

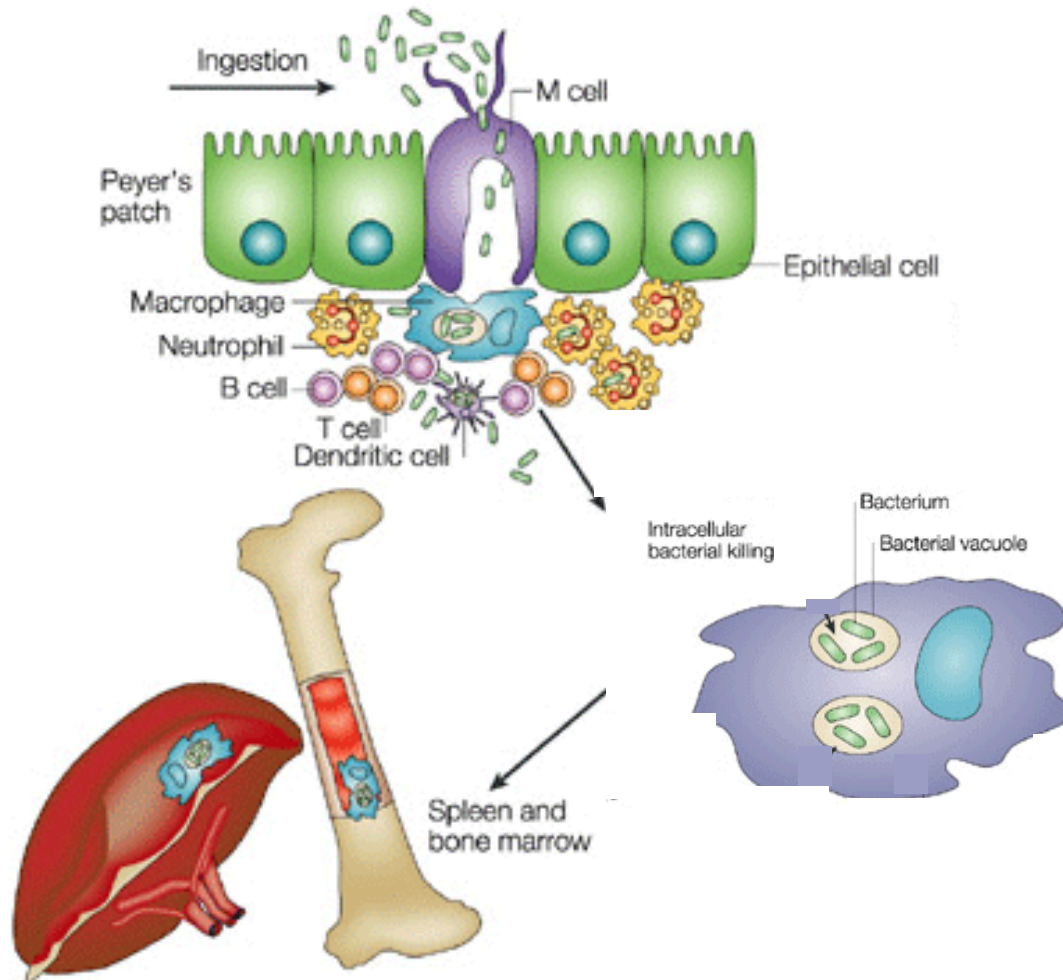
**Identification of immunogenic
Salmonella enterica serotype Typhi
antigens expressed uniquely *in vivo* in
chronic biliary carriers of *S. Typhi*
in Kathmandu, Nepal**

Richelle C. Charles, Tania Sultana, Mohammad Murshid Alam, Yanan Yu,
Meagan Bufano, Sean Rollins, Lillian Tsai, Jason B. Harris, Regina C.
LaRocque, Daniel T. Leung, Sabina Dongol, Buddha Basnyat, Stephen B.
Calderwood, Jeremy Farrar, Farhana Khanam, Firdausi Qadri, Stephen Baker,
Edward T Ryan

Enteric Fever

- Enteric fever is caused primarily by *Salmonella enterica* serovar Typhi and Paratyphi A
- They are both human restricted pathogen that causes an acute illness characterized by high fever, malaise, and abdominal pain.
- Endemic throughout the Asian and African continent
- There are 21 million cases per year resulting in 200,000 deaths

