

Salmonella Typhi bactericidal antibody activity is a correlate of disease severity, but not protection against typhoid fever

After live oral vaccine in the human challenge model

Andrew J Pollard

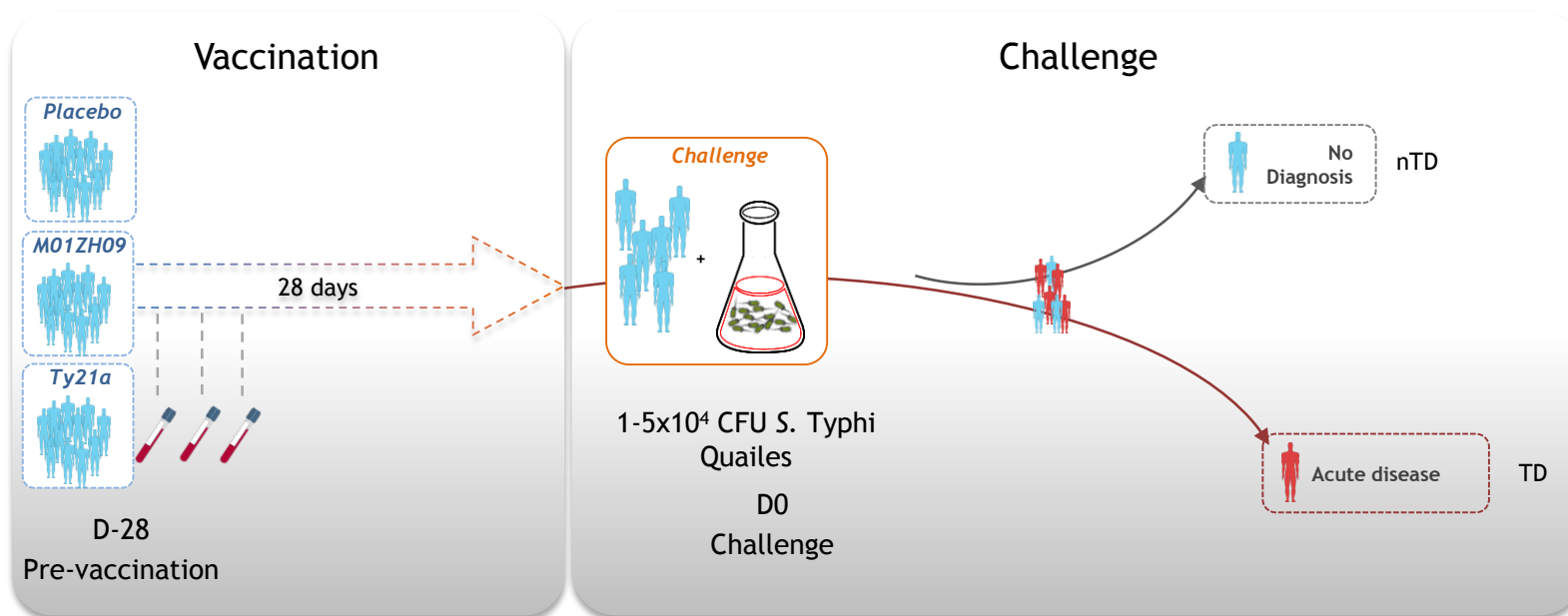
Helene B Juel*, Helena B Thomaides-Brears*, Thomas C Darton, Claire Jones, Elizabeth Jones, Sonu Shrestha, Rebecca Sie, Ushma Galal, Stephen Baker, Christoph J Blohmke



Using a human challenge model of typhoid infection to measure vaccine efficacy



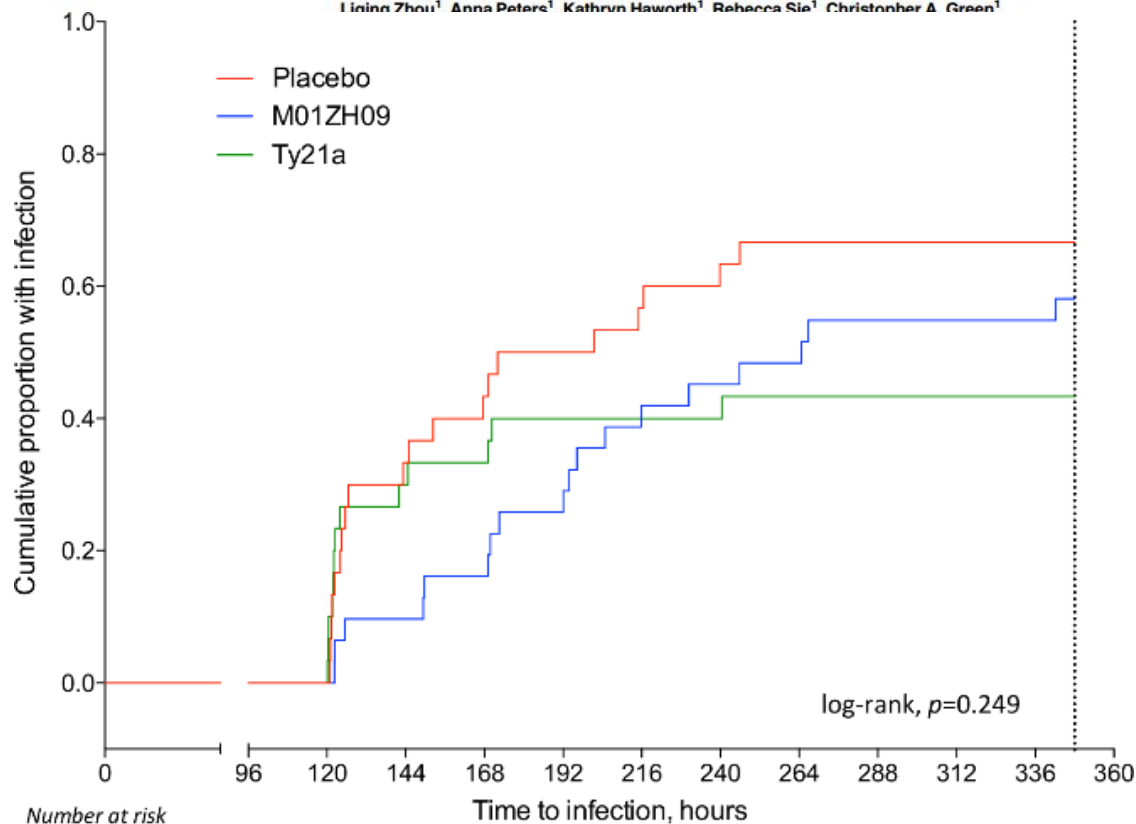
Attenuated *S. Typhi* strain
2 independent, (*ssaV* and *aroC*) which
limit replication and growth



