Innovative enterobactin-specific egg yolk antibodies for controlling Gram-negative pathogens

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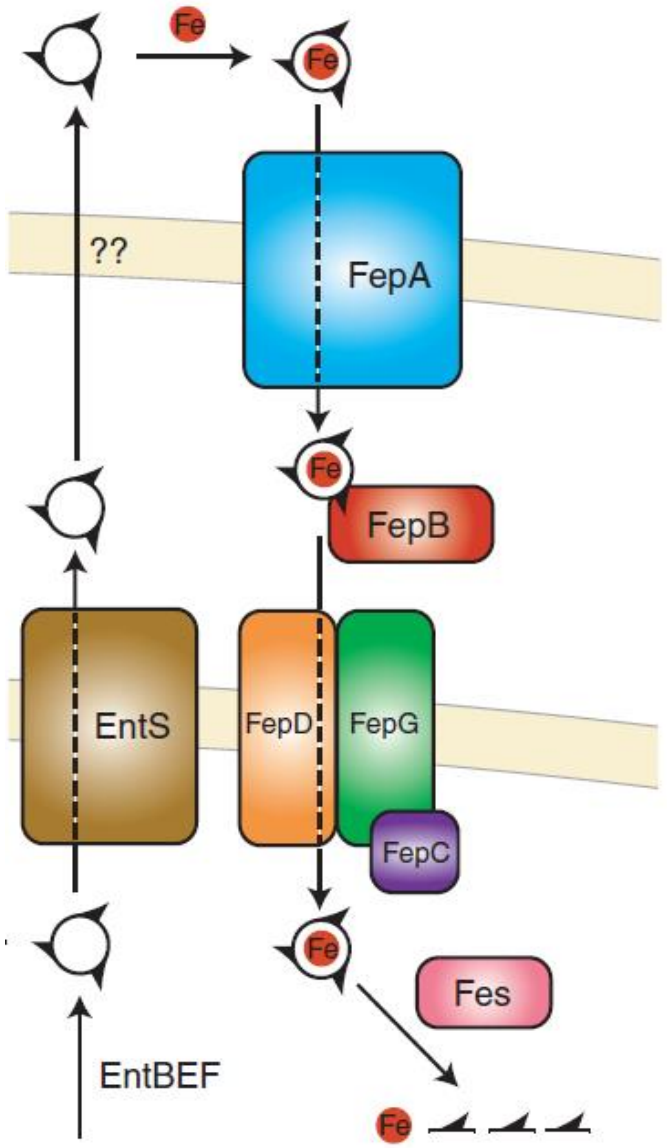
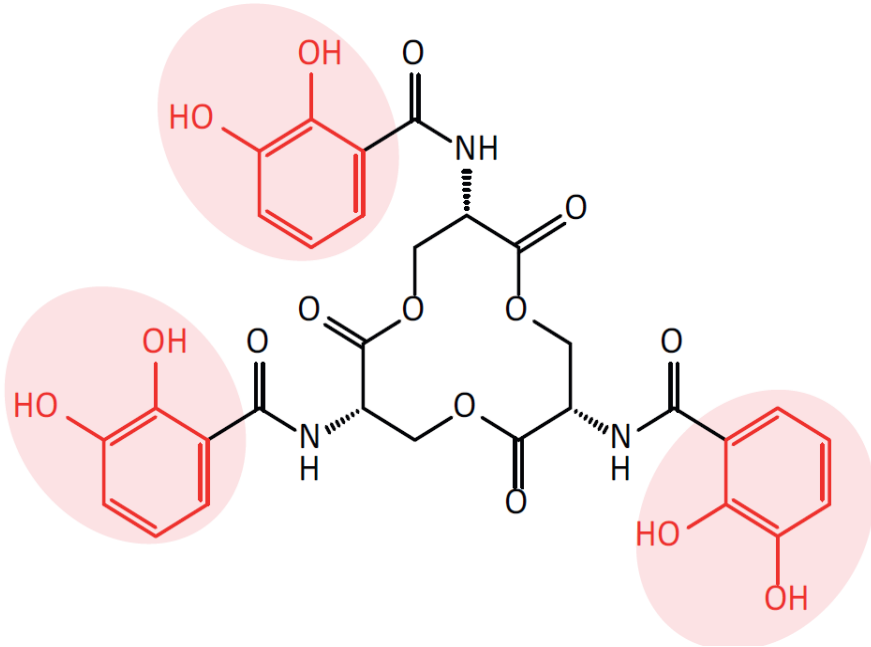
Ferric Enterobactin (FeEnt) Acquisition System



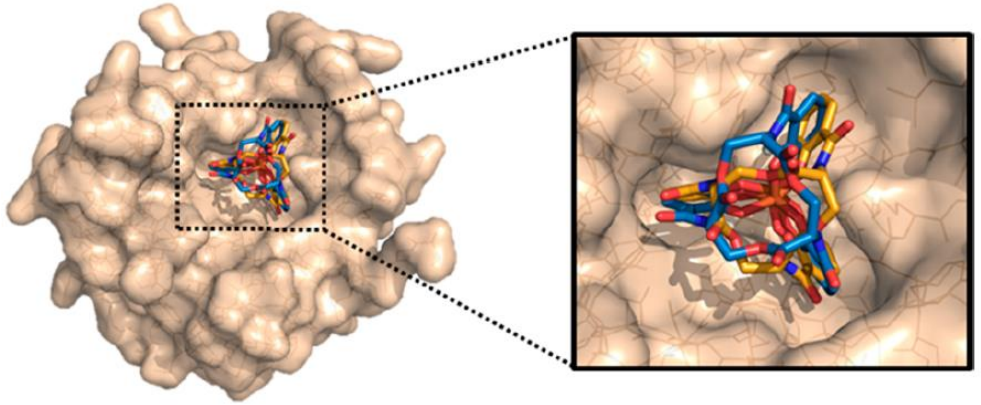
Enterobactin (Ent) has the highest affinity to Fe^{3+} among different iron acquisition systems



