Identification of Disease Diagnostic and Prognostic Biomarkers in the Equine Gastrointestinal Microbiome

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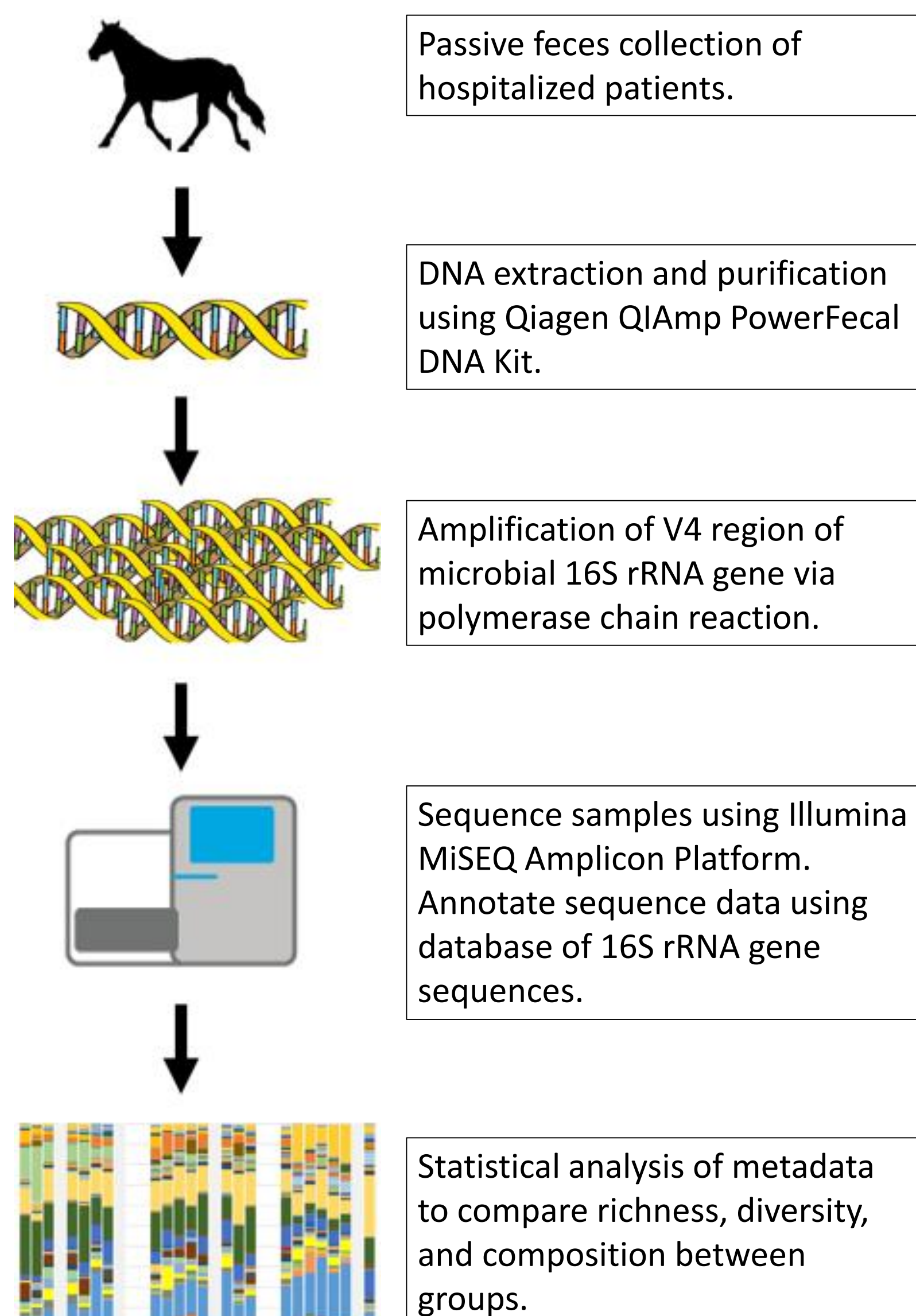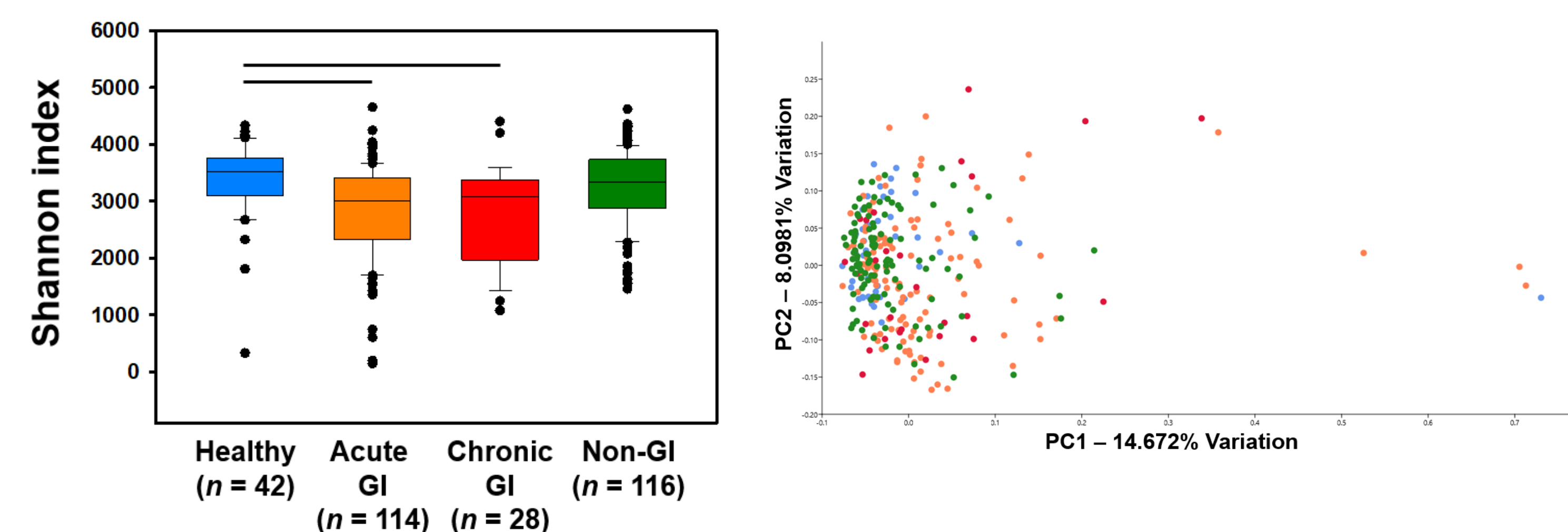[4] MU Metagenomics Center, Columbia, MO

