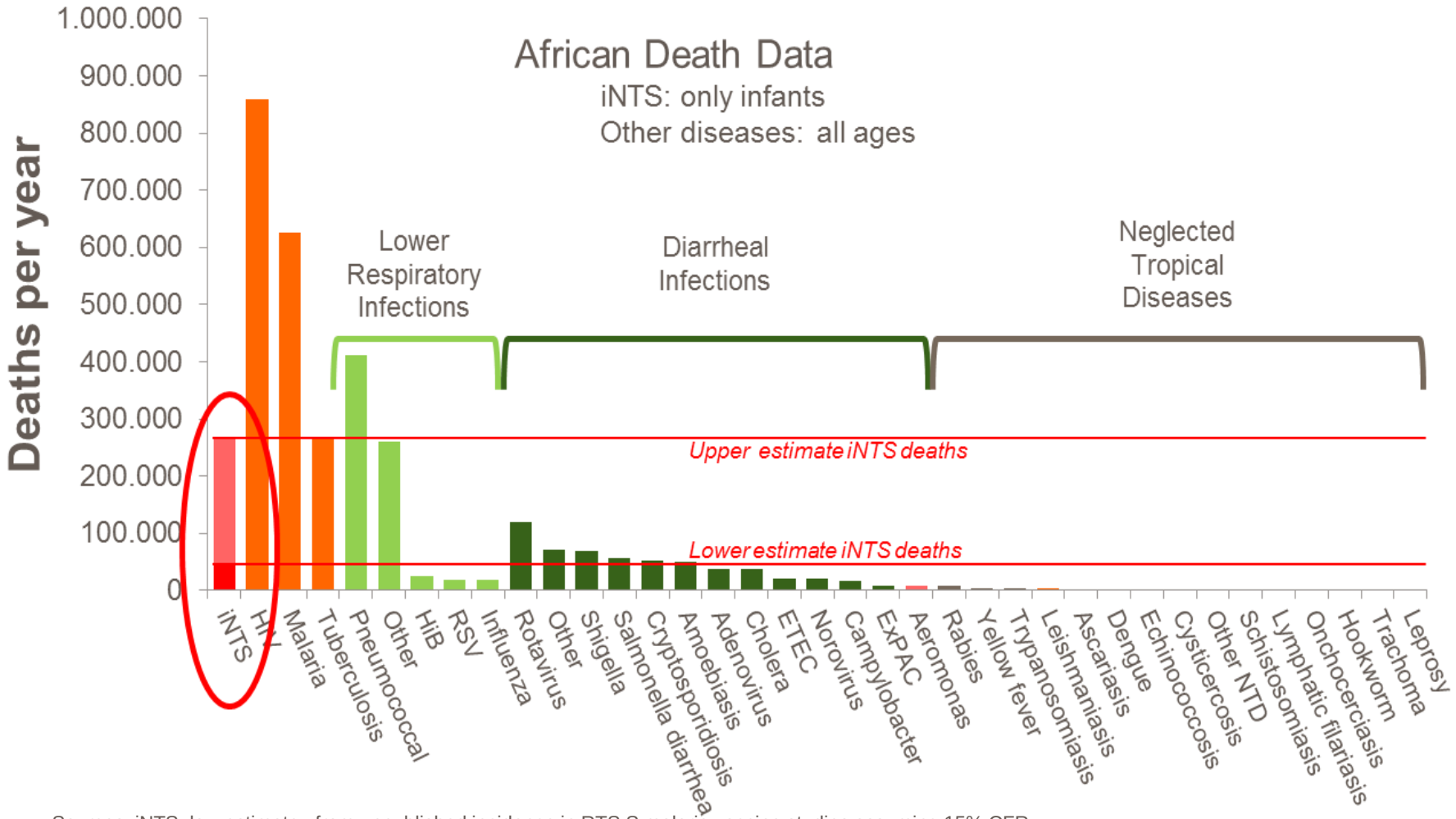
100% owned by GSK but is a separate legal entity from GSK Vaccines

