

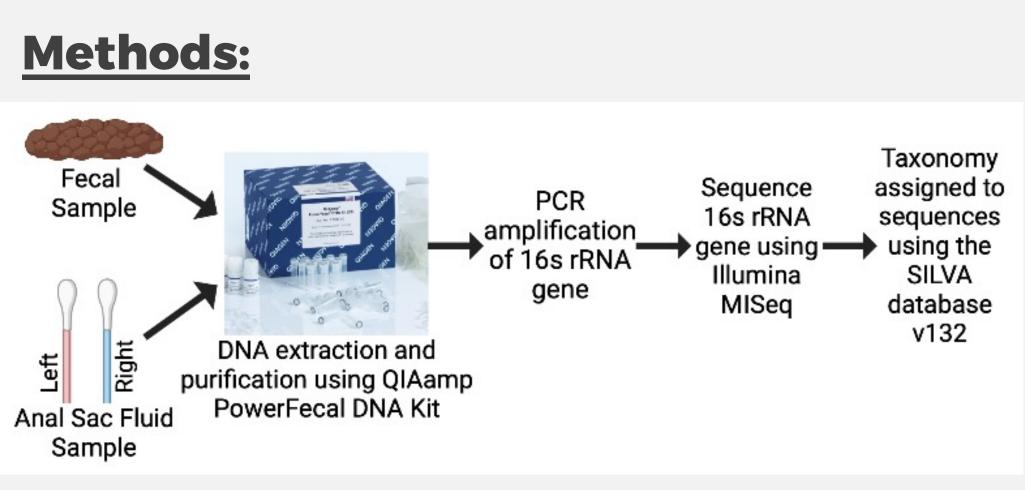


## **Background**:

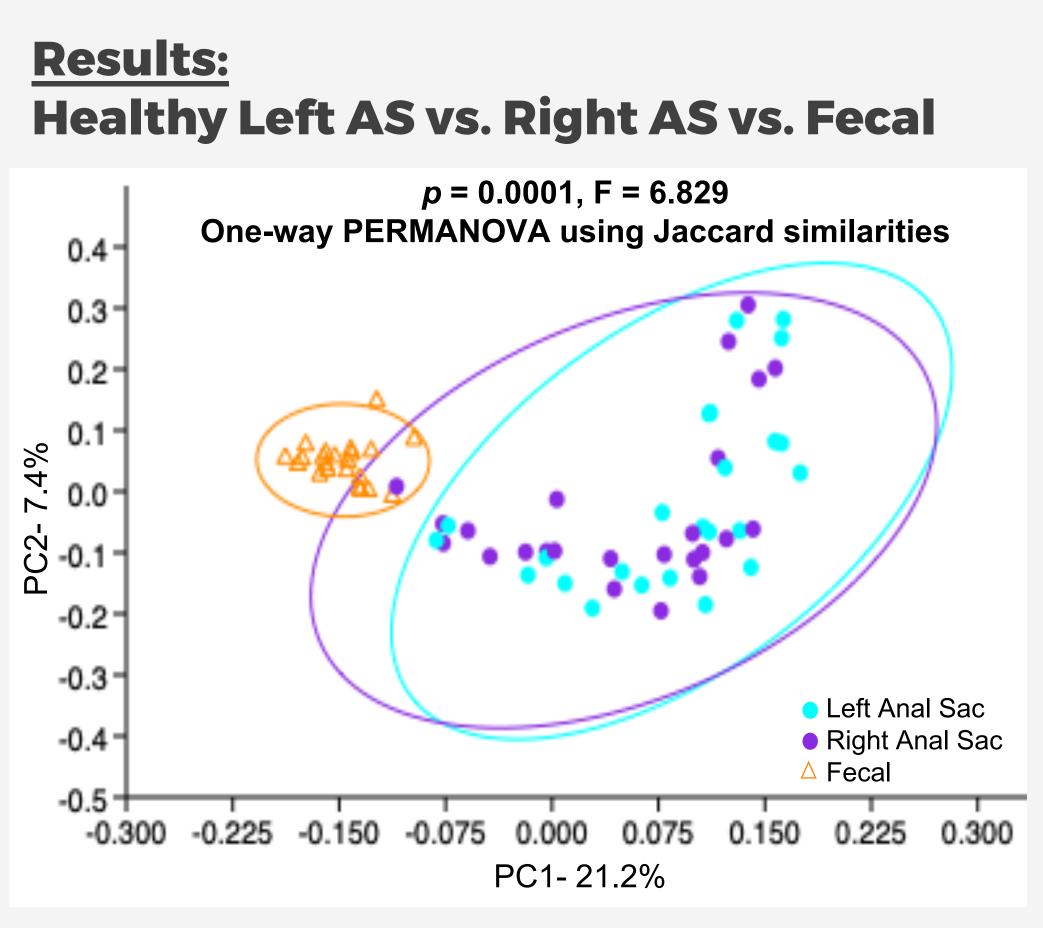
- Canine apocrine gland anal sac adenocarcinoma (AGASACA) are malignant tumors of the anal sacs.
- AGASACA is commonly diagnosed and has an unknown cause.
- Environmental factors including the microbiome may contribute to the pathogenesis.
- The microbiome of anal sacs of healthy pet dogs and dogs with AGASACA have not yet been described.

