# Modeling Human Enteric Dysbiosis and Rotavirus Immunity in Gnotobiotic Pigs


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## Background

Oral vaccines, such as those for rotavirus are less efficacious in children from underdeveloped regions, where most severe disease occurs, than in children from more affluent areas [1,2]. This disparity may be due to altered gut microbiota composition (dysbiosis), environmental enteropathy (EE), high maternal antibody titers, malnutrition, or influence of concurrent enteropathogens [1-4].

Composition of gut microbiota in children is influenced by method of delivery, environmental hygiene and nutritional status [2]. Studies have shown composition of gut microbiota to be significantly different between African and northern European infants and between malnourished and well-nourished children [5-7]. A recent study has shown that EE was associated with failure of the oral rotavirus vaccine Rotarix, and underperformance of the oral polio vaccine [8].

An animal model to study the effects of enteric dysbiosis on oral vaccine immunity is needed to evaluate potential treatments to reverse the dysbiosis and/or improve vaccine efficacy. Pigs and humans have similar immune systems, high genomic and protein sequence homology, omnivorous diet, and colonic fermentation, making pigs valuable models in biomedical research [9]. The neonatal gnotobiotic (Gn) pig is a well-established model of human rotavirus disease and immunity [10].

## Aims and Hypothesis

- Develop a Gn pig model of enteric dysbiosis by colonizing Gn pigs with "unhealthy" human gut microflora (UHGM).
- Use this model to study the influence of UHGM versus "healthy" human gut microflora (HHGM) on attenuated human rotavirus (AttHRV) vaccine immune response.
- Determine which components of HGM correlate with immune responses.

We hypothesized that pigs with HHGM would have a stronger immune response after vaccination than UGHM pigs and, certain components of HGM would be correlated with the immune response.


**References**

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## Experimental Design

### Selection of HGM samples

- 45 Nicaraguan infants (pre-immunization serum & stool, post-immunization serum)
- Compare microbiome & EE scores (ES) between infants with good and poor rotavirus-specific serum IgA response to RV5 (RotaTeq®) vaccine
- HHGM sample from infant with good IgA response to RV5, low ES, high alpha diversity (AD)
- UGHM sample from infant with poor IgA response to RV5, high ES, low AD

### Gn pig study

| Activity | Date |
|---|---|
| Derivation of Gn pigs | PPD0* |
| Oral inoculation with HHGM (n=12) or UHGM (n=12) | PPD5,6,7 |
| Bleeding for detection of serum antibodies by ELISA | Weekly, starting PID0 |
| Oral vaccination with attenuated HRV Wa vaccine (AttHRV 5X10$^7$ fluorescence forming unit [FFU]) | PID0, PID10, PID20** |
| Oral challenge with virulent HRV Wa (G1P1A[8], 1X10$^5$ FFU) | PID28 (PCD0) |
| Record daily fecal consistency scores | PCD0-7*** |
| Rectal swabs for virus shedding (CCIF, ELISA) | PCD0-7 |
| Euthanasia (PID28 n=5-6 and PCD7 n=6-7), collect intestinal contents for antibodies, microbiome and enteropathy biomarker ELISAs; ileum, spleen and blood for T cell responses; serum for antibodies; and duodenum, jejunum & ileum for histopathology | PID28, PCD7 |

*Postpartum day (PPD), **Post-inoculation day (PID), ***Post-challenge day (PCD)

## Results

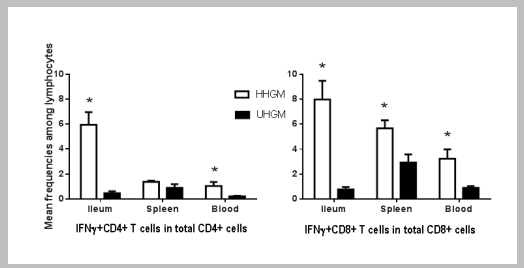


Fig. 1. Frequencies of IFN-γ producing CD4+ and CD8+ T cells among total CD3+CD8+ and CD3+CD4 cells on PID28 in ileum, spleen and blood of HHGM verses UHGM colonized pigs. Error bars indicate standard errors of the means. Asterisks indicate significant differences when compared to UHGM pigs (Kruskal-Wallis rank sum test , P<0.05; n=5-7).