Pathogenesis



Typhi carriers

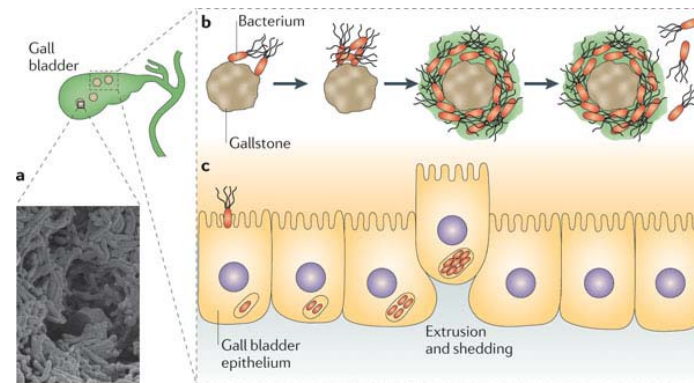
- 1-3% of infected individuals develop chronic infection in the gall bladder which may persist for decades
- May be reservoirs of infection within a community
- May contribute to transmission of infection
- May act as vehicles for introducing *S. Typhi* or *S. Paratyphi A* into previously uninfected communities.

Typhi Diagnostics- chronic carriers

- Microbiologic culture
 - stool – intermittent shedding
- Antibody detection of capsular Vi antigen
 - anti-Vi antibody titers a sensitivity 75% and specificity >95%.
 - In Vietnam 3000 potential carries screened.
 - 3% with positive test
 - no S. Typhi could be isolated from fecal samples

Identification of biomarkers for *S. Typhi* carriage

- We applied an immunoscreening technique, *in vivo*-induced antigen technology (IVIAT) to identify potential biomarkers unique to *S. Typhi* chronic carriers.
- Hypothesis: *S. Typhi* surviving in the biliary tract of humans may express a proteomic profile distinct from that expressed in bacteria grown using standard *in vitro* conditions



Genomic Inducible Expression Library

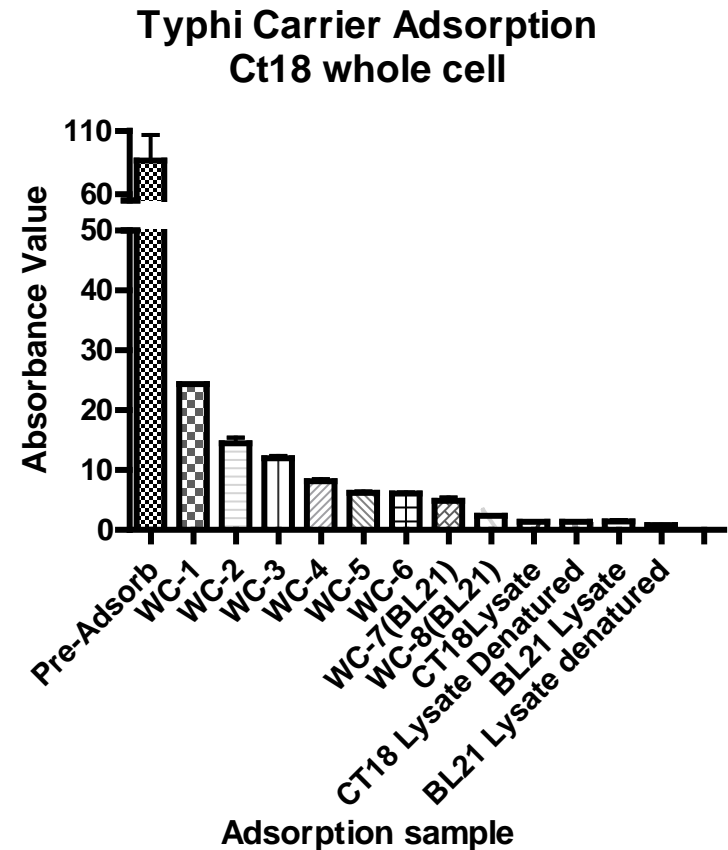
- *Salmonella enterica* serovar Typhi CT18 (5133713 bp, 4753 genes)
 - Chromosome 4,809,037 bp
 - 2 plasmids
 - pHCM1 218,150 bp
 - pHCM2 106,516 bp
- Library size: 120,000 clones (500-1500 bp fragments) in *E. coli* BL21DE3