RESEARCH ARTICLE

Using a Human Challenge Model of Infection to Measure Vaccine Efficacy: A Randomised, Controlled Trial Comparing the Typhoid Vaccines M01ZH09 with Placebo and Ty21a

Thomas C. Darton^{1*}, Claire Jones¹, Christoph J. Blohmke¹, Claire S. Waddington¹, Lining Zhou¹, Anna Peters¹, Kathryn Haworth¹, Rebecca Sie¹, Christopher A. Green¹



Placebo 67% TD

M01ZH09 58% TD; VE 13%

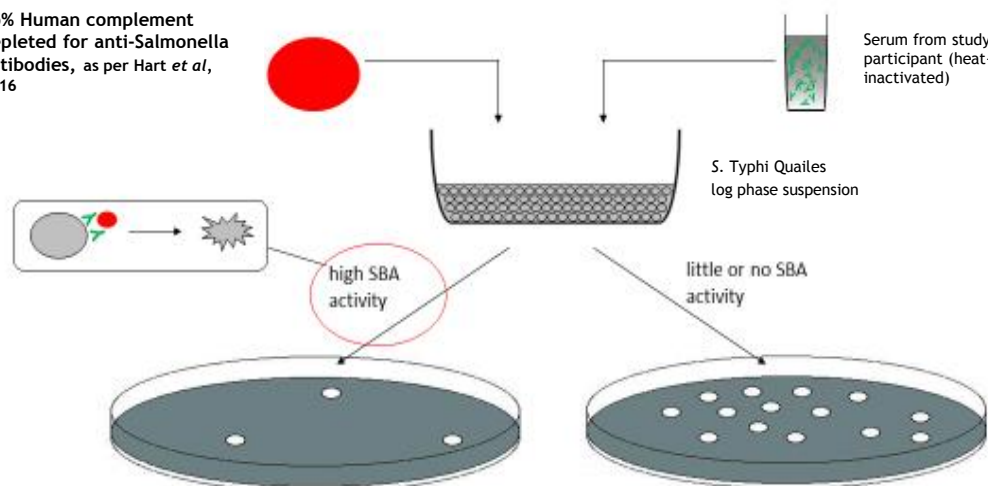
Ty21a 39% TD; VE 35%

Number at risk

	0	96	120	144	168	192	216	240	264	288	312	336	360
M01ZH09	31	31	28	26	23	18	17	16	14	14	14	14	14
Placebo	30	30	20	17	15	13	11	10	10	10	10	10	10
Ty21a	30	30	21	20	18	18	18	17	17	17	17	17	17

Serum bactericidal antibody assay and typhoid

25% Human complement depleted for anti-Salmonella antibodies, as per Hart *et al*, 2016



PLOS ONE

RESEARCH ARTICLE

Differential Killing of *Salmonella enterica* Serovar Typhi by Antibodies Targeting Vi and Lipopolysaccharide O:9 Antigen

Peter J. Hart¹, Colette M. O'Shaughnessy¹, Matthew K. Siggins¹, Saeeda Bobat¹, Robert A. Kingsley², David A. Goulding², John A. Crump^{3,4,5,6}, Hugh Reyburn⁷, Francesca Micoli⁸, Gordon Dougan³, Adam F. Cunningham¹, Calman A. MacLennan^{1,2,9*}

The SBA titre defined as the lowest dilution of test serum to achieve $\leq 50\%$ bacterial killing relative to complement alone

modified from M. Sadarangani

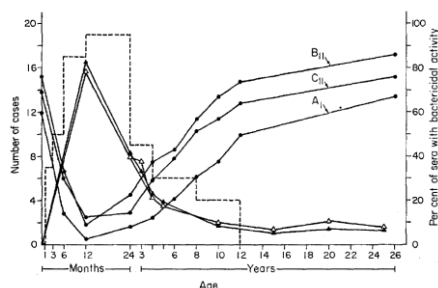


FIG. 1. Age-related incidence of meningococcal disease in the United States and prevalence of serum bactericidal activity against three pathogenic strains of *N. meningitidis*.