Fischbach MA et al. 2006. *Nat Chem Biol.*
Wilson BR et al. 2016. *Trends Mol Med.*

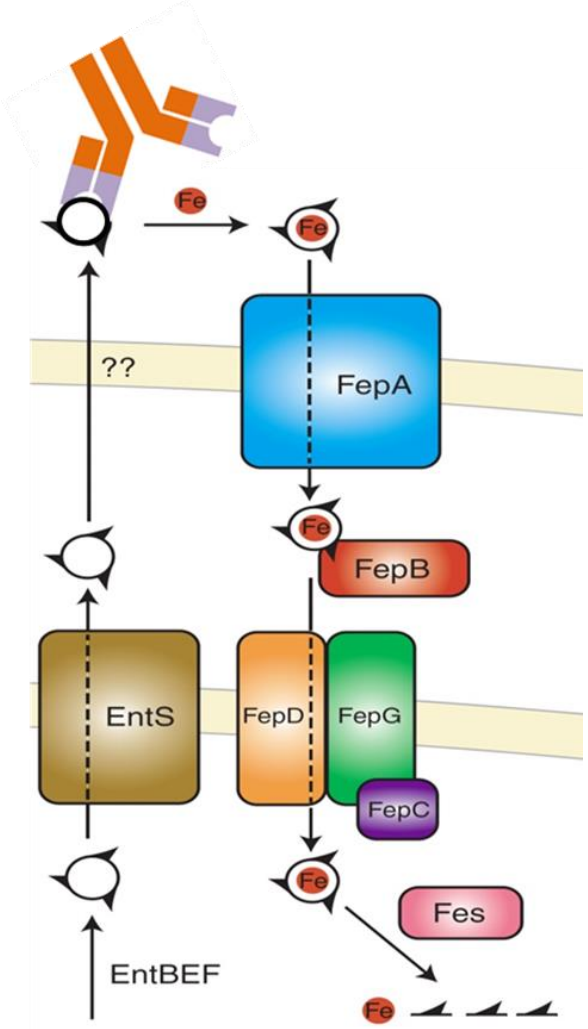
Lipocalin: an innate bacteriostatic agent

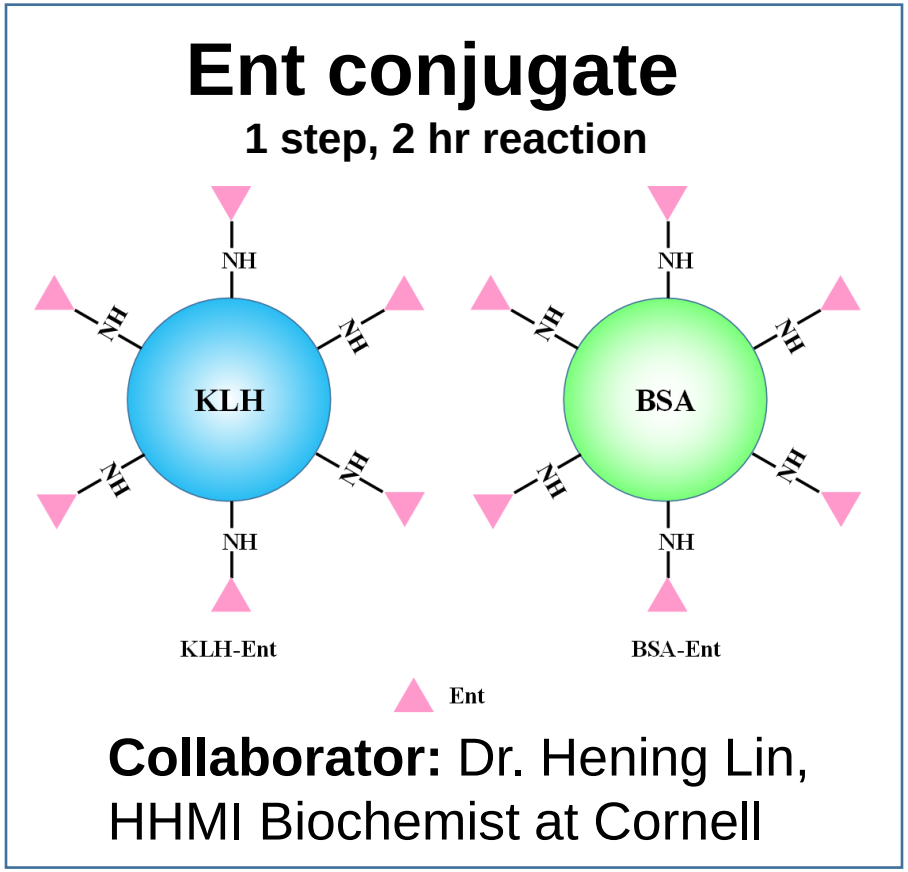
- **Lipocalins: host acute phase proteins**
- **Also function as bacteriostatic agent to interfere with Ent-mediated iron acquisition through potent Ent-binding ability**



Flo *et al.* 2004. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature* **32**:917-921

Hypothesis: Ent specific antibodies may function as a lipocalin-like effective bacteriostatic agent





Collaborator: Dr. Hening Lin, HHMI Biochemist at Cornell

Siderophore-based immunization strategy to inhibit growth of enteric pathogens

Martina Sassone-Corsi[a,b,1], Phoom Chairatana[c,1], Tengfei Zheng[c], Araceli Perez-Lopez[a,b], Robert A. Edwards[d], Michael D. George[e,2], Elizabeth M. Nolan[c,3,4], and Manuela Raffatellu[a,b,3,4]