Sample Collection

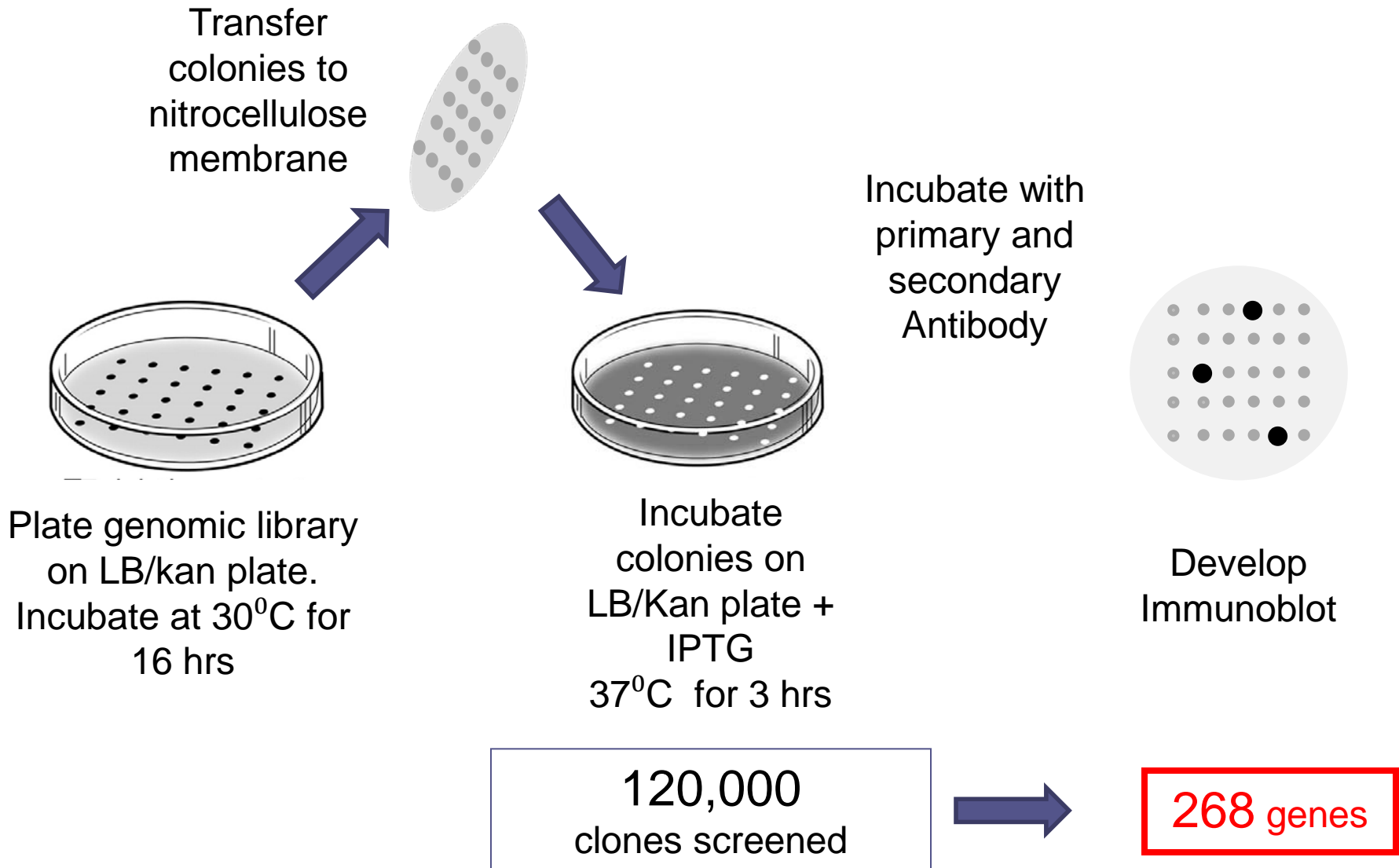
- Individuals undergoing elective cholecystectomy in Nepal were enrolled
 - At the time of cholecystectomy
 - venous blood sample taken and stored
 - bile sample was taken for microbiologic analysis

Sera adsorption

- Selected sera:
 - pool of 5 patients with bile cultures positive for *S. Typhi*
- Sera adsorbed against the following samples
 - *S. Typhi* whole cell
 - *S. Typhi* lysate
 - *E. coli BL21 DE3* (with empty vector, pet30c) whole cell
 - *E. coli BL21 DE3* (with empty vector, pet30c) lysate