A correlate of protection with encapsulated bacteria like *Neisseria meningitidis* Goldschneider, I, Gotschlich, EI & Artenstein MS, 1969



Serum Bactericidal Assays To Evaluate Typhoidal and Nontyphoidal *Salmonella* Vaccines

Mary Adetunke Boyd,^{a,c} Sharon M. Tennant,^{a,b} Venant A. Saague,^{a,b} Raphael Simon,^{a,b} Khitam Muhsen,^{a,b} Girish Ramachandran,^{a,b} Alan S. Cross,^{a,b} James E. Galen,^{a,b} Marcela F. Pasetti,^{a,c} Myron M. Levine^{a,b,c}

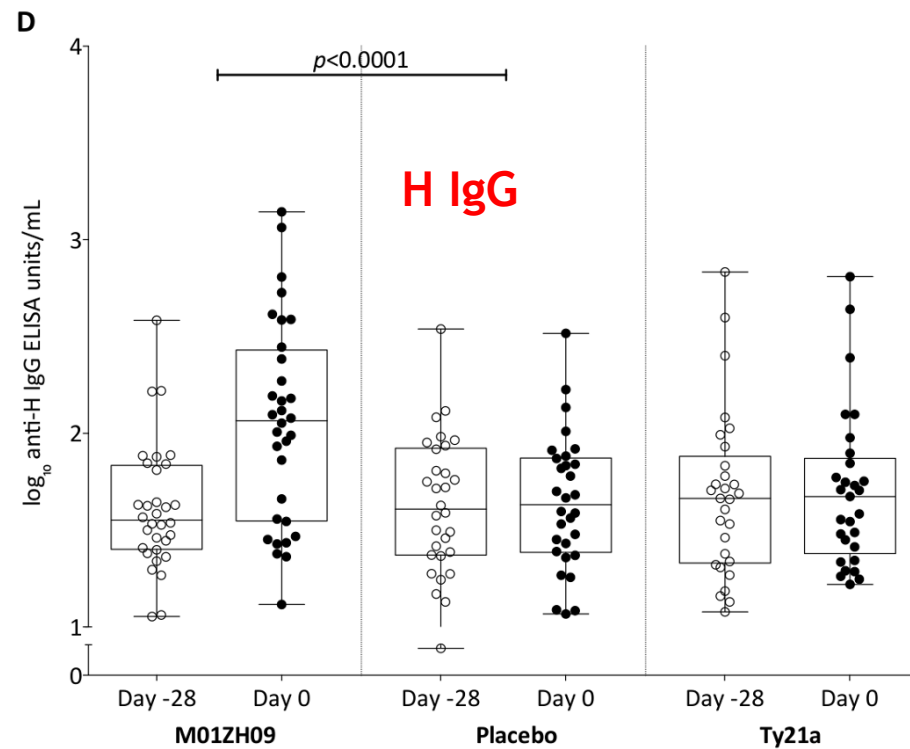
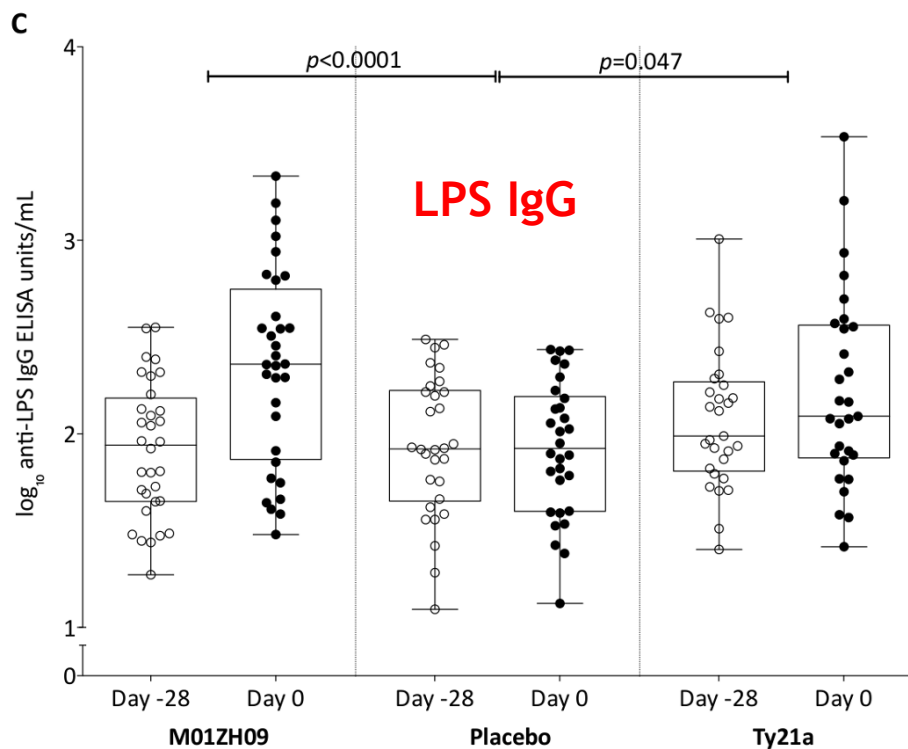
Center for Vaccine Development,^a Department of Medicine,^b and Department of Pediatrics,^c University of Maryland School of Medicine, Baltimore, Maryland, USA