Background

- Horses have unique gastrointestinal (GI) tracts that rely on hindgut fermentation by an extensive microbiome.
- The equine GI microbiome is suspected to influence disease etiology.
- Specifically, idiopathic GI diseases, such as colic, are a leading health problem of horses and yet there is little known about the relationship between the microbiome and the etiology of colic.
- The objectives of this study are to determine if an association between the equine gut microbiome (GM) and primary organ system affected (e.g., respiratory, GI, orthopedic disease, etc.) exists, and whether there is a difference in the GM between decompensated colics that end in euthanasia or colics that resolve and are discharged.
- We hypothesize that the equine GM will form distinct clusters based on general disease etiology or affected organ system, reflecting a relationship between disease and the microbiome population.
- We also hypothesize that decompensated colics ending in euthanasia will possess microbiomes with lower richness relative to colics that resolve.
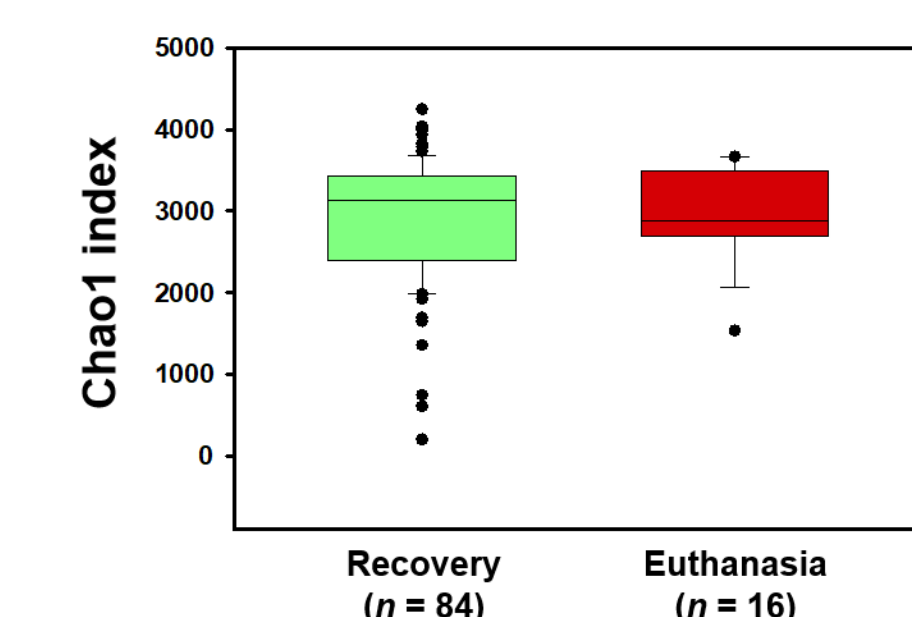
Methods

Passive feces collection of hospitalized patients.

DNA extraction and purification using Qiagen QIAmp PowerFecal DNA Kit.

Amplification of V4 region of microbial 16S rRNA gene via polymerase chain reaction.

Sequence samples using Illumina MiSEQ Amplicon Platform. Annotate sequence data using database of 16S rRNA gene sequences.

Statistical analysis of metadata to compare richness, diversity, and composition between groups.

Comparison of GI to Non-GI Cases

Figure 1: Box plot showing α-diversity results for healthy vs. acute GI vs. chronic GI vs. non-GI cases. Significant differences found between healthy and acute GI, and between healthy and chronic GI (Shannon Index: p<0.005 all [shown]; Chao-1: p<0.002 all).

Figure 2: Principal component analysis of samples shown in Figure 1. Significant differences in β-diversity found between groups (weighted Bray-Curtis, F = 2.874, p<0.0001).

Comparison of Disease Etiologies

Relative Abundance of Phyla per Disease Category

1 - Healthy	(n = 42)
2 - Acute GI	(n = 114)
3 - Chronic GI	(n = 28)
4 - Cardio	(n = 11)
5 - Musc/skeletal	(n = 33)
6 - Neuro	(n = 5)
7 - Ocular	(n = 22)
8 - Repro	(n = 12)
9 - Respiratory	(n = 18)
10 - Systemic	(n = 11)
11 - Urinary	(n = 4)

Figure 3: Bar chart showing the relative abundance of microbiota at the phylum level, group by disease etiology. No significant difference in β-diversity identified between groups.

1 - Healthy	(n = 42)
2 - Acute GI	(n = 114)
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4 - Cardio	(n = 11)
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11 - Urinary	(n = 4)