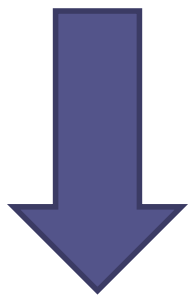


Screening - Immunoblots

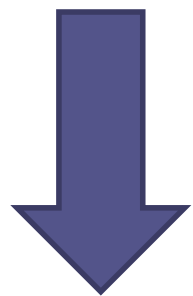


Screening - Immunoblots

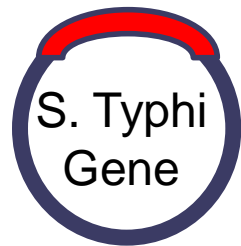
268 genes



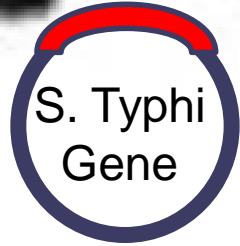
56 genes



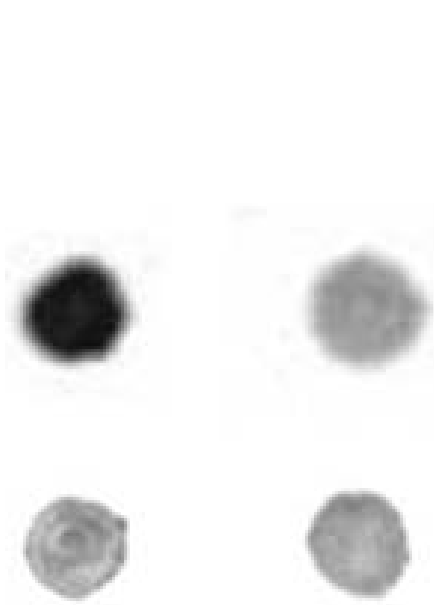
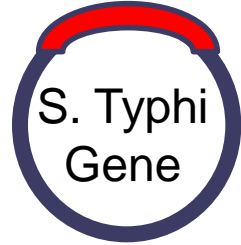
13 genes