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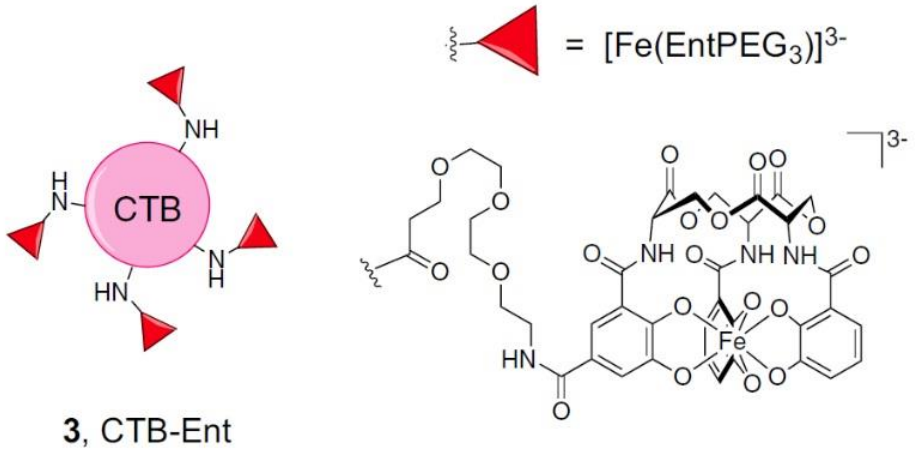
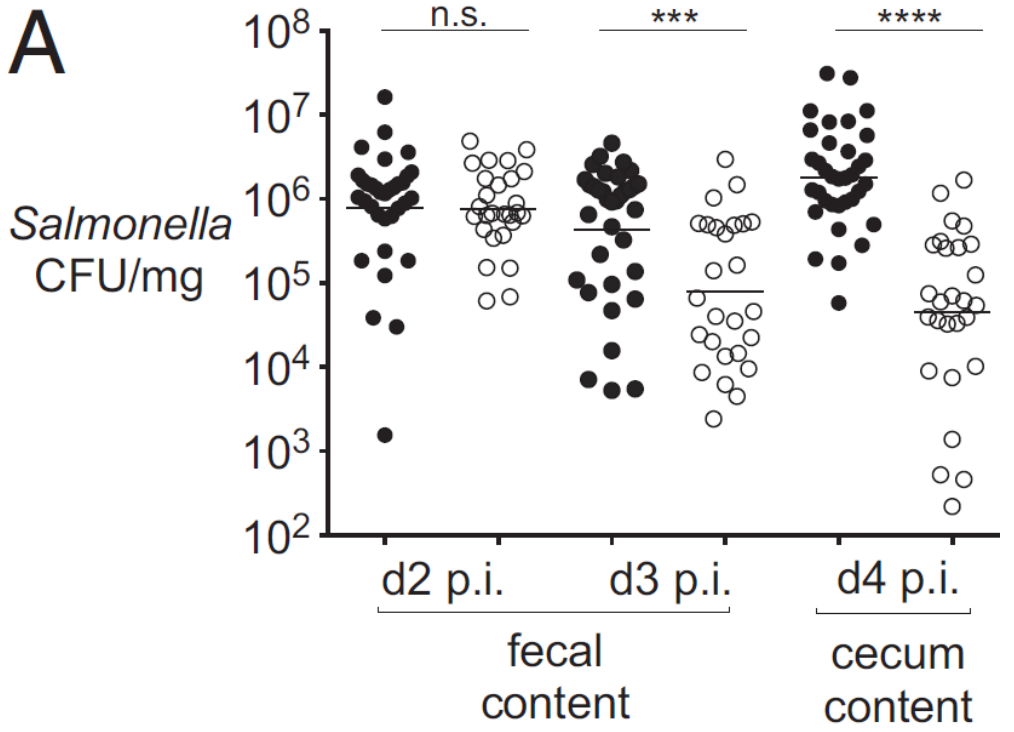
Edited by Ralph R. Isberg, Howard Hughes Medical Institute/Tufts University School of Medicine, Boston, MA, and approved July 11, 2016 (received for review April 25, 2016)



3, CTB-Ent

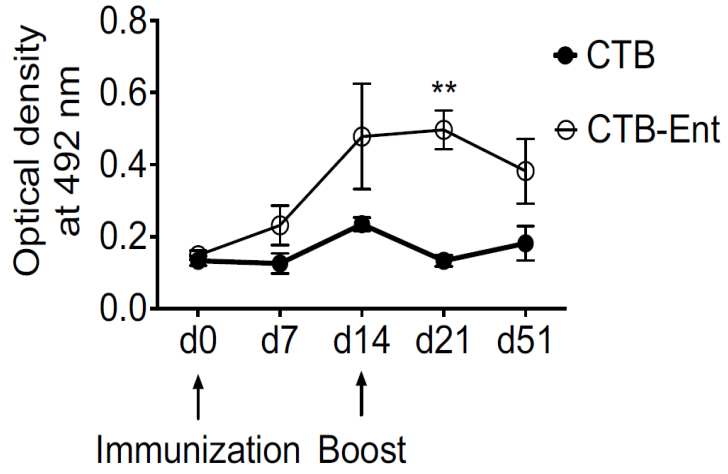
10-step lengthy synthesis of CTB-Ent conjugate



