Comparative Invasion of Salmonella Serotypes

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Introduction:

Salmonella enterica infection is the most common cause of gastroenteritis in humans. . There are over 2500 known serotypes of *Salmonella* with a wide range of hosts and virulence. The morphology of the lipopolysaccharide (LPS) component of the cell wall is variable between *Salmonella* strains and it is an important characteristic that impacts the virulence of the strain. The LPS is known to be involved in many factors facilitating successful infection by the pathogen including motility, adhesion and invasion, colonization, replication and resistance to complement activation and macrophage killing. Elucidating the correlation between the morphological components of the LPS and their respective effects on the pathology of Salmonella infection may provide insight into successful vaccine development against *Salmonella* and other gram negative bacterial pathogens. In this study six subtypes that are known human pathogens were used to infect HeLa epithelial cells and THP-1 differentiated macrophages. A previous study performed in the lab showed that the LPS of these six strains had different molecular weights, indicating differences in morphology. We hypothesize that there will be significant differences in the rate of invasion between these strains. The strains studied include: *Arizonae, Berta, Seftenberg, Tennessee, Thompson O,* and *Typhimurium.* The second focus of this study was to observe how different rates of invasion correlated with IL-8 production. IL-8 is important for chemotaxis of leukocytes in response to infection. We hypothesize that accompanying the differential invasion rates there will be different amounts of IL-8 production.

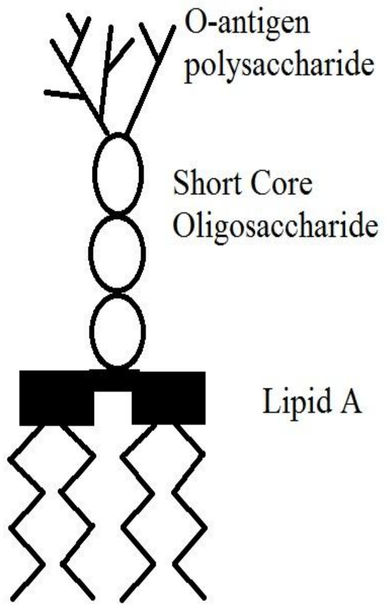

Figure 1: LPS Structure.

- Conserved lipid A region which stimulates activation of Toll Like Receptor 4 (TLR-4) activating transcription of pro-inflammatory cytokines
- Variable short core oligosaccharide
- Variable O-antigen polysaccharide
- Variable regions are the target of modification in vaccine development.
- Particularly the truncation of the O-antigen which seems to be the primary defense of *Salmonella* against serum complement activation

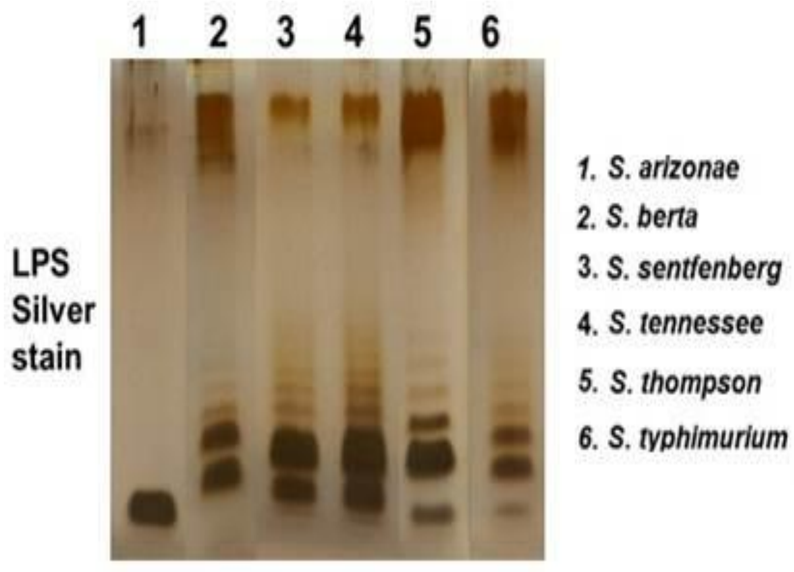

Figure 2: Silver stain showing different LPS MW between *Salmonella* strains

Results:

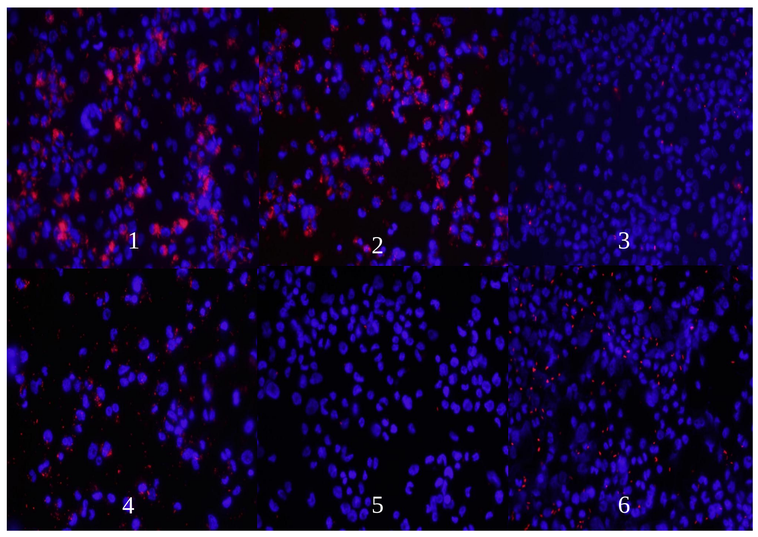

Figure 3: IFA of the six virulent *Salmonella* strains showing infection of HeLa cells. *1) Arizonae 2) Berta 3) Seftenberg 4) Thompson O 5) Tennessee 6) Typhimurium . Note control not shown.*

Methods: HeLa cells were seeded 24 hours prior to infection at a density of 10^5 cells/well on 24-well plates. The 6 strains were previously transformed with PDsRed2 and single colonies were selected and grown overnight statically in 3 mL LB containing ampicillin. Cultures were pelleted down and re-suspended in RPMI 1640 without antibiotics at a MOI of 20/200 uL/well. The RPMI 1640 culture media was aspirated from the wells and 200 uL of the above bacterial suspensions were added to each well and incubated at 37˚ C in 5% CO_2 for 2 hours. Wells were washed 3 times with 500 uL of PBS followed by addition of 300 uL of RPMI 1640 containing gentamycin and the plates were incubated in previously listed conditions for 1 hour. The wells were washed as described before and 200 uL of 2 % paraformaldehyde was added and plates were incubated at room temperature for 15 min. The paraformaldehyde was aspirated and the wells were washed. 300 uL of cold methanol was added and the plate was incubated at 4˚ C for 15 min. Methanol was aspirated and the wells were washed. 10 uL of DAPI fluid was mounted on glass slides and the cover slips were transferred from the wells to the slides.

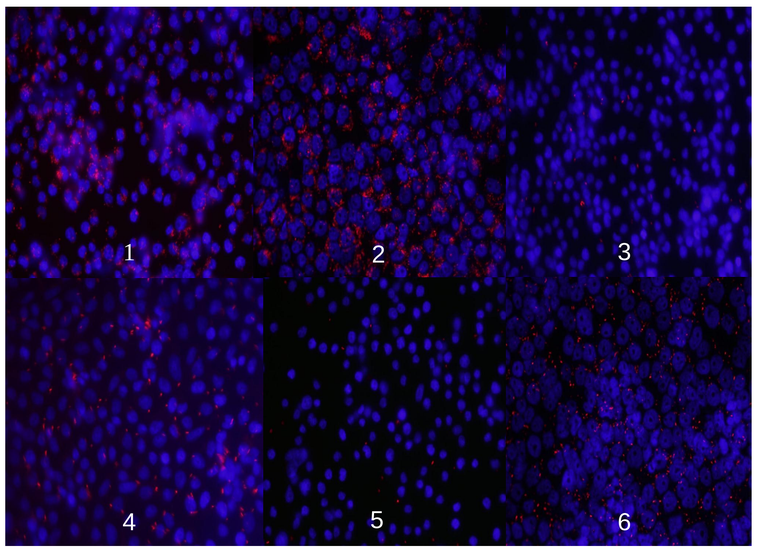

Figure 4: IFA results for infection of THP-1 macrophages. *1) Arizonae 2) Berta 3) Seftenberg 4) Thompson O 5) Tennessee 6) Typhimurium Note: control not shown.*. Methods are described in the previous figure.

Acknowledgments:

This study was supported by NIH Public Health Service grant RO1AI083364 (GZ) from the National Institute of Allergy and Infectious Diseases.
Stipend provided by Idexx-Bioresearch
Thank you to Dr. Zhang and Dr. Peng for their help and support!