M01ZH09 vaccination induces LPS IgG

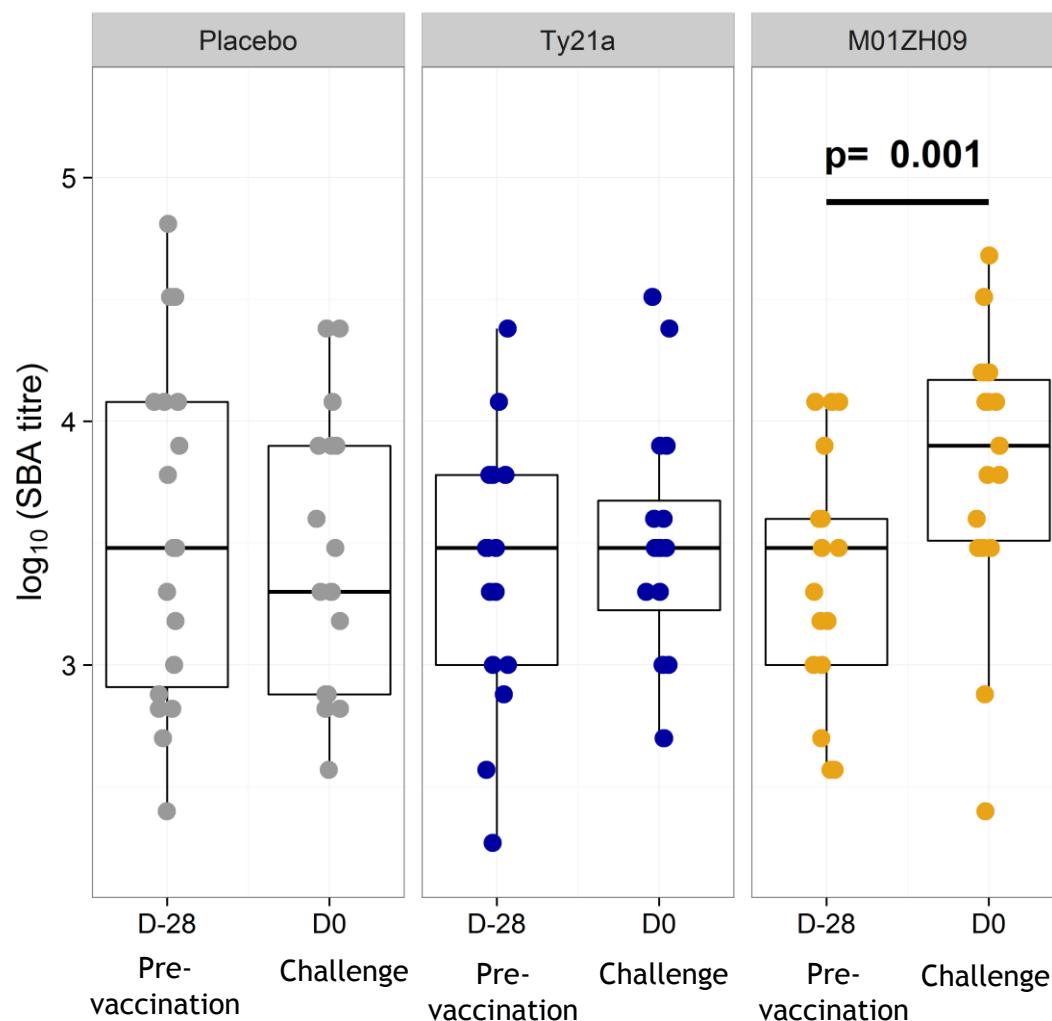


- Darton *et al* 2016: Showed M01ZH09 induced high antibody titres against key antigens



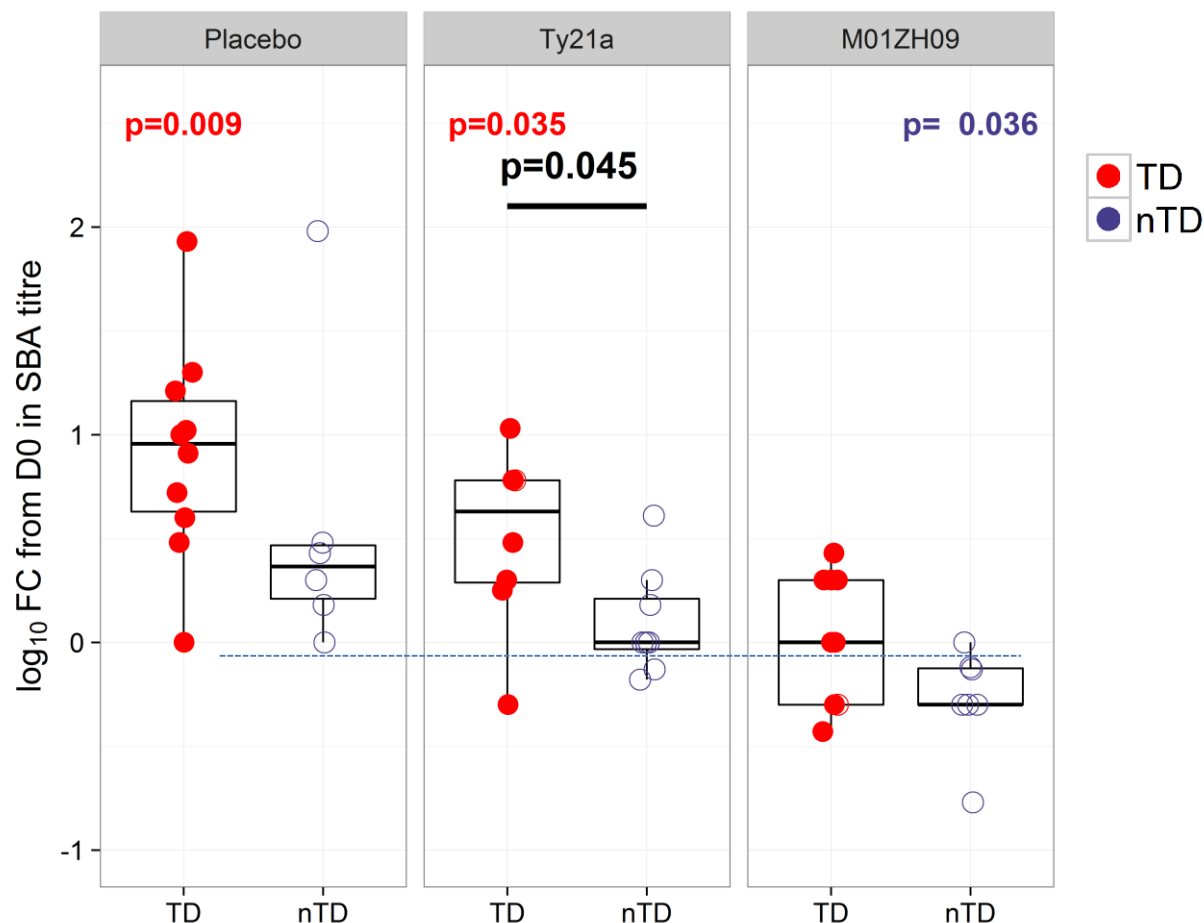
- Significant p value: Wilcoxon Signed Rank test between D-28 and D0

