The canine vaginal microbiome and associations with puppy survival
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Perinatal and neonatal deaths account for a significant financial and emotional loss to breeders each year. Bacterial infection during parturition such as from vaginal group B Streptococcus in humans is associated with increased risk of neonatal septicemia but this has not been evaluated in canines.

Objectives
1) To describe the vaginal microbiome during pregnancy, and in postmortem samples of stillborn and neonatal loss puppies. 2) To determine if there are associations between vaginal microbiome during pregnancy, and likelihood of litters with stillbirths and neonatal puppy losses.

Hypothesis
We hypothesize that (1) the bacterial diversity of the canine vagina is greater than previously thought, (2) that novel bacteria not previously associated with stillbirths are identified, and (3) that there are differences in the vaginal microbiome between bitches that have normal, healthy puppies and puppies that fail to thrive.

Study design
Vaginal samples during the last week of pregnancy were evaluated using metagenomic analysis. Bacterial DNA was extracted and the 16S ribosomal RNA gene amplified by PCR for sequencing. The 16S ribosomal RNA gene was used to identify the bacterial genus of origin. Differences between bacterial populations in the vagina of animals that have high stillbirth rate were evaluated. Samples of liver, and stomach contents from stillborn puppies were assessed using metagenomic analysis and compared with the vaginal sample of the dam to determine if there is an association between the maternal microbiome and stillbirths. Discriminant analysis using JMP Pro 12 was used to evaluate the correlation between the bacterial taxa in the different samples. 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 only variable with a $P < 0.05$ were retained in the final model.

Results
The five most common bacterial genera in the vagina regardless of stillbirths and puppy health conditions are *Bradyrhizobium*, *Streptococcus*, *Gemella*, *Mycoplasma*, and *Avibacterium*. The five most abundant genera in the microbiome of the stillborn puppy liver samples are *Bradyrhizobium*, *Serratia*, *Phenylobacterium*, *Enterococcus*, and *Flavobacterium*. The most abundant five genera in the stomach content samples of the stillborn puppies are *Streptococcus*, *Acinetobacter*, *Bradyrhizobium*, *Enterococcus*, and *Serratia*. The presence of the genera *Biberistinia*, *Staphylococcus*, *Pasteurella*, *Corynebacterium* and *Methyllobacterium* in the vagina of the dam all significantly increased the probability that there would be a stillborn puppy in the litter. However, none of these bacteria are found in significant levels in the stillborn puppy liver or stomach content samples according to discriminant analysis.

Discussion/conclusion
We identified five genera that are correlated with incidence of stillbirth, but further studies are required to determine bacterial causation of stillbirth. However, if the presence of specific bacteria in the vagina is associated with puppy outcomes, treatment options such as antimicrobials may be able to reduce puppy losses and diseases.

Keywords: Canine, microbiome, stillbirths, neonatal losses