

Virulence of invasive
Salmonella Typhimurium
ST313 in animal infection
models

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Background

- *Salmonella* Typhimurium ST313 strains are commonly isolated from the blood of febrile patients
- 50% of ST313 infections present without diarrhea
- ST313 strains differ genetically from ST19
- Show phenotypic differences in:
 - Motility
 - Biofilm formation
 - Macrophage survival



Research questions

Do these genotypic and phenotypic differences reflect a difference in virulence?

- Are ST313 strains more invasive than ST19 strains?
- Do ST313 strains induce less diarrhea than ST19 strains?



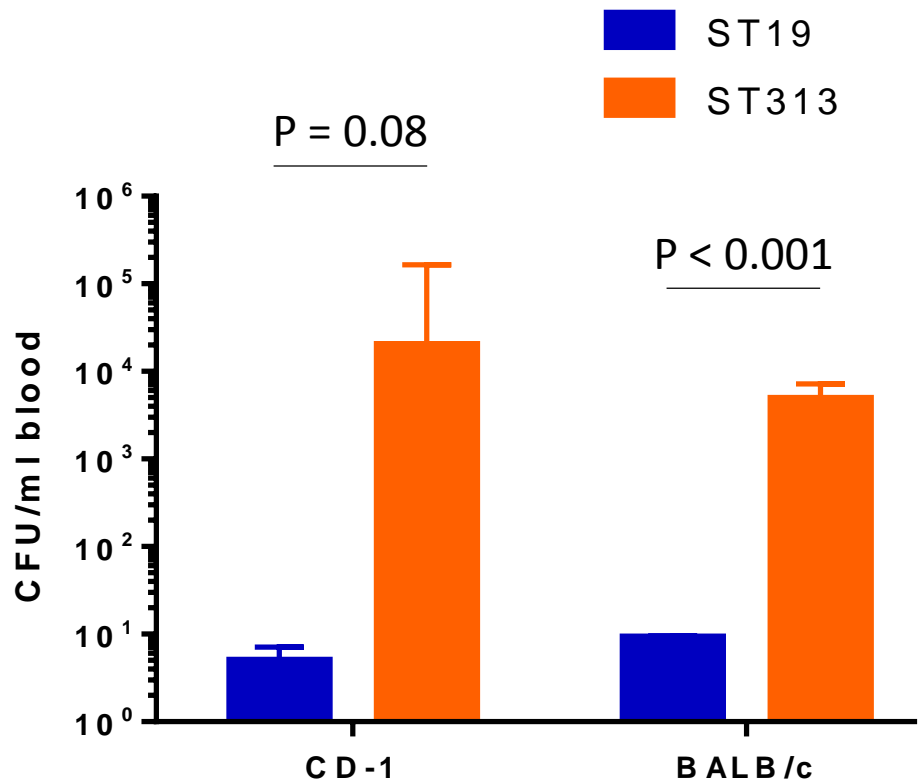
Virulence in mice

- Determined the 50% lethal dose of strains using:
 - 3 ST313 strains and 3 ST19 strains, all isolated from blood cultures in Mali
 - BALB/c and CD-1 mouse strains
 - Adult and juvenile mice
- No significant difference in LD₅₀ between sequence types



Dissemination in mice

- Infected mice perorally with I77 (ST19) or D65 (ST313)
- Sampled spleen, liver and blood at different timepoints
- No difference in organ counts
- Significant difference in blood counts at 24 h post-challenge



Rhesus macaque infection model

- Rhesus macaque studies first carried out in 60s-70s (Kent *et al.*, 1966; Rout *et al.* 1974)
- *Salmonella* infected animals had diarrhea, and showed signs of severe intestinal inflammation
- We recently revived this model to investigate vaccine safety in SIV-positive and healthy rhesus macaques (Ault *et al.* 2013)
- Similar symptoms to humans (diarrhea, fever, lethargy, weight loss, intestinal inflammation)



Rhesus macaque infection model

- 6 Indian rhesus macaques (3/group) were challenged intragastrically with I77 (ST19) or D65 (ST313)
- Monitored for 3 weeks for signs of infection including:
 - Clinical signs (temperature, weight loss, WBC count)
 - Bacterial load in stool
 - Stool grade
 - Blood culture
 - Histopathological analysis of organs
 - Bacterial counts in organs