M01ZH09 vaccination induces bactericidal antibodies



- Significant p value: Wilcoxon Signed Rank test between D-28 and D0

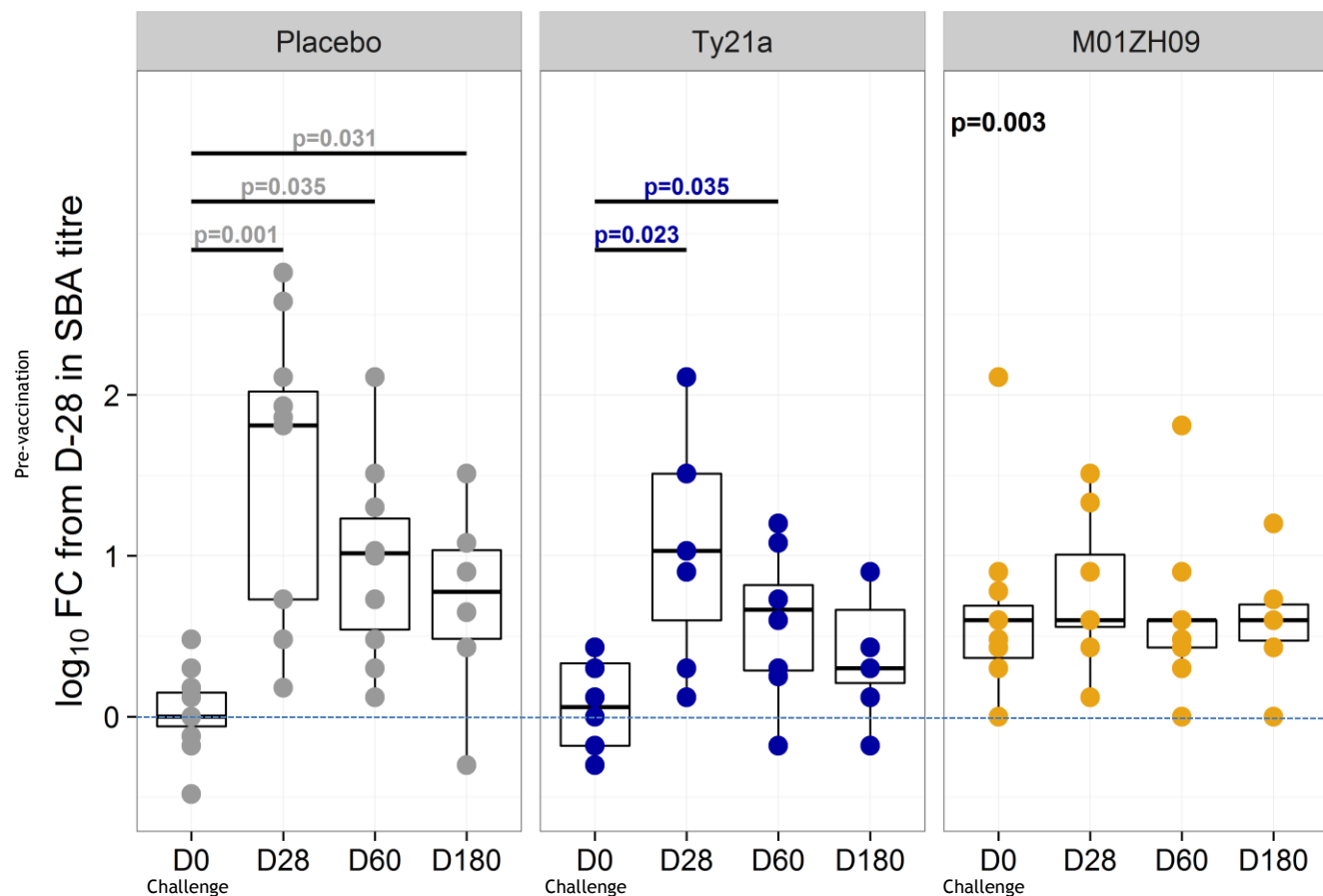
Typhoid challenge induces bactericidal antibodies in placebo and Ty21a



Mainly in TD groups

- Day 60 post-challenge shown
- Significant p values in colour: Wilcoxon Signed Rank test against 0 FC from D0
- Significant p value in black: Mann-Whitney between TD and nTD for that study arm