## **Objective:**

- Characterize the microbiome of the anal sacs of pet dogs with and without AGASACA.
- Compare the fecal microbiome of dogs with AGASACA to healthy pet dogs.



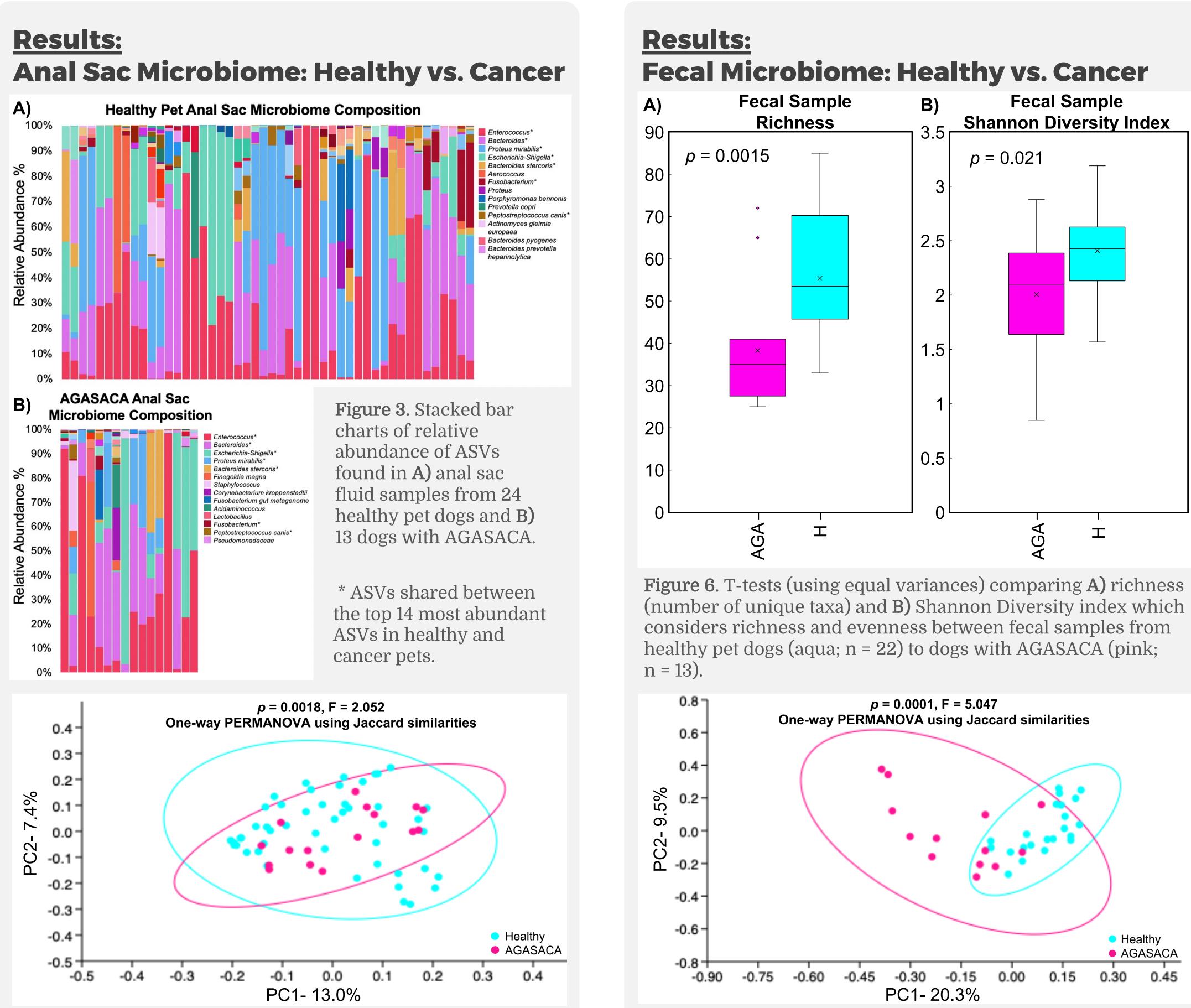
**Figure 1.** Overview of experimental procedure. Steps 3-5 were completed at the University of Missouri Metagenomics Center.