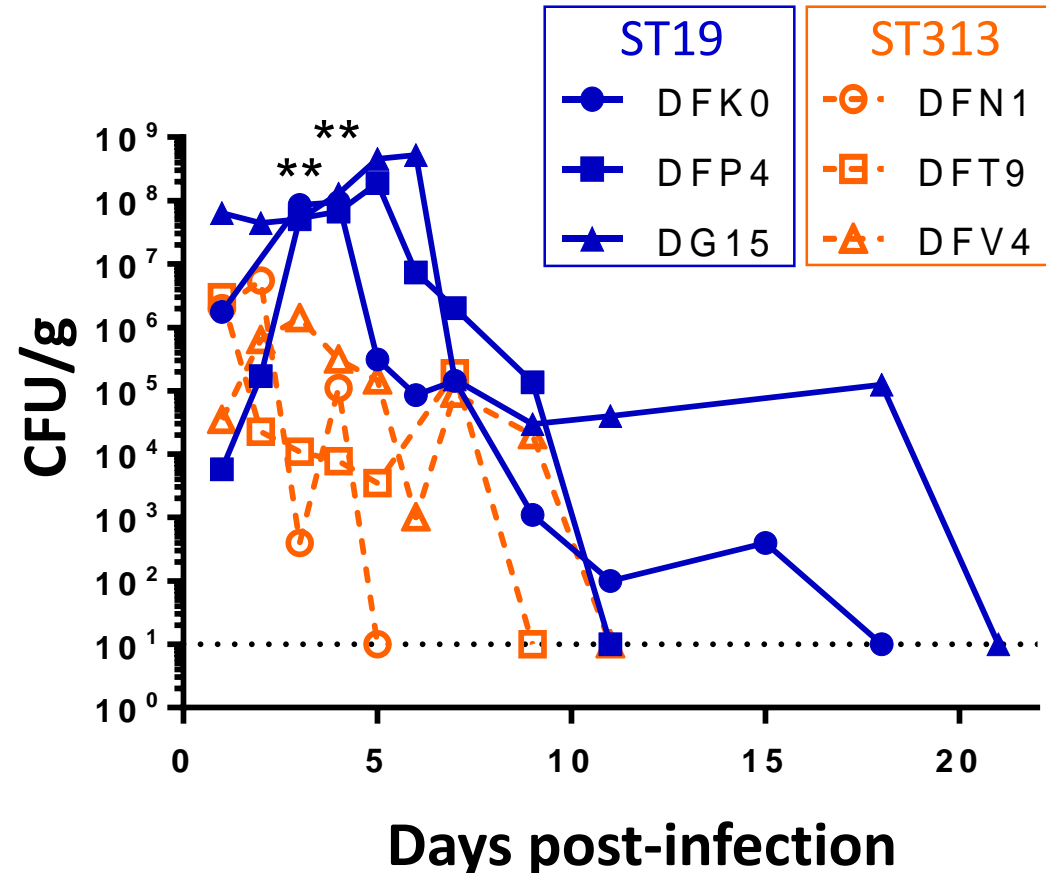
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RM infection – stool counts

- Stool counts were done to determine intestinal burden
- ST19 infected animals:
 - shed more bacteria at days 3 and 4 post-challenge
 - shed for a longer period than ST313 infected RM