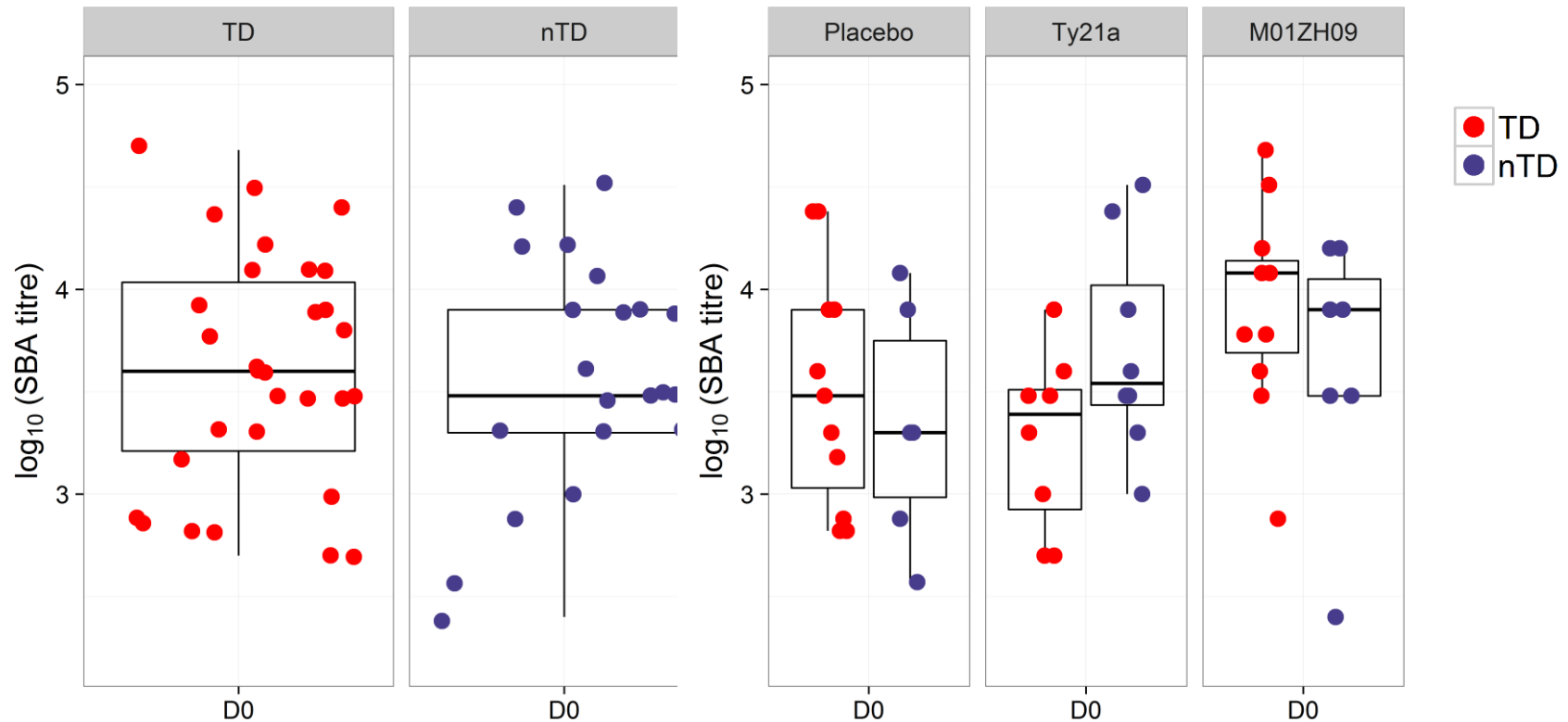
M01ZH09 bactericidal antibody activity persists after infection



- Only TD participants shown
- Significant p values in colour: Wilcoxon Signed Rank test to D0 within study arm
- Significant p value in black: Mann-Whitney test to D0 of Placebo



Bactericidal antibody activity does not protect against typhoid infection



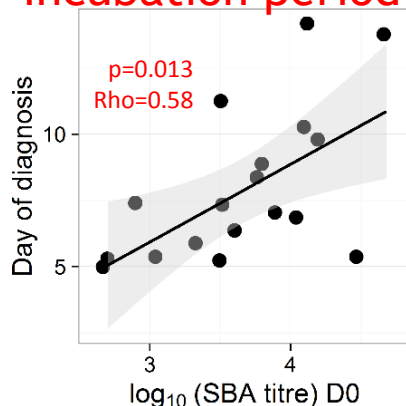
No significant p values following Mann-Whitney test between TD and nTD in combined or individual study arms



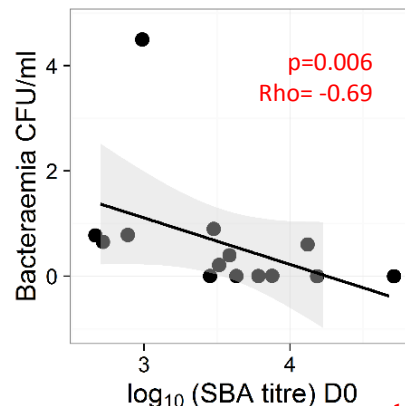
High bactericidal antibody activity reduces disease severity and cytokine response of vaccinated participants