**Figure 2.** PCoA plot comparing the  $\beta$ -diversity between left (aqua; n= 24) and right (purple; n = 24) anal sac fluid samples and fecal samples (orange; n = 22) from healthy dogs using Jaccard (unweighted) similarities.

# **Characterization of the microbiome of anal sacs in pet dogs** with apocrine gland anal sac adenocarcinoma

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**Figure 4.** PCoA plot comparing the  $\beta$ -diversity between anal sac fluid samples from healthy dogs (aqua; n = 48) and cancer dogs (pink; n = 16) using Jaccard Similarities (unweighted) with 95% confidence ellipses.

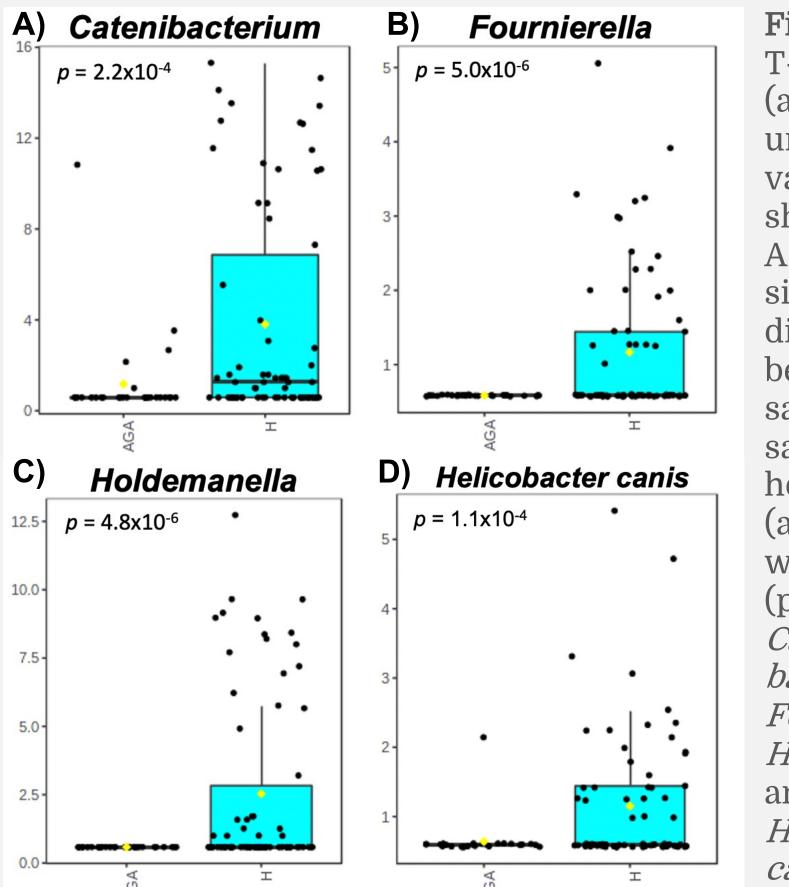
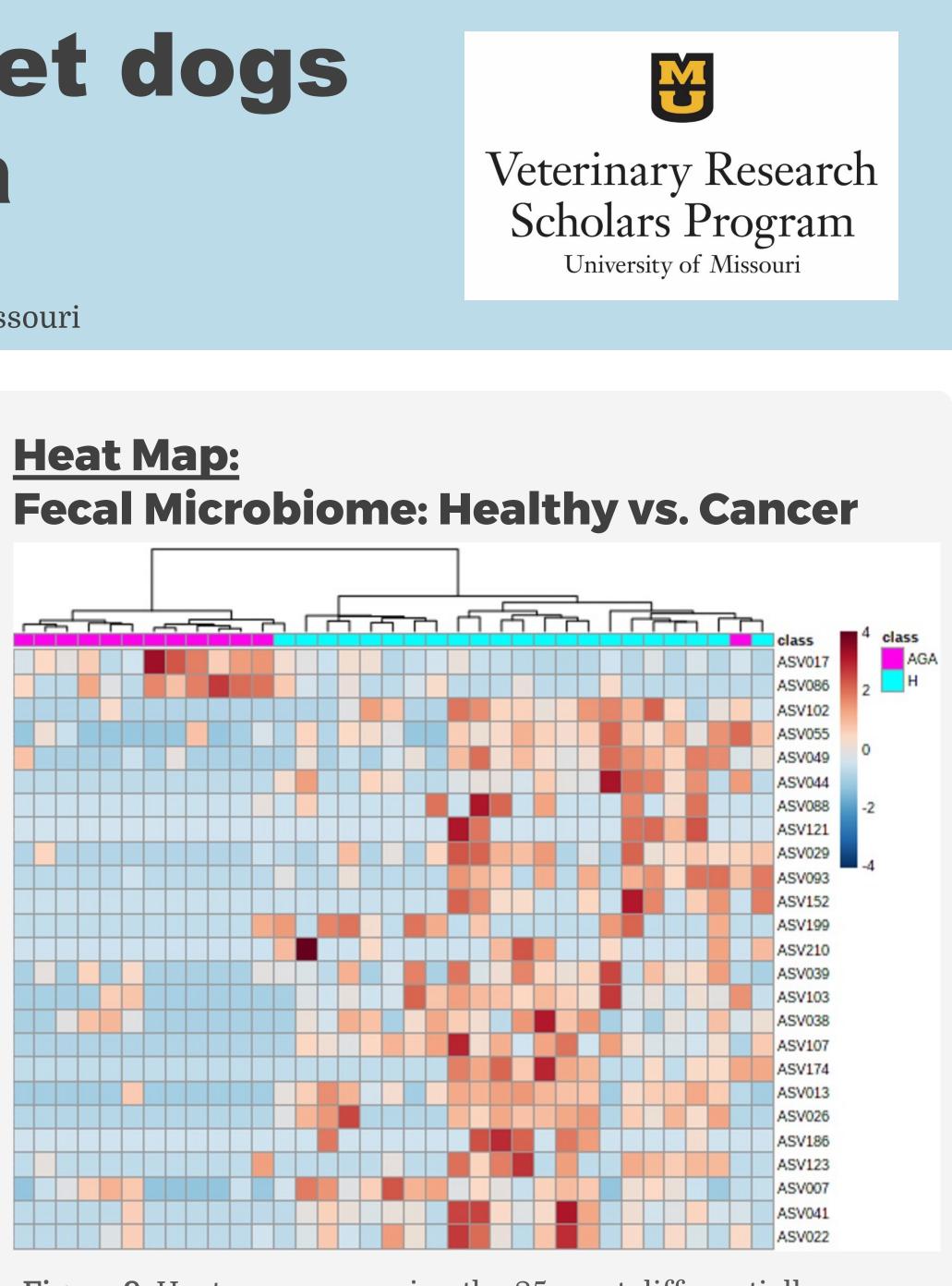