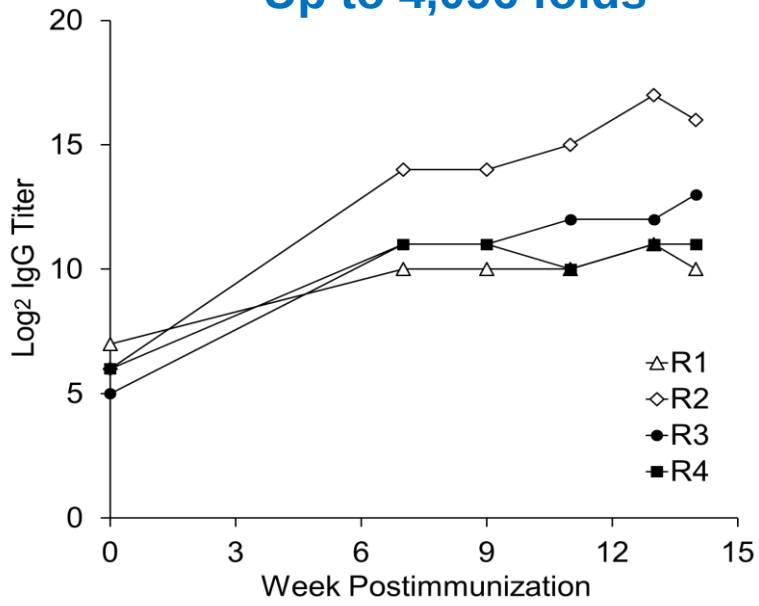
Advantages of our Ent conjugate vaccine

1. Conjugate preparation: simple and easy
2. Induce much higher titer of Ent-specific antibodies

The *PNAS* Study
< 4-fold



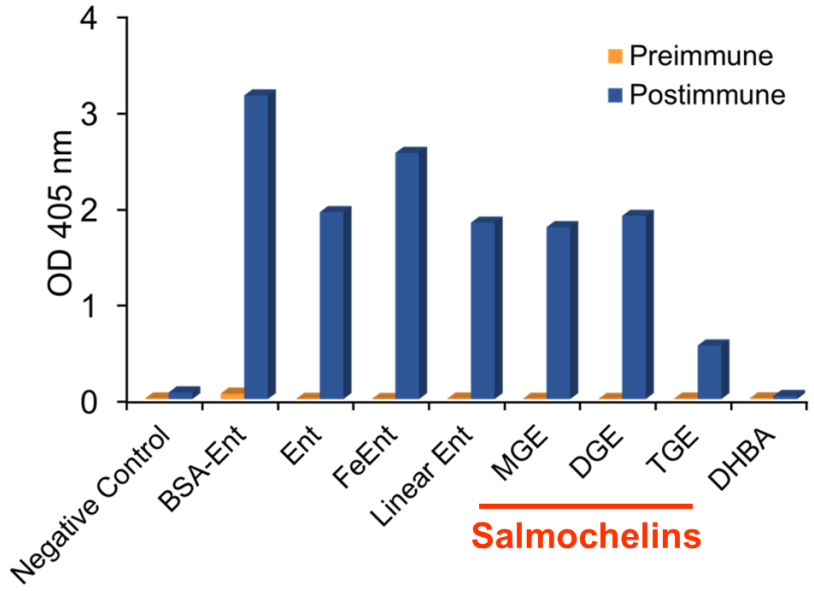
Our Vaccine
Up to 4,096 folds



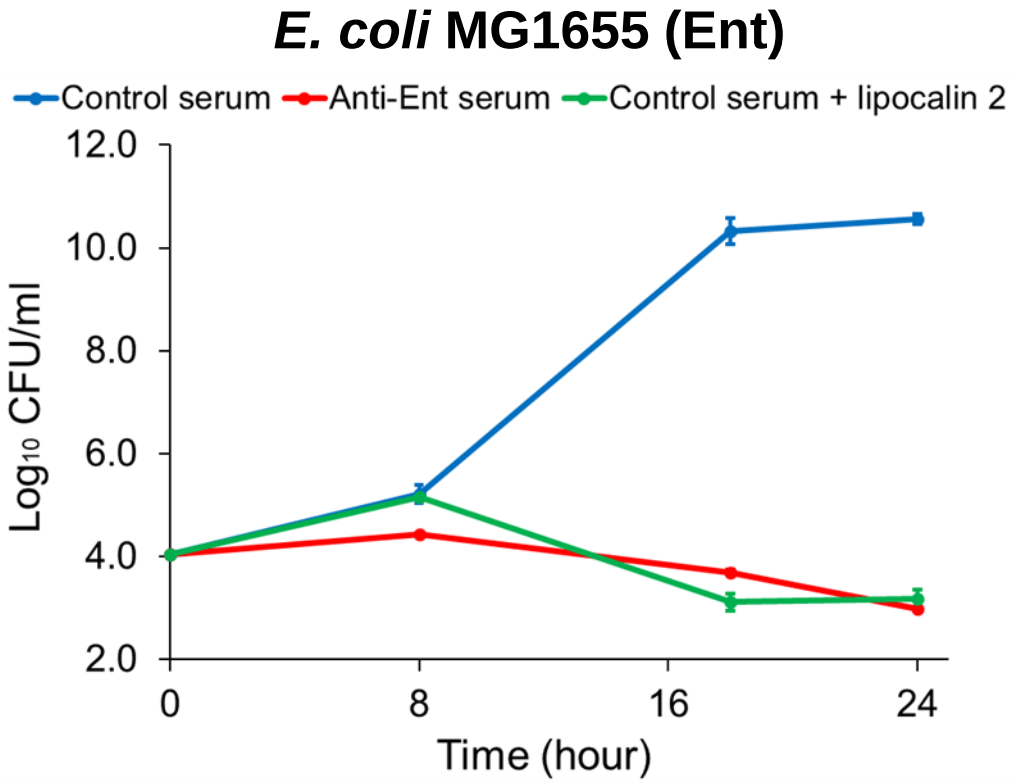
Wang *et al.* 2019. Characterization of the enterobactin specific antibodies raised by a new enterobactin conjugate vaccine. *Applied and Environmental Microbiology*. 85:e00358-19