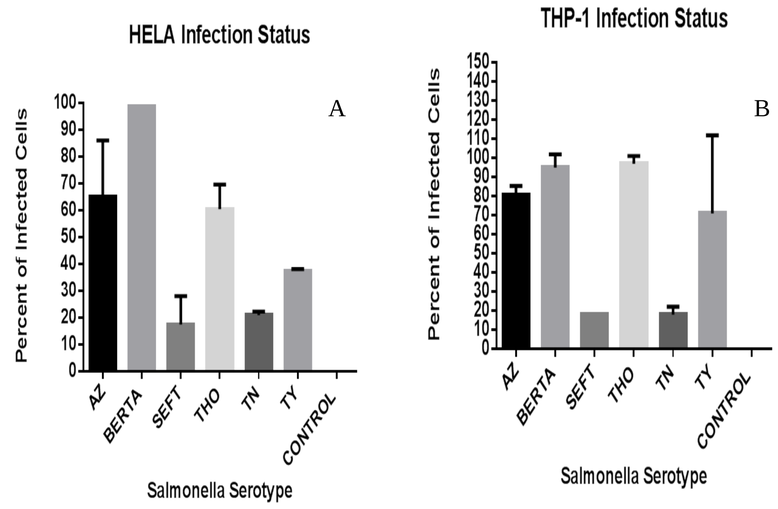

Figure 5: Graphs showing the percent of infected cells two hours post infection. Two 100 cell counts were done for both HeLa and THP-1 infected cells for each serotype. One-way ANOVA and multiple comparisons were performed to determine significant differences in the rate of infection. A) Significant differences were found between Az v. Seft*, Az v. TN*, Berta v. Seft ***, Berta v. THO *, Berta v. TN ***, Berta v. TY **, Seft v. THO * and THO v. TN*. B) Significant differences were found between Berta v. Seft *, Berta v. TN*, Seft v. THO * and THO v. TN*.

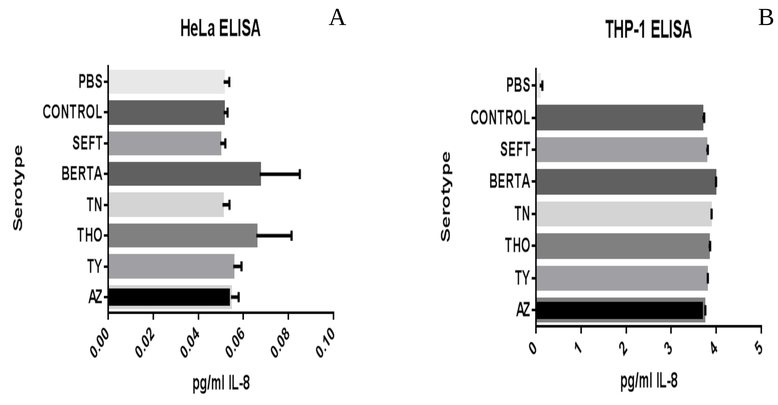

Figure 6: Results of ELISA analysis of IL-8 production by HeLa and THP-1 cells. One way ANOVA and multiple comparisons were performed to detect significant differences in the amount of IL-8 produced by the two different cell types. A) There were no significant differences in IL-8 production between the Salmonella serotypes and there was no detectable IL-8 production by the HeLa cells due to infection with any of the serotypes. B) Significant differences in IL-8 production were observed between AZ v. THO *, AZ v. TN ***, AZ v. Berta ****, TY v. TN *, TY v. Berta ****, THO v. Berta ****, TN v. Berta **, TN v. Seft * and Berta V. Seft ****.

Methods: ELISA was performed according to the protocol provided in the kit. The kit used was Human IL-8 ELISA Ready Set Go! (second generation) produced by affymetrix eBioscience INC.

Conclusions:

The results of this study showed that there were significant differences in the invasion rate between Salmonella serotypes. These differences were more pronounced in the HeLa epithelial cells in which invasion is more dependent on the characteristics of each serotype versus THP-1 macrophages which also phagocytize the bacteria. The specific morphology of the LPS that makes certain serotypes more successful at infecting cells remains to be determined.The analysis of IL-8 production showed that the HeLa epithelial cells did not produce detectable amounts of IL-8. This could be because they are not the true host cell for Salmonella infection. Using intestinal epithelial cells for future studies may provide better results with respect to reaction of the cells to infection. Alternatively, the lack of IL-8 production could be because the infection time was not long enough for the cells to alter transcription and translation to produce detectable levels of IL-8.