16S rDNA-based profiling of canine reproductive tract microbiota reveals a complex microbial ecosystem

C. Lyman,a K. Meinkoth,a U. DeSilva,b E. Coffman,a G.R. Holyoak a
aCenter for Veterinary Health Sciences and bDepartment of Animal Science, Oklahoma State University, Stillwater, OK

While it is established that the cranial vagina of mammals harbors a thriving microbial ecosystem, the uterus was long considered to be a sterile environment in order to sustain a successful pregnancy. This paradigm was recently challenged by the observation that human placentae harbors a diverse microbiome. There is very little information available on canine reproductive tract microbiota and the few published studies were conducted using culture-based techniques that fails to detect >90% of resident microflora. As a proof-of-concept study in an effort to identify the normal flora of healthy canine vagina and uterus, we performed a comprehensive analysis of their resident microbiota. Twenty five young, healthy bitches presented for elective ovariohysterectomy (OVH) were anesthetically induced and samples from the cranial vagina were taken for cytology. Serum samples for progesterone analysis were taken to confirm the stage of estrous. During OVH the uterus and ovaries were removed using sterile technique. Endometrial biopsy samples and vaginal swabs were collected for microbial analysis. Animals were assigned to pre-pubertal, anestrus, pro-estrus, estrus or diestrus stages (n=5 per group). Vaginal swab and endometrial samples were collected under strict aseptic conditions to prevent contamination and a vaginal swab and an endometrial biopsy sample from each animal was used for DNA extraction. Integrity of extracted DNA was verified spectrophotometrically as well as by gel analysis. A 300bp fragment from 16S ribosomal v3/v4 region was PCR amplified. Each DNA sample was barcoded to identify the individual animal and the tissue (vagina/uterus) of origin and over 4 million paired-end reads were generated from the samples. After removing ambiguous sequences, barcodes and misalignments, sequences were further analyzed using MOTHUR and taxonomic assignments were made using the SILVA database. The results showed that the canine reproductive tract consists of a rich and diverse microbial environment with over 300 different operational taxonomic units (OTUs) of organisms identified. A comprehensive analysis of the microbiomes across different stages of estrus as well as a core microbiome of the canine reproductive tract will be provided.

Keywords: Endometrial microbiome, canine, estrous cycle

Reference