Typhi
Carrier Sera



Healthy
control
Sera



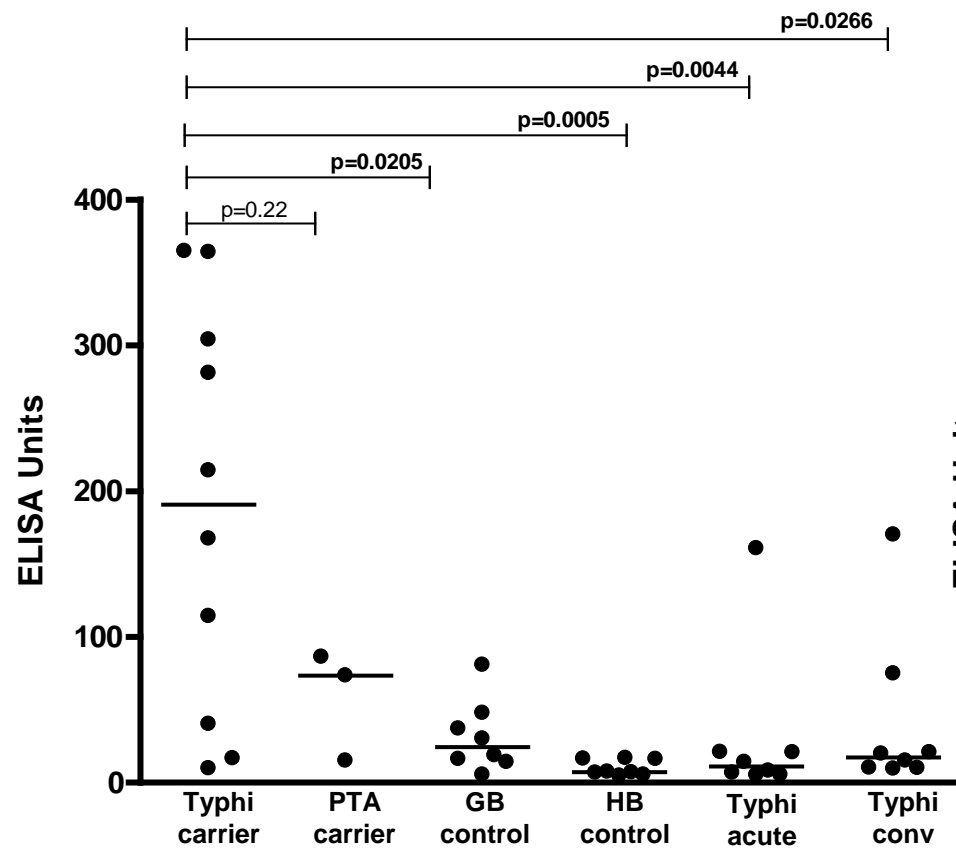
Top 13 Hits

STY Locus	Gene Name	Sequence
STY1364		hypothetical periplasmic protein
STY2657	xapB	xanthosine permease
HCM2.0069c		hypothetical protein
HCM2.0043		hypothetical protein
HCM1.137		Replication initiation protein
STY2386		putative lipoprotein
STY1479	yncE	possible ATP-binding protein
STY2454	yejE	putative binding-protein-dependent transporter
STY2248	pduG	PduG protein
STY3709	purH	phosphoribosylaminoimidazolecarboxamide formyltransferase and IMP cyclohydrolase (bifunctional enzyme)
STY2155	sirA	invasion response-regulator
HCM1.213c		Putative transposase
STY0712		haemolysin-related protein

YncE (STY1479) response

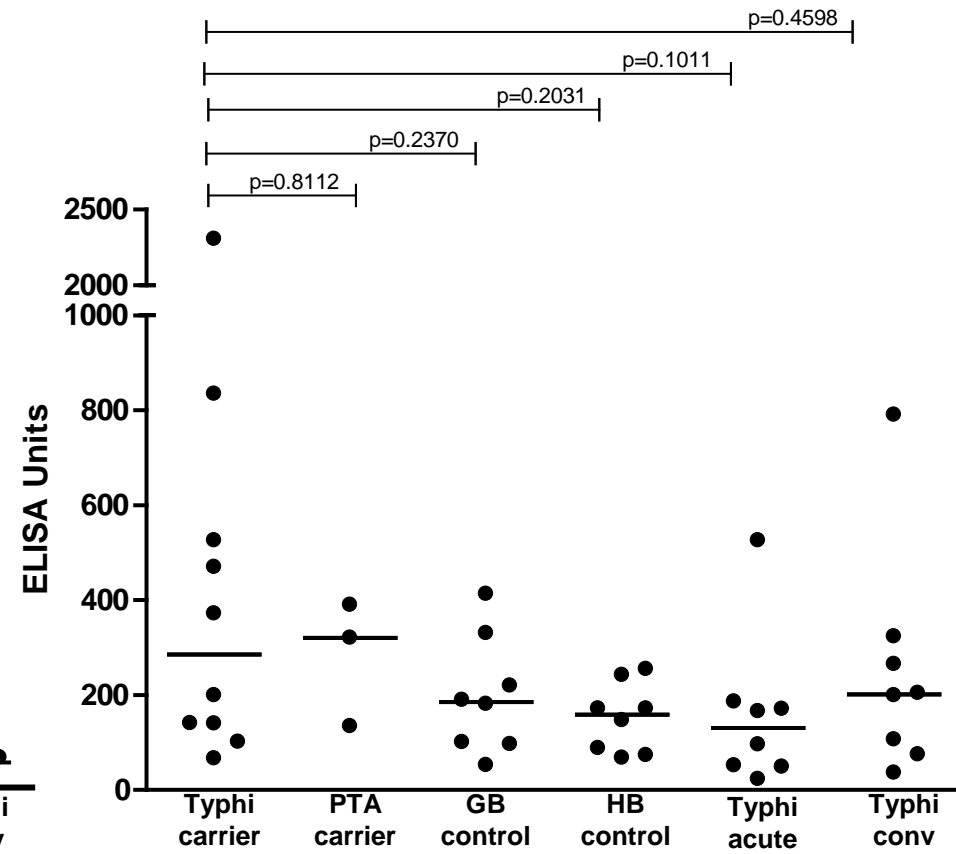
A)