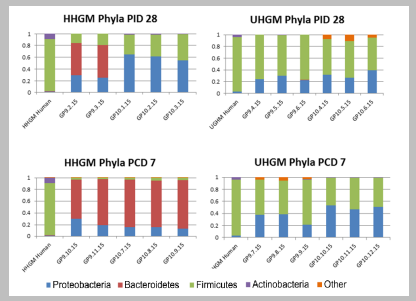


Fig. 2. Relative abundance of phyla in the microbial community of large intestinal contents of individual HGM-colonized Gn pigs before HRV challenge (PID28) and post-challenge (PCD7). The human sample is included for reference in each panel.

| Positive Correlations | OTU | Tissue | T cell | ? | adj. p-value |
|---|---|---|---|---|---|
| PID28 | *Collinsella* | Ileum | CD8+ | 0.91 | 0.001 |
| | *Collinsella* | Blood | CD8+ | 0.83 | 0.016 |
| | *Collinsella* | Blood | CD4+ | 0.89 | 0.002 |
| **Negative Correlations** | **OTU** | **Tissue** | **T cell** | **?** | **adj. p-value** |
| PID28 | *Clostridium* | Ileum | CD8+ | -0.90 | 0.002 |
| | *Anaerococcus* | Ileum | CD8+ | -0.88 | 0.002 |
| | *Propionibacterium* | Blood | CD8+ | -0.88 | 0.002 |
| | Bacillales (unclassified) | Blood | CD8+ | -0.87 | 0.003 |
| | *Blautia* | Blood | CD8+ | -0.81 | 0.009 |
| | *Clostridium* | Ileum | CD4+ | -0.83 | 0.013 |
| | *Anaerococcus* | Ileum | CD4+ | -0.86 | 0.006 |
| | Clostridiales (unclassified) | Blood | CD4+ | -0.89 | <0.01 |
| | Clostridiales (unclassified) | Blood | CD4+ | -0.82 | 0.014 |

Table 1. Spearman's rank correlation coefficients between specified OTUs and rotavirus-specific IFN-γ+CD8+ or IFN-γ+CD4+ T cells among all Gn pigs at PID28.

## Summary

- Significantly more rotavirus-specific IFN-γ producing effector T cells were in the ileum, spleen and blood of HHGM than UHGM pigs at PID28 (Fig. 1).
- Rotavirus-specific antibodies in intestinal contents trended higher in HHGM than UHGM pigs before and after VirHRV challenge (data not shown).
- AttHRV vaccine provided increased protection against rotavirus shedding and clinical signs after VirHRV challenge in pigs colonized with HHGM than UHGM (data not shown).
- Significant correlations existed between a few OTUs and vaccine induced T cell responses (Table 1).
- There were significant differences in AD and relative abundances of OTUs in HHGM pigs before (PID28) and after (PCD7) VirHRV challenge (Fig. 2).

## Conclusions and Future Directions

Gn pigs transplanted with HGM can be used as a model of enteric dysbiosis. The impaired enteric immunity to oral vaccines in human infants was recapitulated in the UHGM pigs. HHGM pigs demonstrated stronger T cell and mucosal immunity compared to the UHGM pigs. Significant correlations between OTUs and effector T cell responses existed. Microbiome differences between groups were present before and after VirHRV challenge. These results show that gut microbiota has a major impact on vaccine immunogenicity.

The HGM transplanted Gn pig model will be a valuable tool to investigate strategies to modulate the intestinal microbiome and enhance immunogenicity and protective efficacy of rotavirus and other oral vaccines. Alteration in microbiome diversity and composition, along with correlations between microbial taxa and T cell response warrant further investigations into the role of specific bacteria on enteric immunity.

Work described in this study has recently been published in *Gut Pathogens*[11].