Actively seeks **partners to fund** research and development activities, particularly for **production and clinical trials**

About **50 people** in **Translational Research**, **Technology Development,** and **Clinical Development & Regulatory Affairs**

Invasive nontyphoidal *Salmonella*

A major threat in Africa



Sources: iNTS low estimate: from unpublished incidence in RTS,S malaria vaccine studies assuming 15% CFR
iNTS high estimate: *Emerg Infect Dis. 2015;2010(1):21*
Other diseases: *Global Burden of Disease 2015 Lancet 2016;388 (10053):1459*

Invasive nontyphoidal *Salmonella*

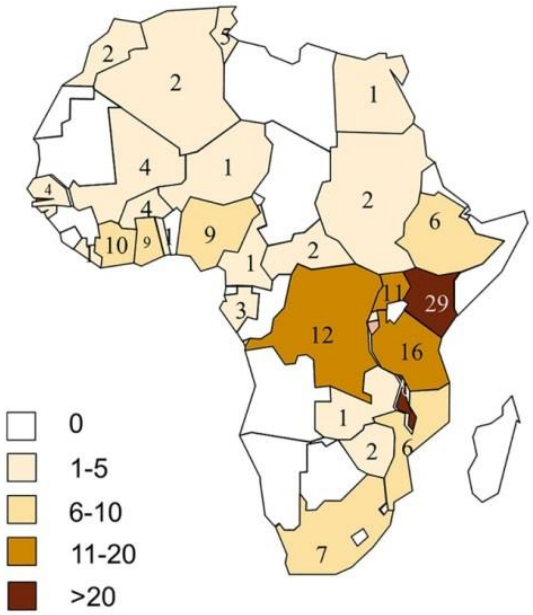
A major threat in Africa

- iNTS disease occurs in whole Africa
- Almost all cases caused by serovars expressing O-antigen O:4,5 or O:9
- Human adapted, genotypes largely distinct from those responsible for gastroenteritis in industrialized countries
- African iNTS strains are highly drug resistant

- **An effective and affordable iNTS vaccine can save many lives.**

- presentation by Gianluca Breghi, Fondazione Sclavo, Siena, Italy

Number of publications reporting NTS blood culture isolates



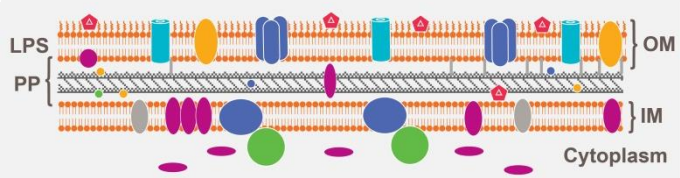
Valentine Uche, Incidence, Risk Factors and CFR of iNTS in Africa (**Poster**)

Uche V. et al., 2017
Marks et al., 2017
Kingsley R. et al., 2009

Bornstein et al., PLOS NTD 2017
Gordon M., et al., 2008

GVGH's GMMA platform

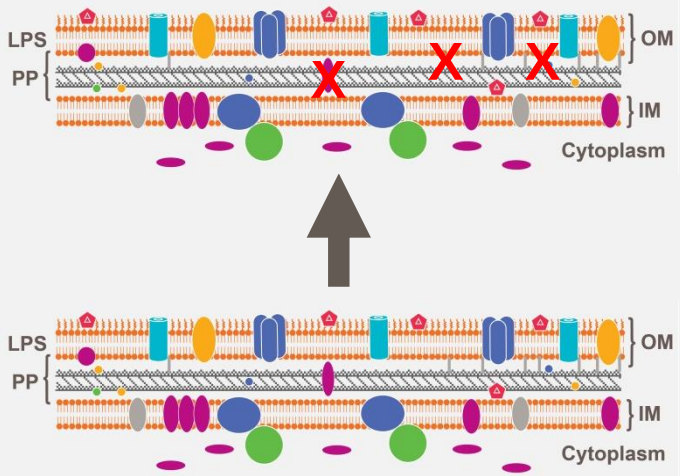
Applied to different vaccines for low and middle income countries