Advantages of our Ent conjugate vaccine

3. Ent antibodies bind to various Ent derivatives

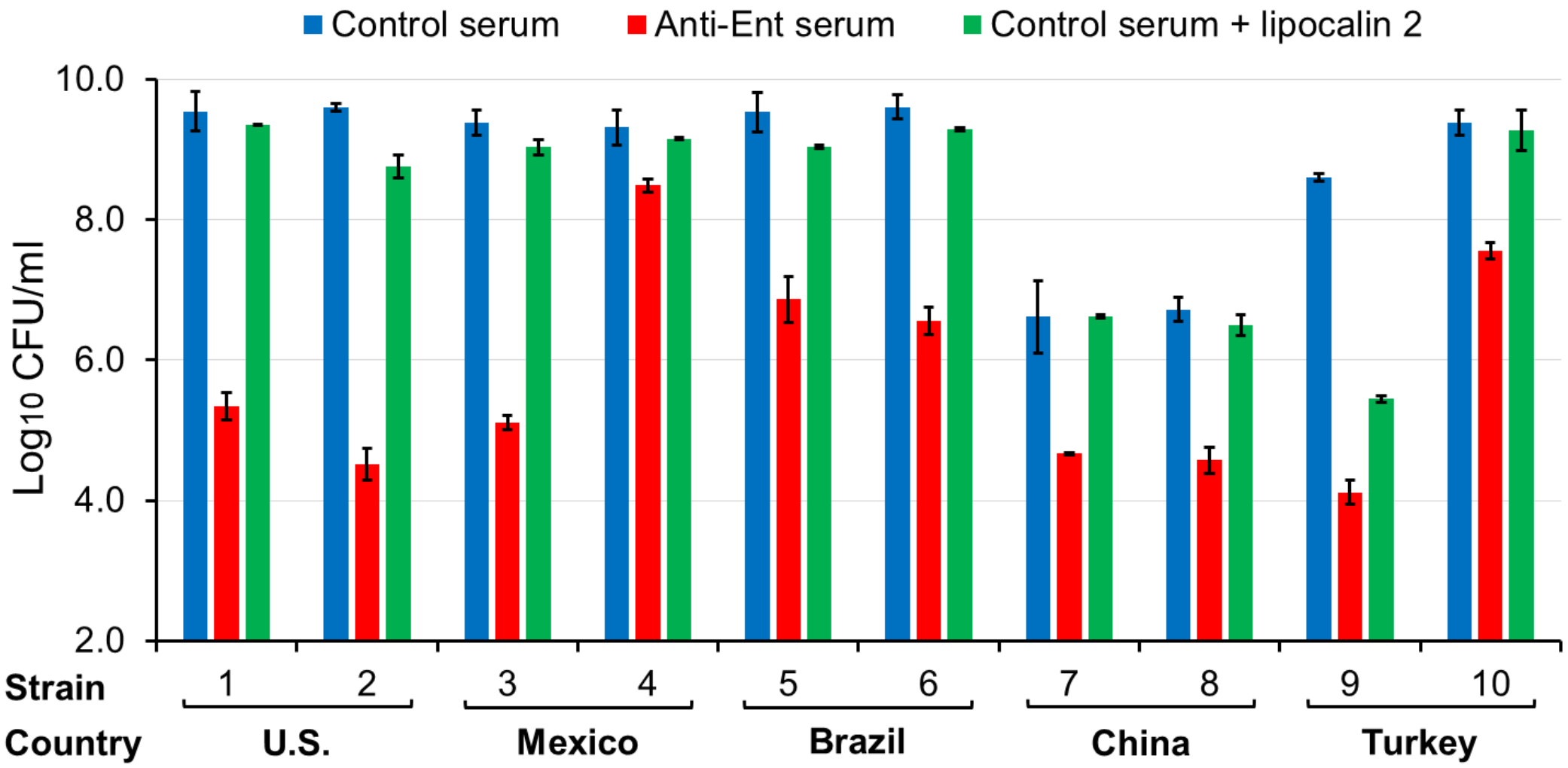


4. Ent antibodies inhibit Ent-dependent growth

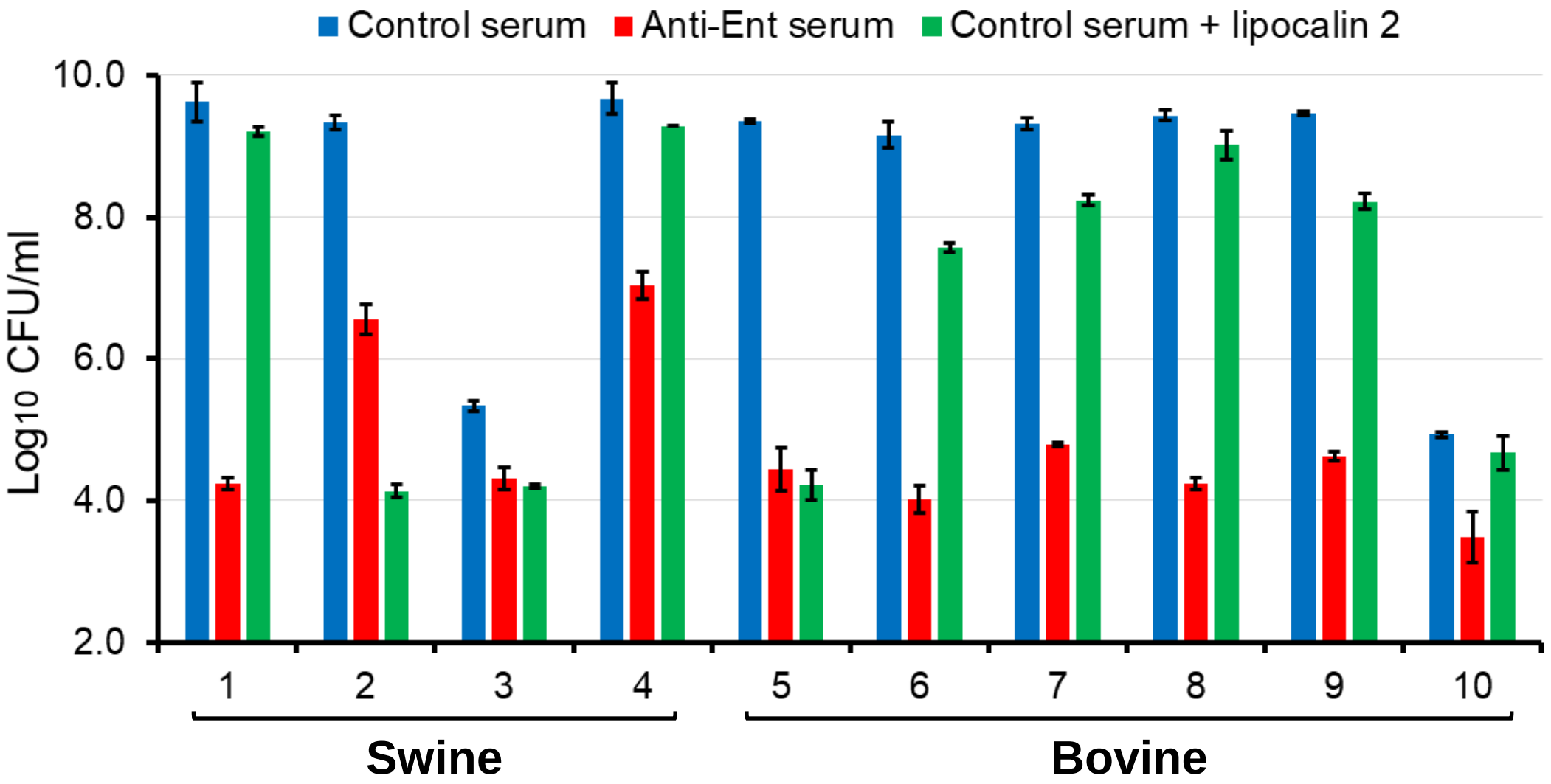


Wang *et al.* 2019. Characterization of the enterobactin specific antibodies raised by a new enterobactin conjugate vaccine. *Applied and Environmental Microbiology*. 85:e00358-19

Avian pathogenic *E. coli* (n=10)

