Metagenomic analysis of the equine placental microbiome

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The placenta is a vital, but transient, organ of pregnancy that has been regarded as sterile in both women and mares. Recent evidence in women has demonstrated that the placenta harbors a unique microbiome. The objective of this study was to determine if the equine placenta harbors a microbiome under healthy conditions, and if so, is there any association with the microbiomes of extraplacental body sites (oral, vaginal, or fecal) of the mares. We hypothesize that the equine placental microbiome has a distinctive metagenomic profile, not yet characterized. Fecal, oral, and vaginal samples were taken from pregnant mares within 30 days of foaling, as well as the gravid and non-gravid regions of the chorioallantois at the time of foaling (n=4). Genomic DNA was isolated from all samples, and the bacterial 16s ribosomal RNA gene was amplified by PCR. Blank extractions were amplified as negative controls, while known tissue samples were amplified as positive controls. Amplified bacterial DNA was sequenced using the Mi-Seq sequencer. Sequence reads were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) program and assigned to Operational Taxonomic Units (OTUs). These OTUs identify the genera of bacteria present in the sample. Discriminant analysis (JMP Pro 12) was used to evaluate the correlation between bacterial taxa and the prevalence of each type of bacteria in the placental samples with the potential source of bacteria from extra-placental body sites. Different prevalence of bacteria in each sample was used as a covariate in a stepwise discriminant analysis model. Variables were removed in a stepwise manner until the only variable with a p< 0.005 were retained in the final model. Relative abundance within the chorioallantois demonstrated 3 main phyla represented in the gravid horn (Firmicutes, Proteobacteria, Bacteroidetes) and those same three phyla plus Actinobacteria in the non-gravid horn. The most abundant phyla in the oral, fecal, and vaginal samples (Firmicutes and Proteobacteria) were also detected in the chorioallantois. The gravid horn and feces of the mare have different populations of bacteria. Bacillus, Mycoplasma, and Gemella are all higher in relative abundance in the fecal samples of the mare (p<0.0001). Conversely, Clostridium and Moraxella are higher in the gravid horn samples (p<0.0001). The most abundant bacterial phyla in gravid and nongravid chorioallantois share significant overlap, suggesting similar, but not identical, environments within different compartments of the chorioallantois. Phyla of relatively high abundance in oral and vaginal samples correspond to those found in the chorioallantois, indicating possible associations between placental and extra-placental microbiota, yet there are significant differences between the gravid horn and the fecal samples. To the authors’ knowledge, this is the first report to characterize the equine placental microbiome by metagenomics. Further studies include pyrosequencing of equine placentae from mares with adverse pregnancy outcomes, i.e. placentitis and fetal growth restriction, to determine if the equine placenta has a unique microbiome in health and disease.

Keywords: Placenta, microbiome, metagenomics, pregnancy