Gram-negative bacteria naturally release small portions of the outer membrane

GVGH's GMMA platform

Applied to different vaccines for low and middle income countries

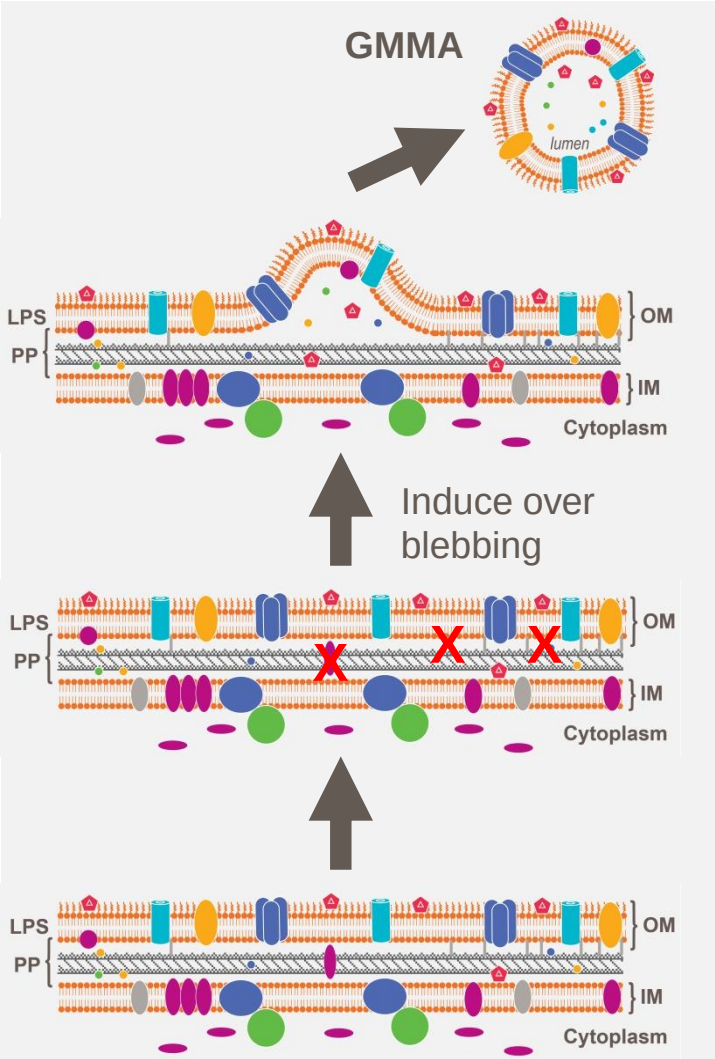


Genetic modification to break membrane links

Gram-negative bacteria naturally release small portions of the outer membrane

GVGH's GMMA platform

Applied to different vaccines for low and middle income countries



GMMA

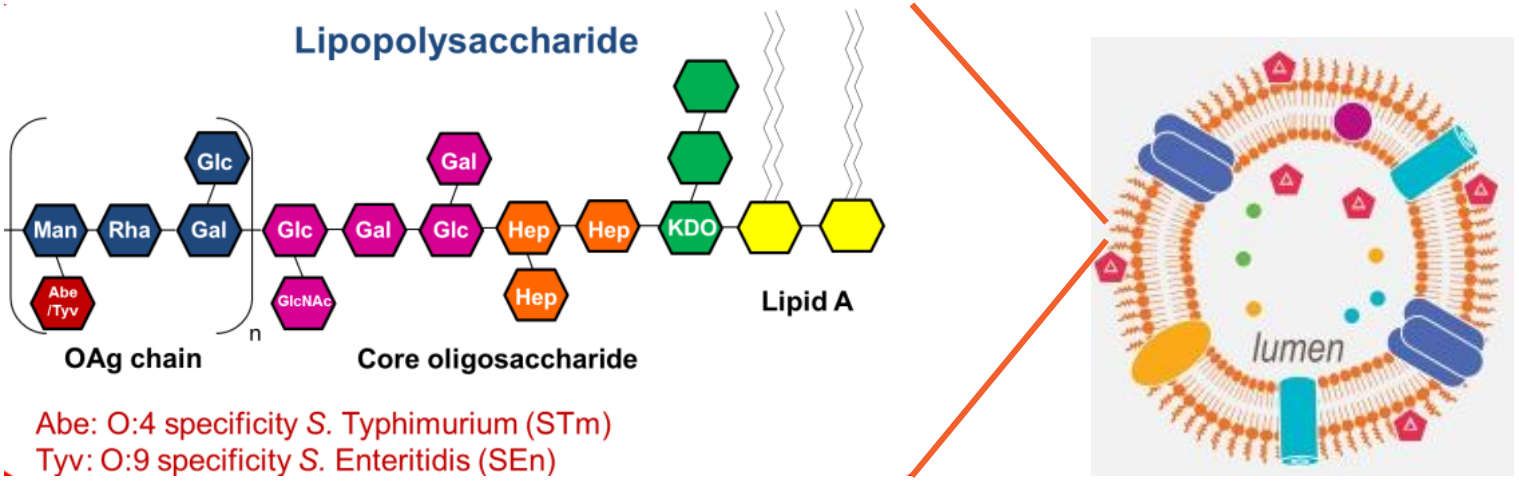
- Just the outer bacterial layer
- Antigens presented in native environment for optimal immunogenicity
- Additional genetic modifications allow targeted vaccine design
- Simple and affordable to manufacture
- Technology applied to different pathogens (***Salmonella***, *Shigella*, Meningococcus)

Genetic modification to break membrane links

Gram-negative bacteria naturally release small portions of the outer membrane

O-Antigen constitutes the active component of iNTS GMMA

Induces production of functional antibodies in mice



A bivalent formulation of STmGMMA (O:4,5) and SEnGMMA (O:9) has the potential to protect against vast majority of iNTS cases in Africa