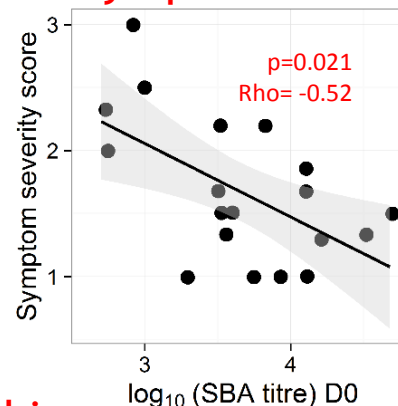
Incubation period



Bacteraemia



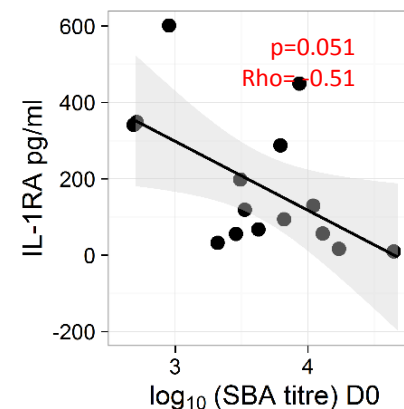
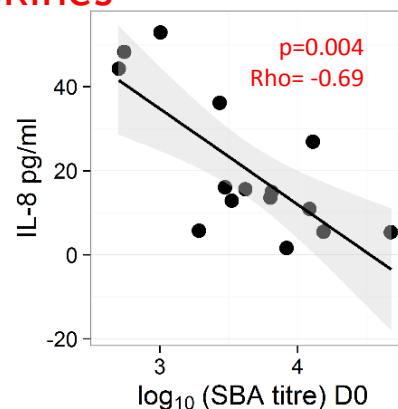
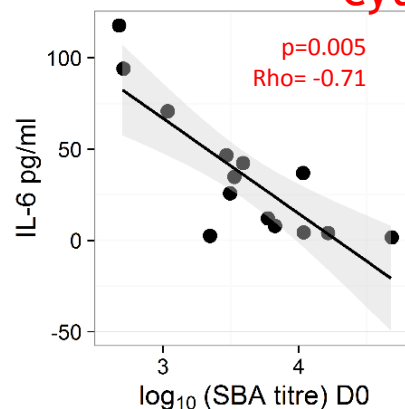
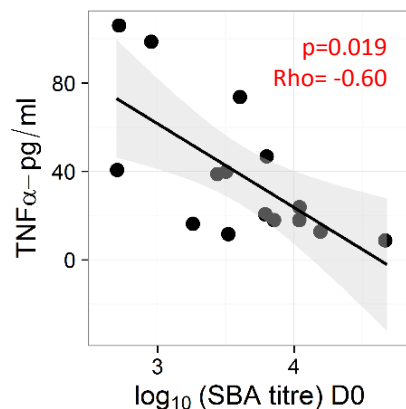
Symptom severity



No such correlations seen with placebo

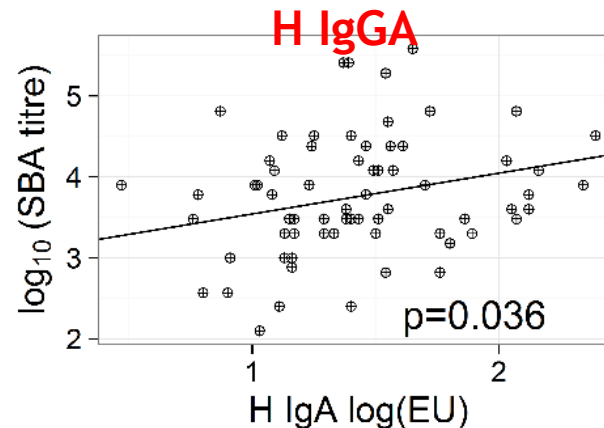
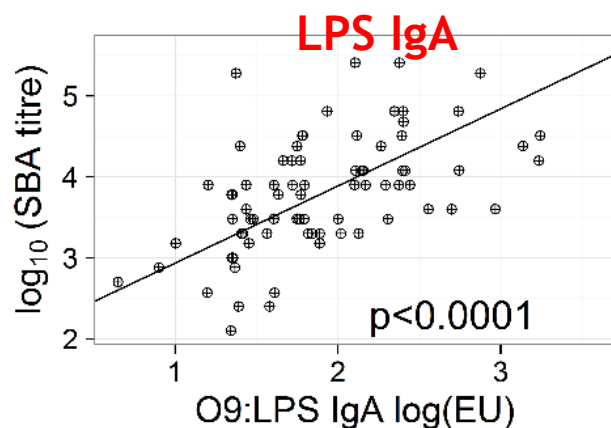
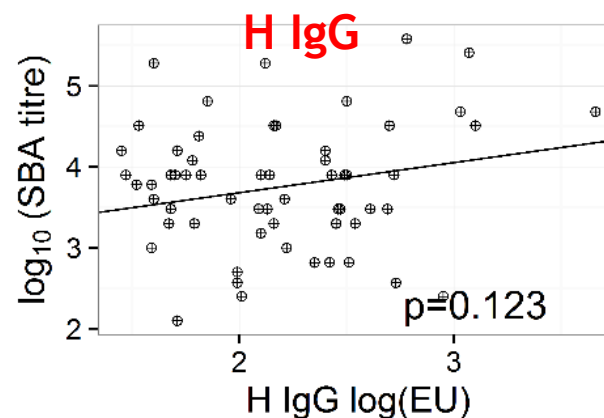
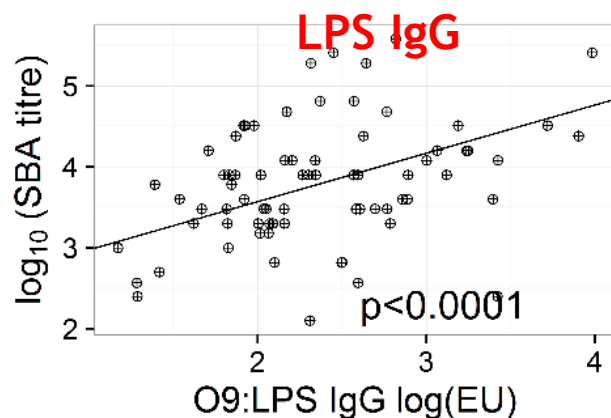
● M01ZH09 & Ty21a TD

