

# Impact of Prophylactic Antibiotics on Canine Fecal Microbiome in Orthopedic Emergency Cases

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## Background & Rationale

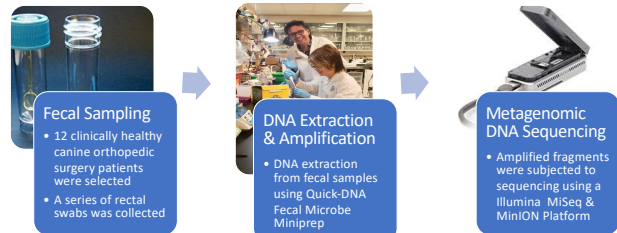
Growing evidence in human medicine supports that hospitalization and administration of antibiotics is associated with adverse changes in the intestinal microbiome (Iapichino, *et al.*, 2008). Selective digestive microbial decontamination (antibiotic use targeted at potential Gram-negative pathogens to protect critically ill patients) has been rejected (Benus, *et al.*, 2010), and it is now known the intestinal microbiome plays a key role in maintenance of systemic health. Antibiotic-induced intestinal microbiota disruption is therefore of clinical concern as it may impact host metabolism and immunity adversely, and inhibit patient recovery (Lankelma, *et al.*, 2017). Extreme intestinal microbiome imbalances may also serve as prognostic indicator in emergency medicine cases (Ojima, *et al.*, 2016). Appropriate antibiotic use (antimicrobial stewardship) and symbiotic therapy (prebiotics/probiotics) are other key areas of significant interest and importance in context of emergency medicine management. In dogs, scientific studies on intestinal microbial population have focused primarily on bacterial identity in more simple comparisons of healthy versus diseased animals. Antimicrobial resistance and zoonotic concerns have also been reported in dogs discharged from an intensive care unit (Ghosh, *et al.*, 2011). A critical knowledge gap exists of how intestinal microbiome abundance and diversity may correlate with relevant clinical parameters in context of small animal medicine, and thus serve as sensitive indicators to guide antibiotic treatment. Consequently, detailed studies linking the intestinal microbiota profile, commonly measured clinical parameters, and canine health status are needed. Canine patients presenting to emergency room settings with non-infectious disease are of major interest in this context. Prophylactic antibiotic use is often considered standard care in canine orthopedic emergency medicine; however, the practice is increasingly contentious in light of growing antimicrobial resistance concerns. Studies in human subjects suggest changes in the intestinal microbiome secondary to antibiotic use, and it is reasonable to expect canine patients will be subject to similar intestinal microbiota disruption. Here, we proposed to investigate canine microbiome composition changes in canine orthopedic trauma patients from admission to discharge and evaluate the microbiota profiles of dogs in context of antibiotic use.

## Hypothesis & Objectives

**Hypothesis:** Prophylactic antibiotic use in canine orthopedic emergency patients adversely impacts the intestinal microbiome and abundance. **Objectives:** 1) Collect rectal swabs from canine orthopedic trauma patients at specific time points; 2) Conduct DNA purification and library construction from samples; 3) Use MiniON technology (third generation, real-time, metagenomic DNA sequencing) and Illumina MiSeq (MiDOG LLC) to characterize the canine fecal microbiome and identify differentially represented taxa; 4) Identify changes in diversity and relative abundance of specific taxa in canine patients on a temporal scale, and in context of antibiotic administration status.

## Design & Methods

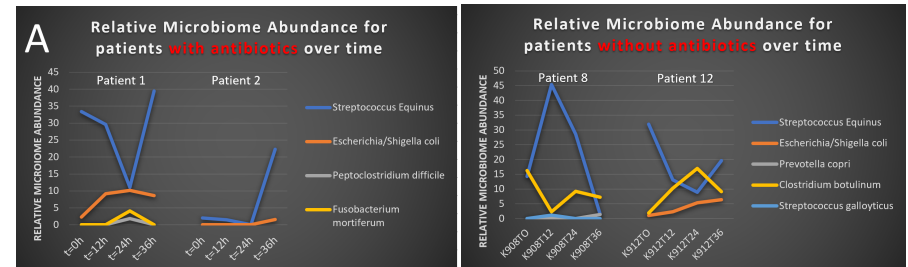
A total of twelve (12) client-owned adult dogs presenting with acute orthopedic trauma (but otherwise clinically healthy) were enrolled in the study. Six (6) dogs received standard care including prophylactic antibiotics, while the remaining six (6) dogs received standard care excluding prophylactic antibiotics. Inclusion criteria: closed, non-complicated fractures. Exclusion criteria: open, complicated fractures, co-morbidities (expected discharge longer than 48 hrs. post admission)