Figure 4: Comparison of α-diversity (Shannon Index) between disease etiology groups. Using Chao-1 and Shannon Index, significant differences identified between healthy horses and acute GI and chronic GI horses (p < 0.015 all).
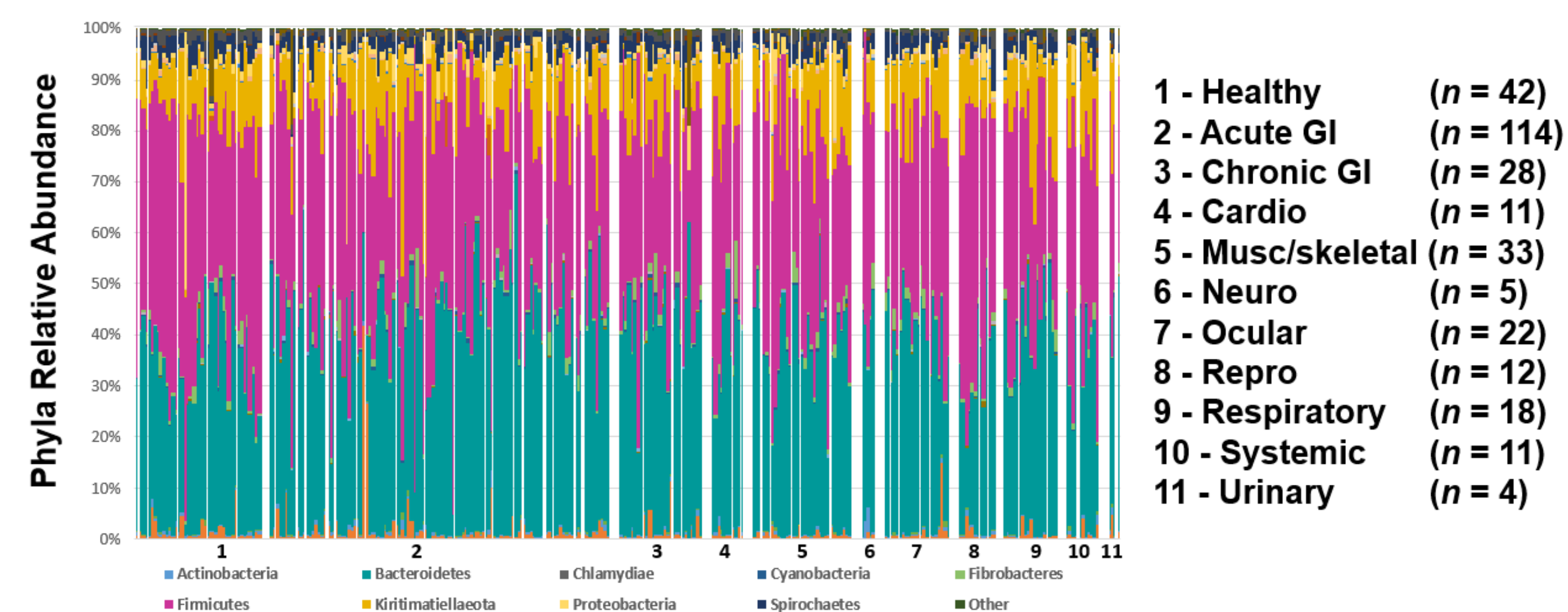
Comparison of Colic Subset