cytokines



Anti-O9:LPS antibodies are the key mediators of bactericidal antibody activity

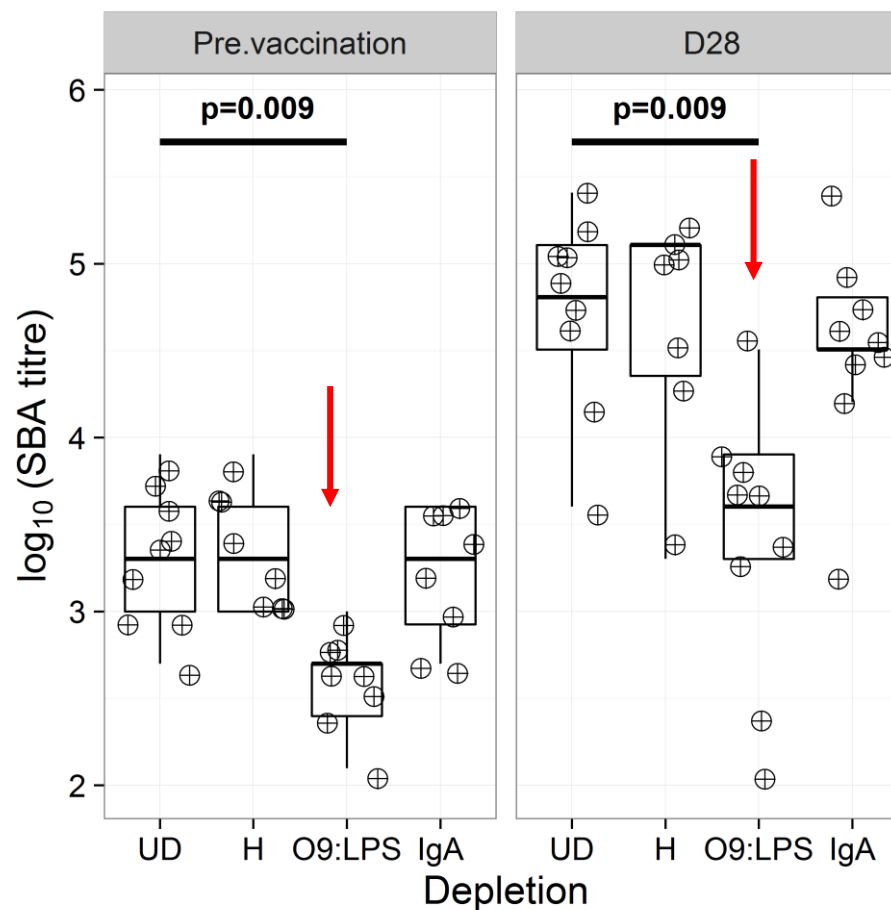
⊕ All study cohorts



HlyE, CdtB and PiiL IgG and IgA titres also tested for fit in mixed effects model.

Shown are fits to a mixed effects model that takes into account the multiple time points from each participant

Anti-O9:LPS antibodies are the key mediators of bactericidal antibody activity



Significant p values: Wilcoxon Signed Rank test against UD



Conclusions



In the challenge model:

- Bactericidal antibodies do not protect against typhoid infection
-But do reduce disease severity
- Most detectable bactericidal antibodies after live oral vaccines are directed at LPS
- Field?
- Paratyphoid?

Acknowledgements



SBA assays



ELISA assays



SBA assays

Helene B Juel*, Helena B Thomaidis-Brears*, Thomas C Darton, Claire Jones, Elizabeth Jones, Sonu Shrestha, Rebecca Sie, Ushma Galal, Stephen Baker, Christoph J Blohmke, Andrew J Pollard, (manuscript in prep)

*Joint 1st authors

- Prathiba Kurupati (Weatherall Institute of Molecular Medicine, University of Oxford) purified the HlyE antigen.
- Matthew K Siggins, Peter Hart, and Calman A MacLennan (School of Immunity and Infection, College of Medicine and Dental Sciences, University of Birmingham) and Leanne Marsay (OVG) advised on SBA assay optimisation
- Elizabeth Bateman (Dept. of Clinical Immunology, Churchill Hospital, Oxford) performed the sheep erythrocyte lysis assay on sera for complement in the SBA.

The Wellcome Trust logo, featuring the text "wellcome" in a bold, lowercase font, followed by "trust" in a smaller, lowercase font.

Supplementary

Estimate and p values in mixed effects models that correlated SBA data from all arms and time points to ELISA titres against specific antigens, taking into account random effects from grouping multiple time points from the same participants.

Correlation with	Estimate (95% CI)	p value
O9:LPS IgG	0.5957 (0.3, 0.9)	7.3e-5
O9:LPS IgA	0.9531 (0.7, 1.2)	1.6e-9
O9:LPS IgM	0.8876 (0.7, 1.1)	1.1e-13
H IgG	0.3703 (-0.1-0.8)	0.123
H IgA	0.5031 (0.1-1.0)	0.036
H IgM	0.3078 (0.2, 0.4)	1.4e-6
Vi IgG	0.2538 (-0.1, 0.6)	0.109
HlyE IgG	0.6144 (0.1, 1.2)	0.037
HlyE IgA	0.3073 (-0.2, 0.8)	0.223
CdtB IgG	0.0978 (-0.4, 0.6)	0.718
CdtB IgA	-0.0678 (-0.8, 0.6)	0.855
PiL IgG	-0.0433 (-0.5, 0.4)	0.843
PiL IgA	-0.1738 (-0.8, 0.5)	0.619