Figure 5. T-tests (assuming unequal variance) showing the 4 ASVs that were significantly different between anal sac fluid samples from healthy pet dogs (aqua) and dogs with AGASACA (pink): **A)** Cateni*bacterium*, **B**) *Fournierella*, C) Holdemanella, and D) Helicobacter canis.

**Figure 7.** PCoA plot comparing the  $\beta$ -diversity between fecal samples from healthy dogs (aqua; n = 22) and cancer dogs (pink; n = 13) using Jaccard similarities (unweighted) with 95% confidence ellipses.

Figure 8. A) Catenibacterium Fournierella B) T-tests  $p = 1.8 \times 10^{-7}$  $p = 3.7 \times 10^{-7}$ (assuming unequal variance) • • : • showing the top 4 ASVs (by p-• value) that were . •• significantly different between fecal samples from D) C) Blautia glucerasea Holdemanella healthy pet *p* = 2.9x10<sup>-4</sup> dogs (aqua) and  $p = 3.0 \times 10^{-6}$ dogs with AGASACA (pink): A) Cateni*bacterium,* B) *Fournierella*, C) *Holdemanella*, and **D)** *Blautia* . . . . glucerasea.





- dogs.

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**Figure 9.** Heat map comparing the 25 most differentially abundant ASVs (rows) between fecal samples (columns) from healthy pet dogs (aqua) and dogs with AGASACA (pink).

## **Conclusions:**

• There was significant difference between the microbiome composition of anal sac fluid samples and fecal samples.

• There was no significant difference in richness or  $\alpha$ diversity in anal sac fluid samples between pet dogs with and without AGASACA.

• There was significant difference in the taxa shared between anal sac fluid samples of healthy and AGASACA dogs. However, there was no significant difference in the relative abundance of taxa shared, indicating that the bulk of the microbiome is the same.

• 4 ASVs were present in significantly different amounts between anal sac fluid samples of healthy and AGASACA dogs: *Catenibaterium*, *Fournierella*, *Holdemanella*, and Helicobacter canis.

• There was significant decrease in richness and  $\alpha$ -diversity in fecal samples of AGASACA dogs compared to healthy

• There was significant difference in taxa shared and relative abundance of taxa shared between fecal samples of healthy and AGASACA dogs.

• 10 ASVs were present in significantly different amounts between fecal samples of healthy and AGASACA dogs, 3 of which were shared with the anal sac microbiome.

### **cnowledgements**:

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**Metagenomics Center University of Missouri**