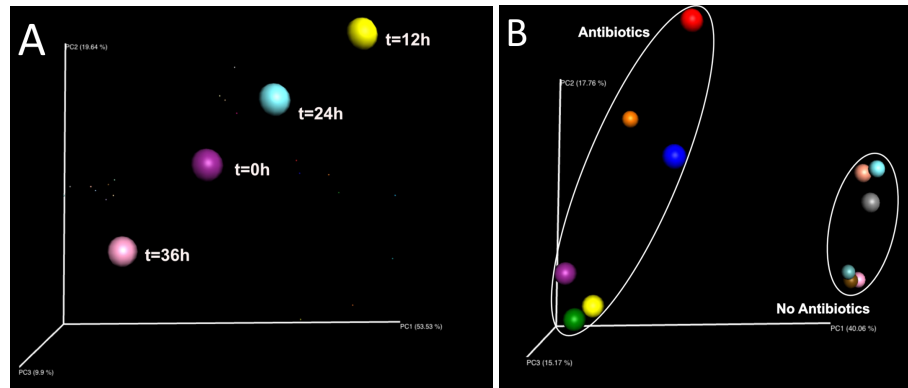
## Results

A.										B.										
Bacterial Pathogen	K901 O	K901 12	K901 24	K901 36	K902 O	K902 12	K902 24	K902 36		Bacterial Pathogen	K908 O	K908 12	K908 24	K908 36	K912 O	K912 12	K912 24	K912 36		
<i>Streptococcus equinus</i>	33.4	29.6	11.1	39.5	2.03	1.45	0	22.3		<i>Streptococcus equinus</i>	14.2	45.4	28.6	1.48	32	13.1	8.94	19.6		
<i>Escherichia/Shigella coli</i>	2.32	9.21	10.2	8.64	0	0	0	1.6		<i>Escherichia/Shigella coli</i>					1.05	2.34	5.31	6.41		
<i>Peptoclostridium difficile</i>	0	0	1.83	0						<i>Prevotella copri</i>	0	0	0	1.34						
<i>Fusobacterium mortiferum</i>	0	0	4.07	0						<i>Clostridium botulinum</i>	16.2	2.26	9.21	7.2	1.85	10.3	17	9.11		
<b>Sub Total</b>	<b>35.72</b>	<b>38.81</b>	<b>27.2</b>	<b>48.14</b>	<b>2.03</b>	<b>1.45</b>	<b>0</b>	<b>23.9</b>		<b>Sub Total</b>	<b>30.4</b>	<b>48.79</b>	<b>37.81</b>	<b>10.02</b>	<b>34.9</b>	<b>25.74</b>	<b>31.25</b>	<b>35.12</b>		

**Table 1** – Timeseries (0, 12, 24, and 36 hrs.) of relative microbial abundance for two select patients treated with a single dose of antibiotic (cefazolin) intraoperatively (A) versus two untreated control cases (B). The data was used to also illustrate differences in treated vs. controls over time in Figure 1.



**Figure 1** - Comparative assessment of temporal dynamics of pathogenic bacteria (Relative Abundance) for select cases post-antibiotic administration (A) vs. select control cases (B). Cefazolin administration resulted in a gradual decrease of *Streptococcus equinus* (gram-positive) at 12 and 24 hrs., followed by an increase at 36 hrs. (A). In untreated controls, microbial analysis indicated presence of different pathogenic bacteria with zoonotic potential, as well as an inverse relationship in the relative abundance of *Streptococcus equinus* and *Clostridium botulinum* over time (B).



**Figure 2** - Principal Coordinate (PCoA) Analyses using Bray-Curtis ecological distances displayed in three-dimensional space. Percentages describe total variance of the dataset explained by each axis. Panel A) Time series of microbiome structure of a single canine patient (control, without antibiotics) sampled at 12h intervals for 36hrs. Panel B) Microbiome of twelve (12) individual canine patients clustering as a function of antibiotic administration.

## Discussion & Conclusion

Treatment of canine orthopedic emergency cases with cefazolin changes the relative abundance of certain fecal pathogens over time. Strains such as *Streptococcus equinus* decrease up to 24 hrs. after hospitalization, followed by an increase. This is consistent with the clinical expectations for a first-generation cephalosporin antibiotic. Microbial analysis also documented clinically relevant pathogens with zoonotic potential (*Streptococcus gallolyticus* and *Clostridium botulinum*) in fecal samples from asymptomatic untreated dogs. This could also constitute a concern for immunocompromised individuals.

Bacterial community structure, based on Principal Coordinate Analyses of Bray-Curtis distances (Fig.2), shows temporal changes in the microbiome structure in fecal samples from a single canine patient in the absence of antibiotic administration (control case). This pattern of microbial structural changes is complex and regressive. We observe the following points: firstly, definite change is seen in bacterial community structure as a function of time without antibiotic administration. This indicates significant temporal fluidity in fecal microbiome composition irrespective of antibiotic treatment. Secondly, the community structure of the fecal microbiome changes most drastically in the time frame immediately following surgery (t=0-12hrs.) in both treated and untreated cases, which may in part be explained by exacerbation of stress load in recently hospitalized patients. Finally, the fecal microbiomes of a cohort of 12 patients shows clustering patterns as a function of antibiotic administration. Patients with no antibiotic administration (controls) show tight clustering patterns. Conversely, antibiotic administration increases both the intercommunity diversity amongst antibiotic administered patients and distance from the control (non-antibiotic) cluster.

In conclusion, these findings indicate that the fecal microbiome in dogs is complex with the ability of extreme biological fluidity. Insights on changes in the microbial diversity and composition in response to antibiotics is important in orthopedic emergency cases because such fluctuations could impact immune function and speed of recovery. It is furthermore a concern to identify fecal pathogens with zoonotic potential in otherwise clinically healthy orthopedic emergency cases.

## Acknowledgements & References

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Comparative data using the MiniON technology will become available in the forthcoming weeks.

References are available upon request.

