**Background**

- Studies of human enteric colonization (EC) in patients with recurrent CDI have demonstrated profound effects on a sustained clinical response to the recovery of Firmicutes bacteria in the GI tract.
- Enterohemorrhagic (EPEC) and Shigatoxin (STEC), also known as E. coli O157:H7, are examples of enteric pathogens that may be present years after primary infection.
- The persistence of enteric pathogens, including Salmonella enterica and Shigella spp., has been attributed to the formation of biofilms, which provide a protective niche for bacterial survival.

**Methods**

- **Candidate viruses for inactivation testing, and selection of Porcine Epidemic Diarrhea Virus (PEDV)**
  - The selected system consists of cultured PEDV and a viral detection assay using Virus coils in a tissue culture infective dose (TCID) assay, with the mpv records maintained independently.

**Candidate bacteria for inactivation testing, and selection of Salmonella enterica**

- The selection criteria for bacteria testing were as follows:
  - **E. coli pathogenic**
    - S. enterica serotype Typhimurium and S. enterica serotype Cholerae were selected as representative model organisms based on biological similarity, risk group, and ability to promote the development of a mAb-based therapeutics.
  - **S. enterica serotype Typhimurium**
    - S. enterica serotype Cholerae was selected as a representative model organism based on biological similarity, risk group, and ability to promote the development of a mAb-based therapeutics.

**Approach to the selection of model viruses for inactivation testing and companion donor criteria assessment**

- The following inclusion criteria were evaluated for selection of a representative model virus for each study:
  - **Well-characterized viral system**
    - The cell type used to produce the virus should be well-characterized and the viral vector should be of known origin.
  - **Comprehensive donor screening**
    - The donor should undergo comprehensive donor screening, including medical history and physical examination.
  - **Culturability**
    - The virus should be culturable in a suitable medium.
  - **Detectability of viral RNA**
    - The viral RNA should be detectable in the donor stool sample.
  - **Safety of operators performing the inactivation studies**

**Spike of S. enterica and Inactivation by SER-109 Process**

- Rapid inoculation to limit of detection (LOD) is demonstrated by the scale-down process at any spike level achieving LOD.

**Summary**

- FMT and FMT-like products are vulnerable to pathogens, which may go undetected during donor screening.
- The selection of representative enteric Gram-negative bacteria (E. coli) as a model for safety testing of frozen product given by enema for recurrent CDI.
- The manufacturing processes and product testing for SER-109, an investigational microbiome therapeutics, are designed to enrich for beneficial Firmicutes spp., while inactivating potential pathogens. Product testing for contaminants adds a third layer of protection.

**References**