GMMA Manufacturing – Generic, Simple and Robust

Production process established at GVGH from Shigella sonnei *1790GAHB*

Genetic modifications

Fermentation

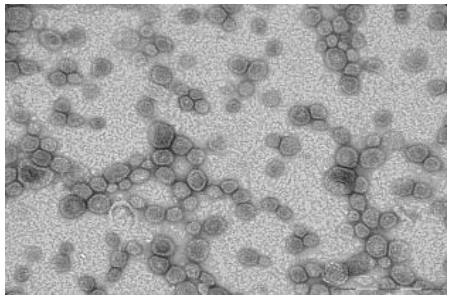
Purification

S. Typhimurium
S. Enteritidis
production strains

1. Increase GMMA production (*tolR* KO)
2. Decrease innate system over stimulation (*msbB* and *pagP* KO)

Micro-filtration
Collect 0.22 µm permeate

Ultra-filtration
Collect 300 kD retentate



Berlanda Scorza F et al PLoS One. 2012
Gerke C et al. PLoS One. 2015 Aug

Sterile filtration
0.22 µm

Formulation
Adsorption on Alhydrogel

GMP lots of GMMA from both strains have been produced

Generic Panel of GMMA Release Tests

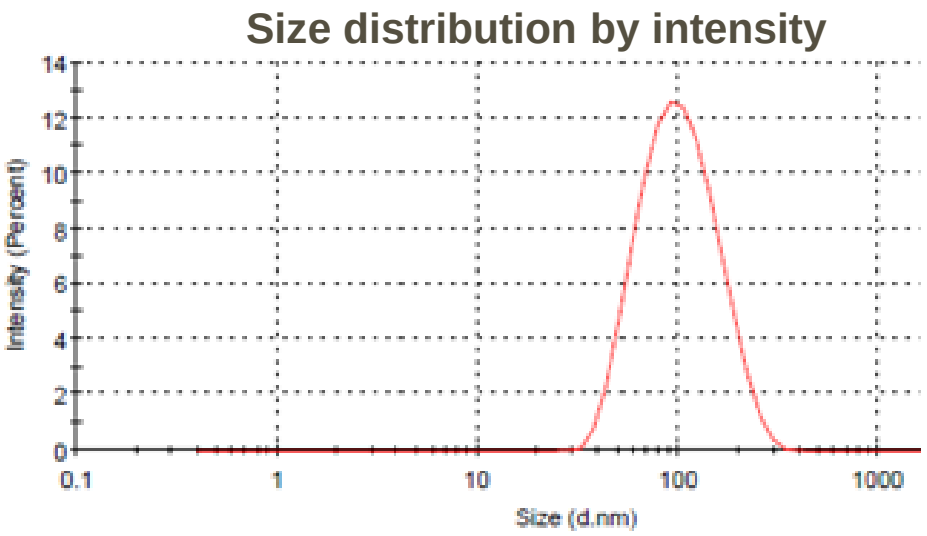
Developed for Shigella GMMA and applicable for GMMA from different organisms

Release tests

Test category	Test
Identity	O-Antigen (OAg) identification
	Lipid A structure
Quantification	Total protein
	Soluble protein
	OAg
	OAg molecular size distribution
	OAg O-Acetyl
	Lipid A
Aggregation, Integrity	Particle Size
Physico-chemical properties	Appearance
	pH
	Osmolality
Purity	DNA
	PPG
	Microbial status

Diameter and OAg molecular size of GMMA

Method	*S.* Typhimurium	*S.* Enteritidis
Particle Size Z-Average diameter (DLS)	107.6 nm	92,2 nm
OAg Molecular size (HPLC-SEC)	34.6 kDa	30 kDa

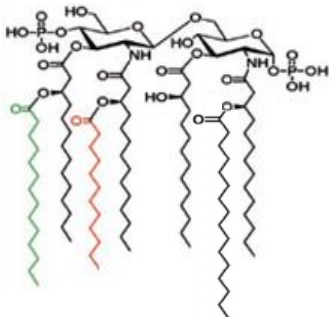


GMMA with engineered Lipid A have decreased potential for induction of pro-inflammatory response

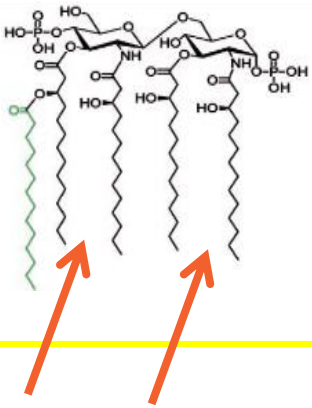
As measured by IL-6 release from human blood cells