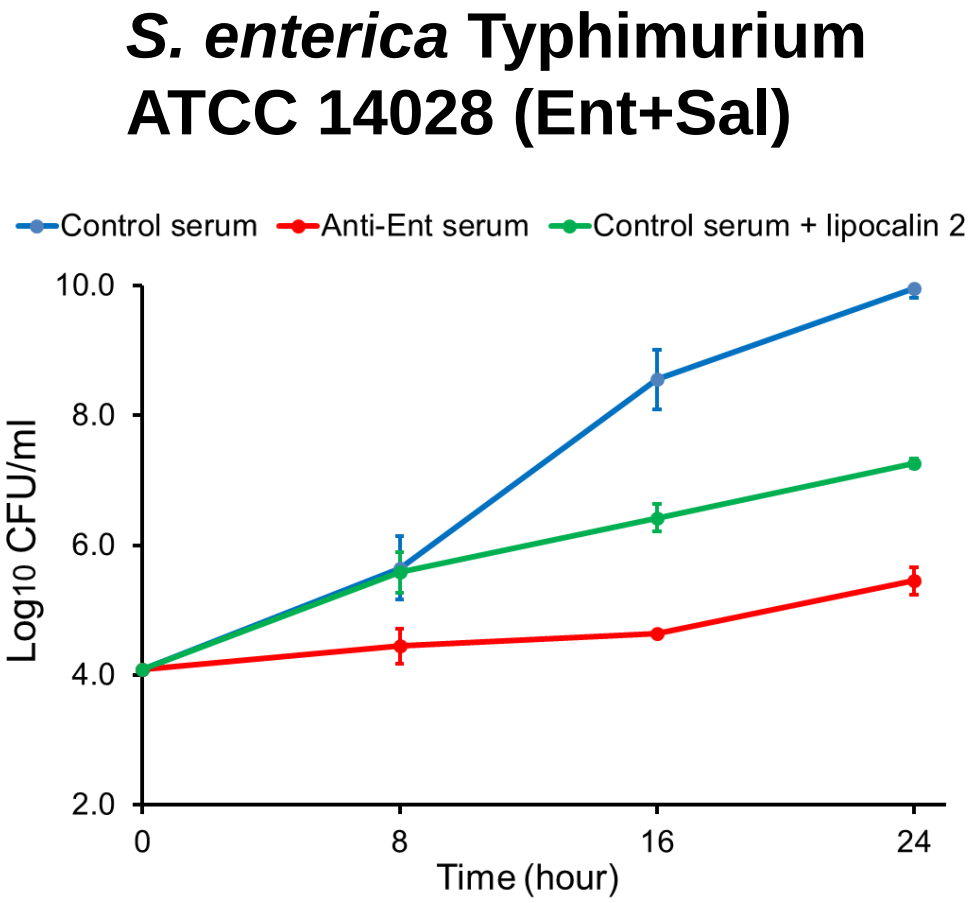


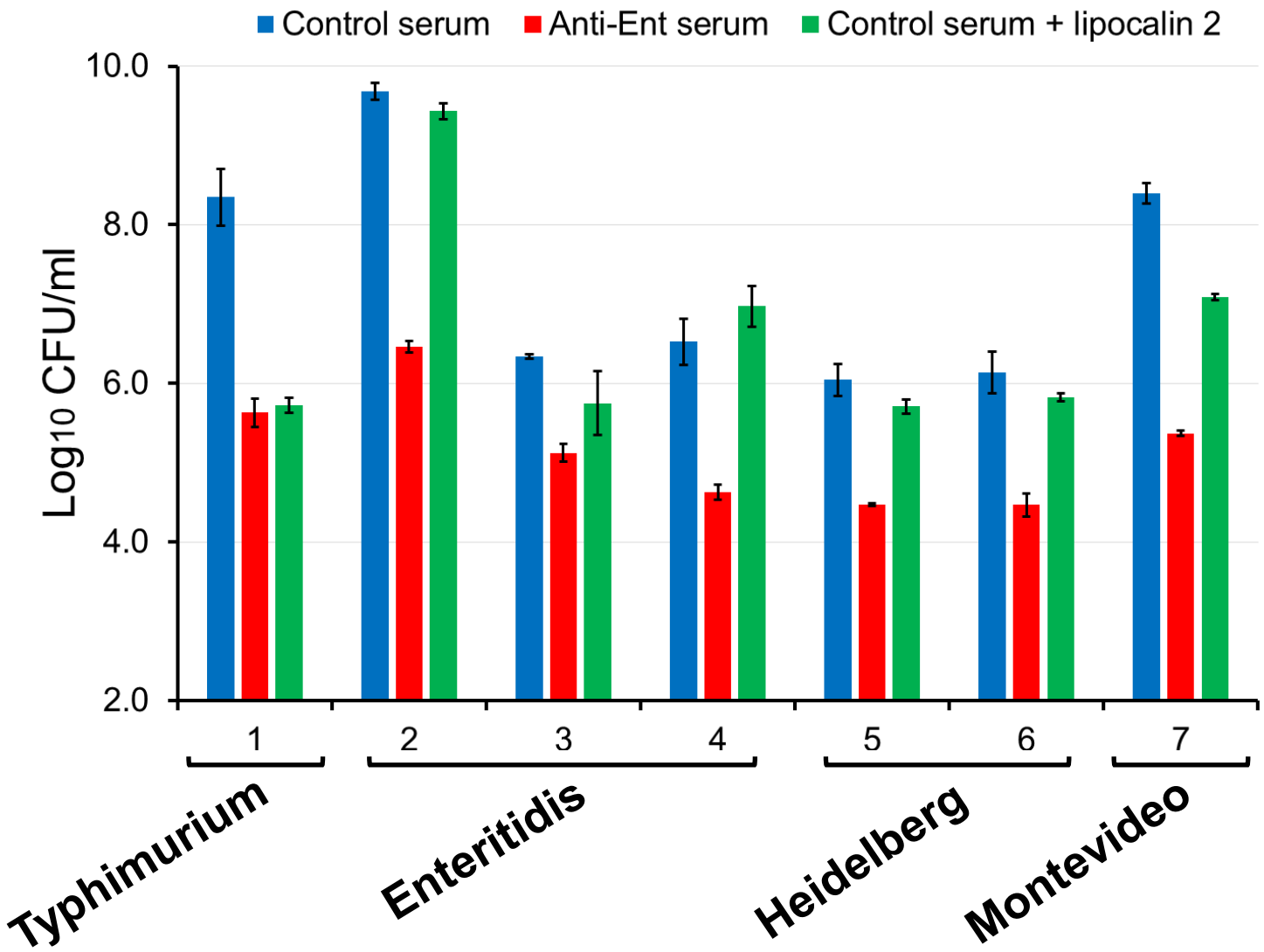
Swine and bovine *E. Coli* isolates (n=10)



Different serotypes of *Salmonella enterica* (n=8)

S. enterica Typhimurium ATCC 14028 (Ent+Sal)





Production of Ent-specific Egg Yolk Antibodies for Passive Immunization

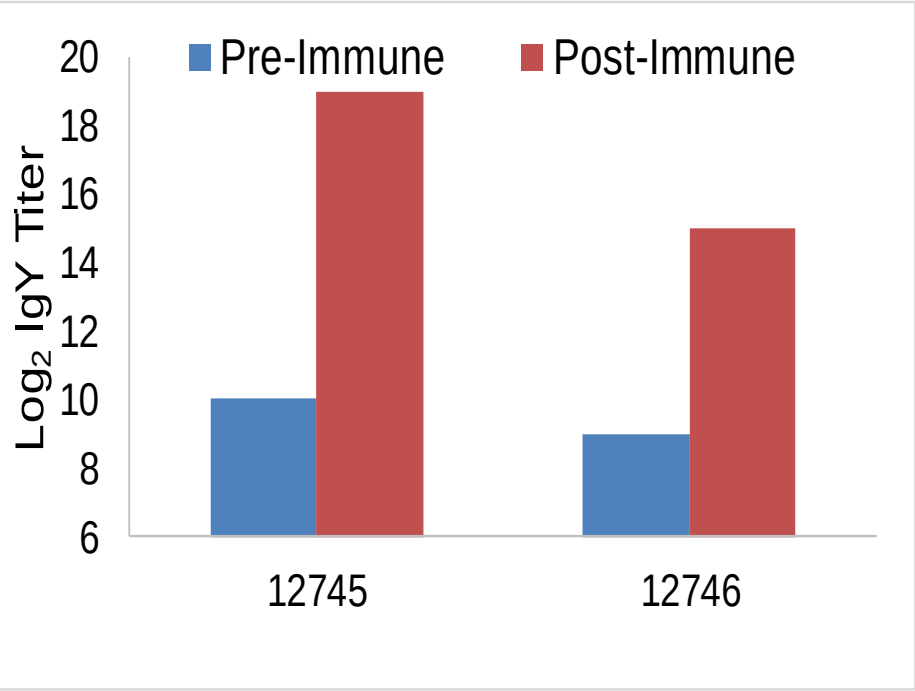
- Cost-effective: a laying hen is a “small factory” for producing large quantities of egg yolk antibodies (IgY)
  - ✓ More than 20 g of IgY per year
- Non-invasive
- IgY antibodies are fairly stable during various processing steps and under physiological conditions

Gadde *et al.* Passive immunization with hyperimmune egg-yolk IgY as prophylaxis and therapy for poultry diseases--A review. *Anim Health Res Rev.* 2015 Dec;16(2):163-76