YncE-IgG

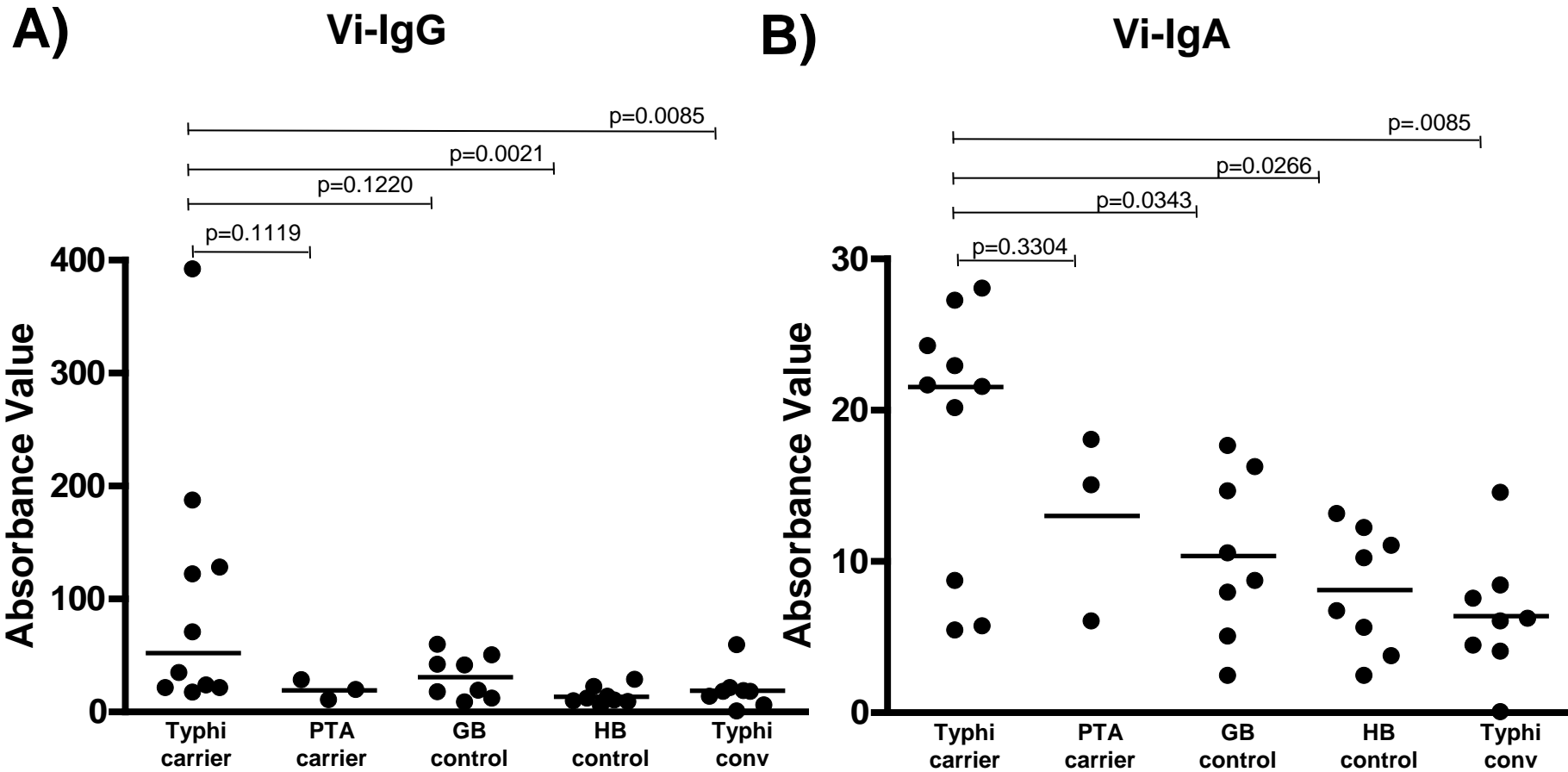


B)

YncE-IgA



Vi Antigen Response



Conclusion

- We have identified a number of immunoreactive antigen in *S. Typhi* carriers, including YncE.
- Further evaluation of YncE and other antigens could lead to development of an improved diagnostic assay and improved understanding of *S. Typhi*'s survival within the biliary tract of carriers

Acknowledgements

- Massachusetts General Hospital
 - Ed Ryan
 - Steve Calderwood
 - Jason Harris
- ICDDR,B
 - Firdausi Qadri
 - Tania Sultana
- OUCRU
 - Stephen Baker
 - Buddha Basynat
 - Sabina Dongol
- **Funding Support**
 - NIAID U01 AI058935
 - NIAID U01 AI077883
 - NIAID K08 AI089721
 - D43 TW005572 NIH/Fogarty International Center: Training program in vaccine development
 - MGH Physician-Scientist Career Development Award