Salmonella Lipid A structure

Wild type LipidA
Hepta-acylated

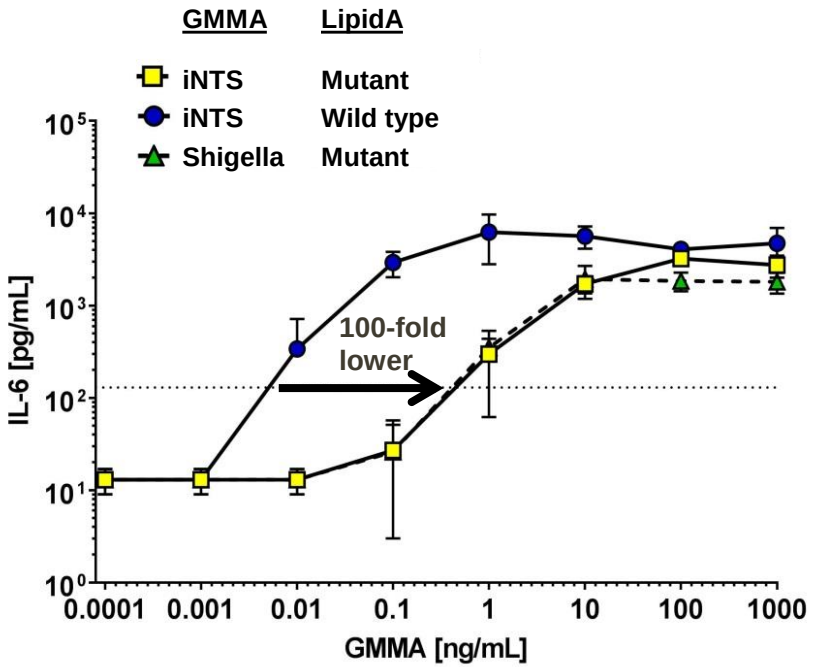


Mutant LipidA
msbB, *pagP* KO
Penta-acylated



Rossi O. et al CVI 2016
Rossi O. et al, JBC 2014

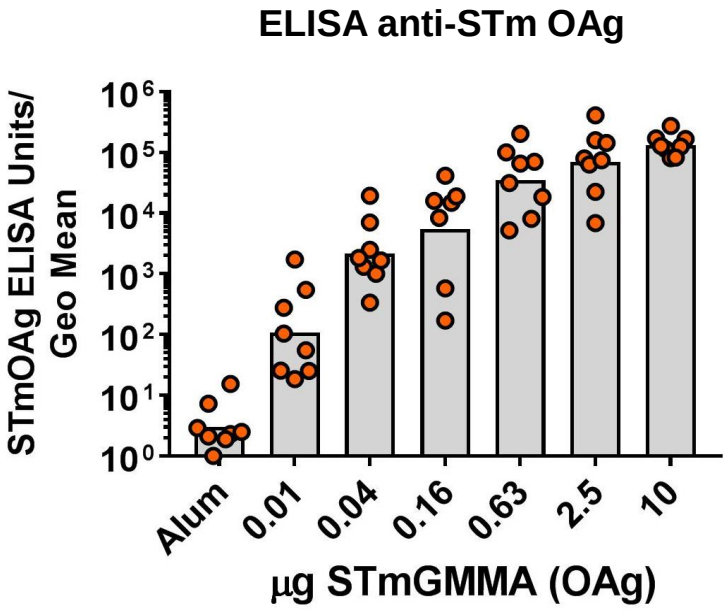
IL-6 (proinflammatory cytokine) release

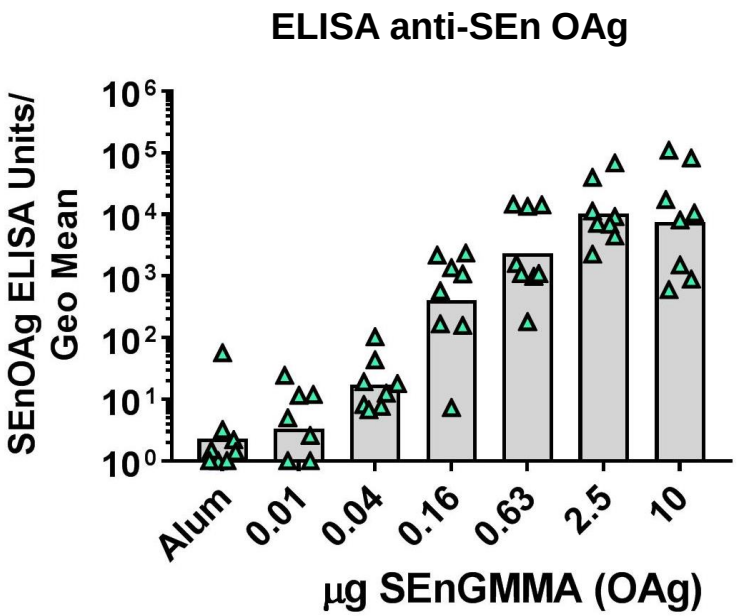


iNTS-GMMA with engineered lipidA

- Approximately 100- fold decreased potential to stimulate IL-6 release than GMMA with wild type LipidA
- Similar to detoxified *Shigella sonnei* GMMA shown to be well tolerated in clinical trials