Production of hyperimmune Ent-specific Egg Yolk

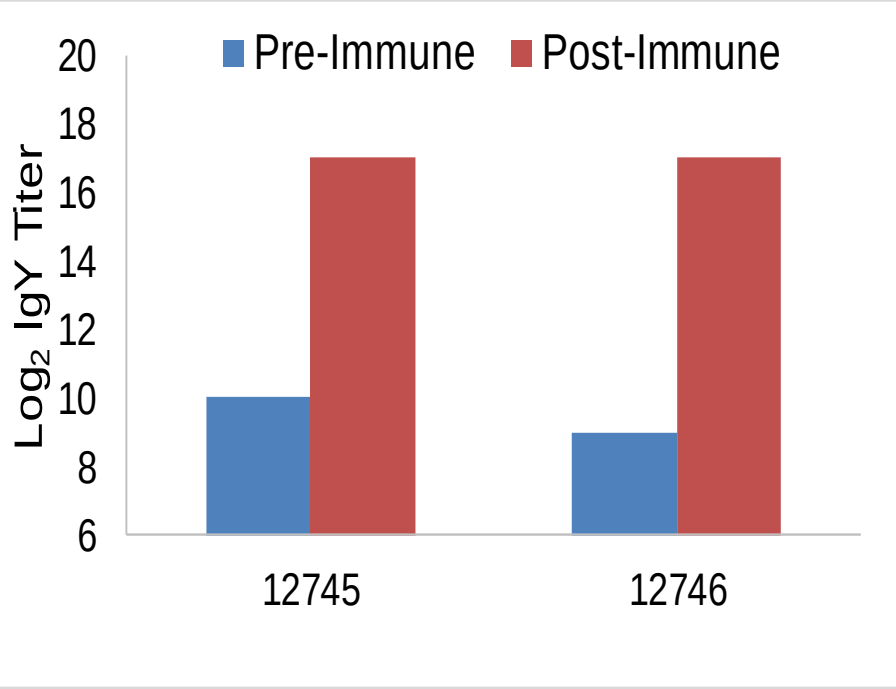
Serum

↑64-512 fold



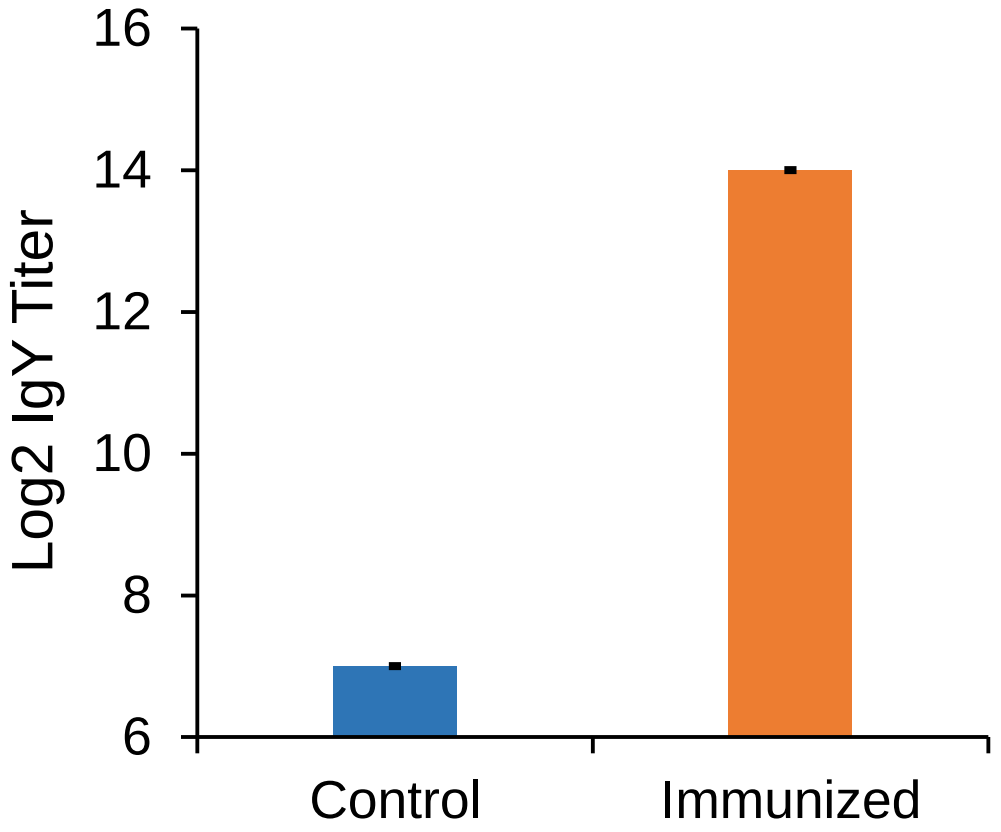
Egg yolk

↑128-256 fold



High-level of Ent-specific IgY in Egg Yolk Powder

Egg yolk powder



Conclusions

- We have developed an innovative Ent conjugate vaccine that can trigger high titer of Ent-specific antibodies
- The Ent-specific antibodies inhibited iron-dependemt growth of diverse Gram-negative pathogens
- We also optimized vaccination regimen to produce high-level of Ent-specifc hyperimmune egg-yolk IgY
- The high level of Ent-specific IgY in egg yolk was still sustained after lyophilization

Acknowledgment

Key Lab Personnel

Dr. Ximin Zeng

Huiwen Wang

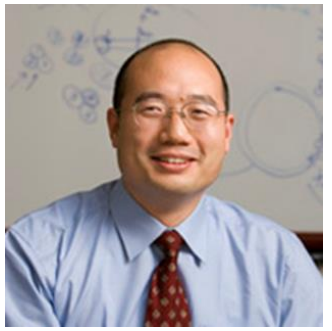
Collaborator

Dr. Hening Lin

Department of Chemistry

& Chemical Biology

Cornell University



National Institute of Allergy and Infectious Diseases

USDA United States Department of Agriculture

National Institute of Food and Agriculture

Questions?