Figure 5: Box plot showing no significant statistical difference in richness (Chao-1 Index) between recovered colics and euthanized colics (p = 0.862).
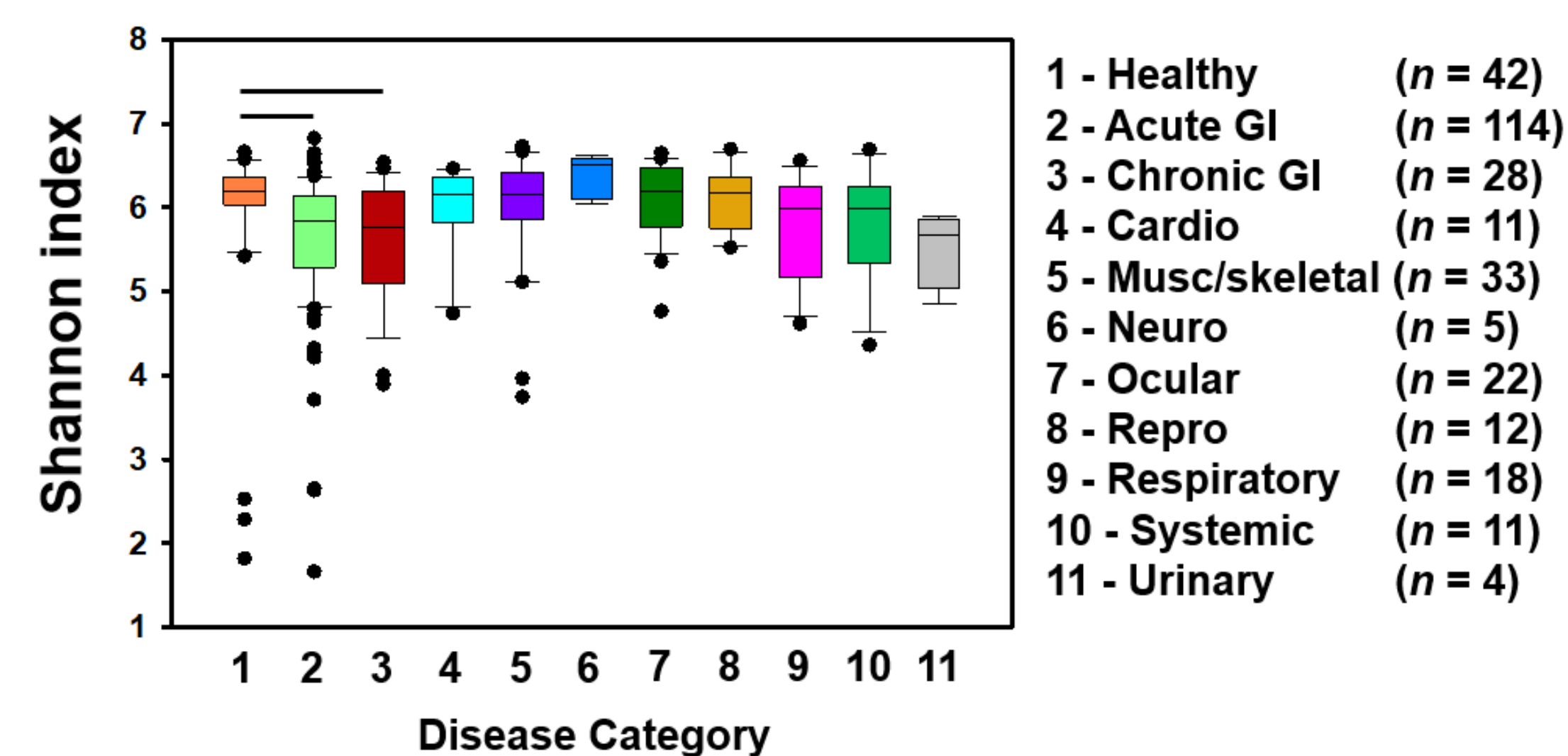
Conclusions

- Kruskal-Wallis one-way ANOVA on ranks, with Dunn's post hoc, using 'healthy' as control showed a significant difference in α-diversity between healthy equine GMs and GMs of horses presenting for gastrointestinal disease.
- Bray-Curtis PERMANOVA also showed significant differences in β-diversity between healthy GMs, GI GMs, and non-GI GMs.
- Other than GI disease, no significant association between disease group/affected organ system and GM diversity or richness was found.
- Contrary to our hypothesis, no difference in richness in the GM between decompensated colics that end in euthanasia or colics that resolve and are discharged was found.
- Limitations of this study include variation in sample size, and no ability to control for diet, breed, treatments, or other patient factors

Equine Gut Group

- ID compositional and functional characteristics of the equine GM associated with health and disease
- Participating Universities:
  - University of Missouri
  - Auburn University
  - Louisiana State University
  - Minnesota University
- 1st fecal sample passed by every horse entering clinic and additional samples every 24-48 hr

Acknowledgements