Formulated GMMA induce high Anti-OAg IgG responses in mice





- STmGMMA or SEnGMMA produced under the industrial process
- Vaccines were adsorbed on Aluminium hydroxide
- CD1 mice were immunized twice 4 weeks apart
- Sera obtained two weeks after the second immunization

Bivalent GMMA induce high Anti-OAg responses in mice

Comparable to individually formulated GMMA

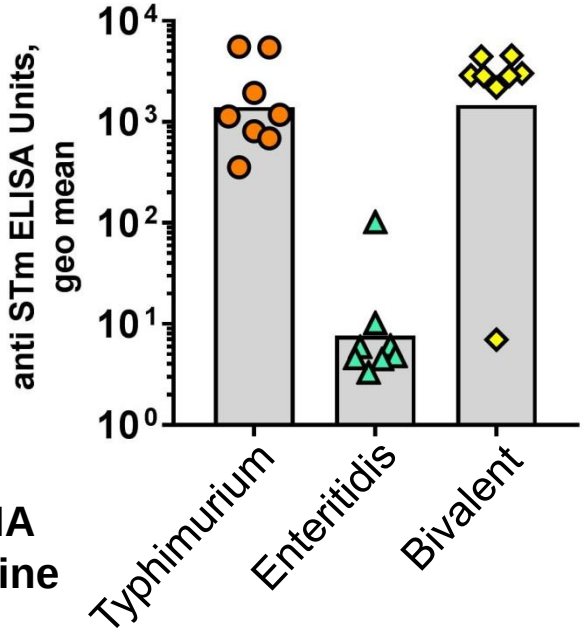
GMMA Dose (OAg)