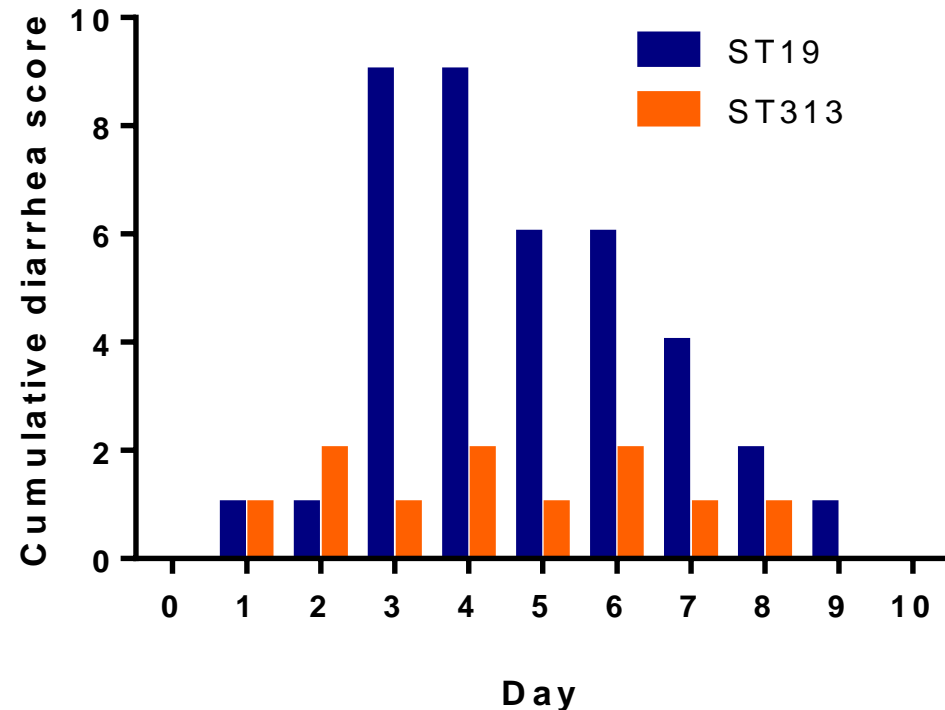


** P < 0.01



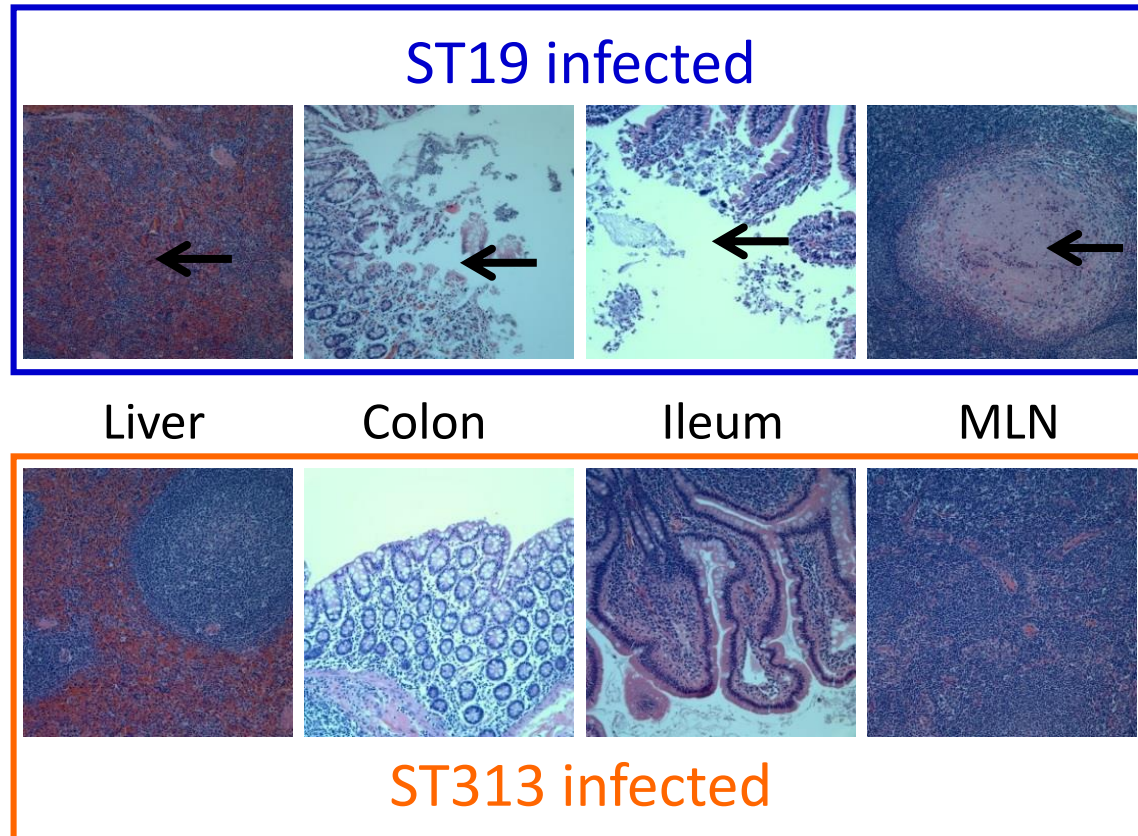
RM infection – stool grade

- Animals were scored each day for severity of diarrhea
 - 1 – mild
 - 2 – moderate
 - 3 – severe
- ST19 infected RM had more severe diarrhea than ST313 infected RM
- No ST313 infected RM had moderate or severe diarrhea



RM infection - histology

- Histology was done on organs at necropsy
- Higher levels of inflammation seen in the organs of ST19 infected rhesus macaques



Summary of RM data

- ST19 strain I77 induced significant levels of diarrhea in infected animals
- ST313 strain D65:
 - Caused less diarrhea
 - Showed decreased colonization of the intestines and reduced shedding
 - Induced less histopathology in organs (liver, colon, ileum and MLN)



Conclusions

- The mouse model is limited in its ability to model virulence of *Salmonella* Typhimurium ST313
- Rhesus macaques are a good model for *Salmonella* Typhimurium induced gastroenteritis
- As postulated from epidemiological studies, ST313 strains are less able to induce diarrhea in a RM model of infection
- Whether or not ST313 are inherently more invasive will require further analysis in the RM model using earlier timepoints



Acknowledgements

- Dr Girish Ramachandran
- Dr Aruna Panda
- Dr Sharon Tennant

Funding:

Center for Excellence in
Translational Research - NIH



- Clinical Microbiology
 - Jasnehta Permala-Booth
 - Sunil Sen
- Comparative Medicine
 - Dr Louis DeTolla
 - Dr Eugene Ateh
 - Dr Michael Lipsky

