Characterizing Bacterial Trafficking After Intravaginal Administration in a Mouse Model

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BACKGROUND

- Preterm birth (PTB) affects 1 of every 10 infants born in the United States[1]
- The vaginal microbiome is closely associated with PTB and other adverse health outcomes[2,3,4]
- It is previously believed that certain bacterial strains ascend from the vagina into the uterus to induce PTB[5]

Hypothesis: Vaginal microbes colonize the vaginal and cervical space but do not ascend to the uterine cavity during pregnancy.

OBJECTIVES

1. Characterize bacterial trafficking through the female reproductive system in a pregnant mouse model
2. Develop a protocol to visualize trafficking of bacteria through the reproductive tract

EXPERIMENTAL METHODS

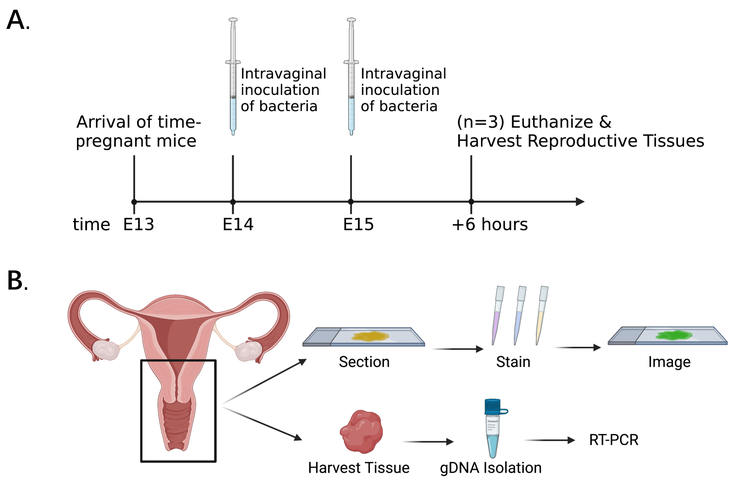

Figure 1. Experimental Design. A. C56/BL6 in-bred, time-mated mice were shipped on embryonic day 12 (E12) and received on E13. Intravaginal inoculations of bacteria *Gardnerella vaginalis* (GV) and/or *Mobiluncus mulieris* (MM) were given on E14 and E15. Animals were euthanized (CO2 overdose) 6h after the second inoculation (n=3). Reproductive tissues including the cervix and vagina were collected. **B**. After euthanasia, reproductive tissue including the cervix and vagina were collected. Tissues were either sectioned and stained following a fluorescent in situ hybridization (FISH) protocol or digested for gDNA isolation followed by Real-Time (RT) PCR. Stained images were imaged on a Zeiss LSM 880 Confocal Microscope and qualitatively assessed. PCR data was analyzed in GraphPad Prism.

RESULTS

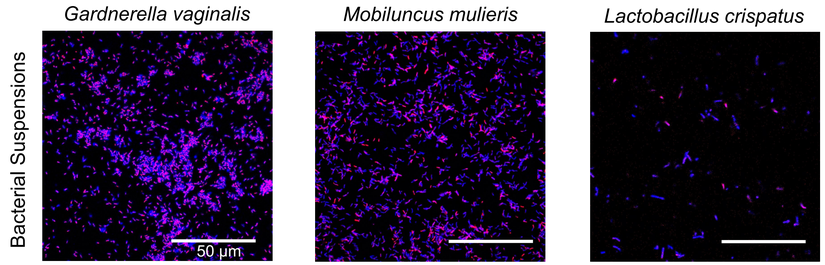

Figure 2. FISH-stained Bacterial Suspensions. Confocal microscope images at 63x magnification. *G. vaginalis* (GV), *M. mulieris* (MM), and *L. crispatus* (LC) bacterial suspensions were stained following a FISH staining protocol. Blue stain is DAPI and the pink stain is the GV, MM, or LC bacterial probe, respectively.