160 ng Typhimurium
or Enteritidis

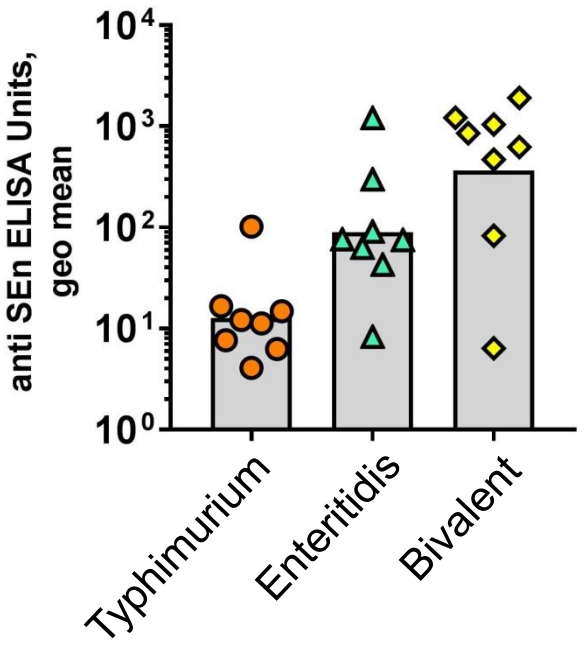
160 ng + 160 ng bivalent

Anti-IgG ELISA

Anti STm OAg antibody responses



Anti SEn OAg antibody responses



GMMA Vaccine

No evidence for interference between the two GMMA administered in combination

Antibodies against Bivalent GMMA show high functionality

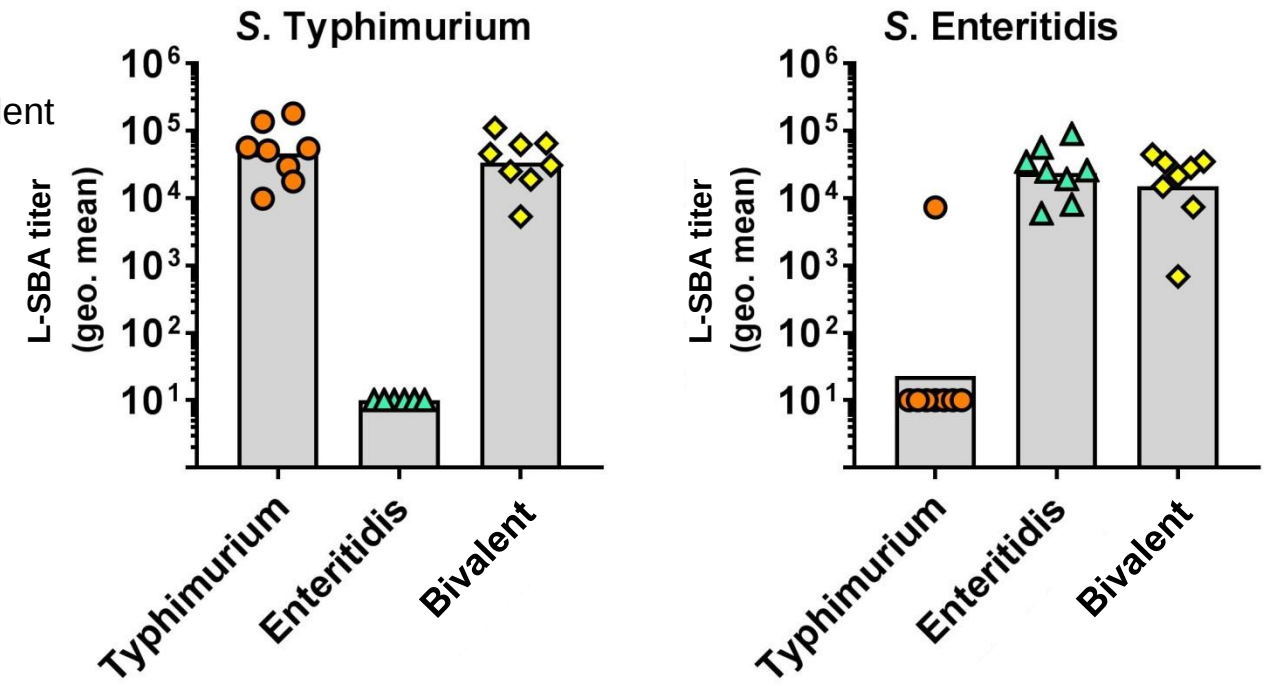
Serum bactericidal assay against S. Typhimurium and S. Enteritidis

GMMA Dose (OAg)

160 ng Typhimurium
or Enteritidis

160 ng + 160 ng bivalent

High Throughput SBA (Necchi et al., 2017)



GMMA Vaccine

- Small quantities of formulated bivalent GMMA induce antibodies with high functional activity against both, Typhimurium and Enteritidis
- Previous studies have shown that antibodies against OAg in GMMA have superior quality than antibodies against similar conjugate vaccines

Summary and Conclusion

- **High burden of disease caused by iNTS in Africa**
- **GMMA technology**
  - Applicable to different vaccines, especially suitable for low and middle income countries
  - Simple and production process
- **iNTS-GMMA**
  - In mice small quantities of the bivalent formulation induce high levels of antibodies with high functionality against Typhimurium and Enteritidis
  - No evidence of interference
  - Plan to proceed to GLP Toxicology study in place

- **Bivalent iNTS-GMMA represent a very promising approach towards an effective and affordable vaccine against iNTS disease**
- **Ready for clinical proof of concept in humans**

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GVGH Project Team - current

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Backup slides

GMMA induce antibodies with superior functionality

Compared with OAg conjugate