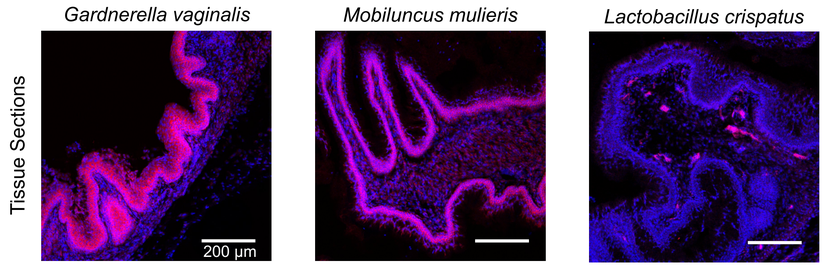

Figure 3. FISH-stained Vaginal and Cervical Tissue Samples. Confocal microscope images at 20x magnification. *G. vaginalis* (GV), *M. mulieris* (MM), and *L. crispatus* (LC) bacteria-treated tissues were sectioned, stained, and imaged. Blue stain is DAPI and the pink stain is the GV, MM, or LC bacterial probe, respectively.

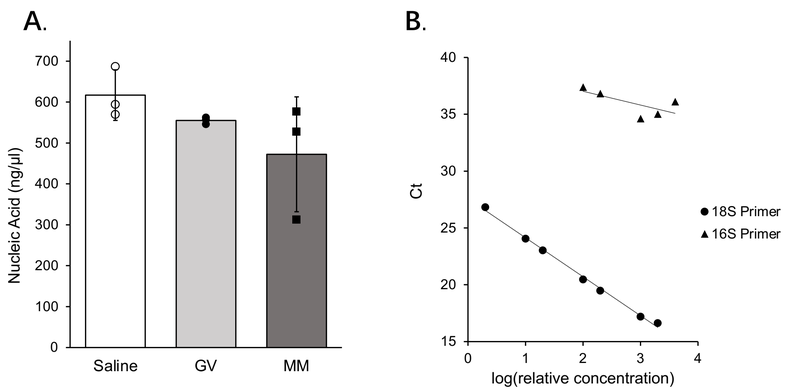

Figure 4. Detection of Bacterial and Mammalian DNA. A. Nanodrop samples of gDNA isolated from saline, GV, or MM treated cervicovaginal samples. Nucleic acid concentrations above 100ng/µL are considered sufficient evidence of the presence of DNA. **B**. RT-PCR Ct values from cervicovaginal samples. A standard curve was successfully achieved using the 18S mammalian primer, such that higher gDNA concentrations resulted in lower cycle numbers to reach the amplification threshold. In contrast, an appropriate standard curve was not developed with the 16S bacterial primer.

DISCUSSION

- A protocol was successfully developed to visualize GV and MM and used to image these bacteria
- Further refinement of the protocol for LC bacteria is need to improved staining efficiency observed
- Sufficient 16S was not detected across biological samples to create a standard curve, preventing further analysis
- To quantitatively confirm bacteria in the cervix, validation with a 16S primer must be conducted

IMPLICATIONS & FUTURE EXPERIMENTS

- The staining, imaging, and analysis techniques established in this project will continue to be used to evaluate bacterial ascension into the uterus during pregnancy
- We will use these techniques to assess the roles of bacterial extracellular vesicles (EVs) on the reproductive tract[6,7]
- Our collective studies have the possibility to uncover novel pathways leading to a broad range of adverse reproductive outcomes and identify potential therapeutic targets

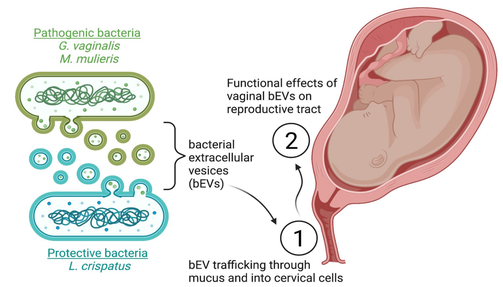

Figure 5. Hypothesized Interactions of Extracellular Vesicles with Female Reproductive Tract. Pathogenic bacterial EVs from GV and MM first enter the female reproductive system through the vagina where they are then trafficked through mucus and into cervical cells. As a result, the EVs negatively affect the surrounding environment. Protective bacteria, LC, is believed to follow the same pathway and provide protective measures.

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References: [1] "Preterm Birth." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention [2]Elovitz, M.A., et al. *Nat Commun (2019) [3]* Stout, M. J. et al. *Am. J. Obstet. Gynecol. (2017) [4]* Brown, R. G. et al. *BMC Med.* (2018) *[5]* Suff, N., et al. *Am. J. Pathol.* (2018) *[6]* Nahui Palomino, R.A., et al. *Nat Commun* 10, 5656 (2019). *[7]* Shishpal, P., et al. *Anaerobe* 61, 102090 (2020).

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