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Two Pyrantel Anthelmintics Alter Equine Fecal Microbiota

Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for Graduation with Honors Research Distinction

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2017

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Abstract

Horses house a dynamic population of microbes within their hindgut that can be disrupted by diet, stress, and medication. Anthelmintic drugs are regularly administered in the horse industry to reduce internal parasites. Although anthelmintic modes of action are well known, there is a lack of knowledge regarding the interaction between the anthelmintic and the intestinal microflora in horses. The objective of this study was to monitor changes in hindgut microbiota after treatment with two pyrantel anthelmintic formulations. Ten Quarter Horse mares (8.0 ± 6.0 yr) were randomly assigned to one of two anthelmintic treatment groups: paste or pellet. All mares continued to receive their basal diet of 0.5% BW of a 12% CP pelleted concentrate with mixed grass hay and water *ad libitum*. Mares in the paste treatment group received one dose (0.9 g per 136 kg BW) of pyrantel pamoate paste. Fecal samples were collected immediately before treatment (d 0) and on d 1, 2, 3, 7, 10, and 14 post-treatment. Mares in the pellet treatment group received pyrantel tartrate pellets (28.3 g per 113 kg BW) once daily for 14 d. Fecal samples were collected immediately before treatment (d 0) and on d 1, 2, 3, 7, 10, and 14 of treatment as well as d 1, 2, 3, 7, 10, and 14 post-treatment. DNA was extracted from fecal samples and subjected to PCR-DGGE with universal primers specific to the V2-V3 region of the 16S rRNA gene. PCR-DGGE images were analyzed with BioNumerics software to generate dendrogram comparisons with further evaluation using principal coordinate analysis (PCA). Dendrograms and PCA revealed clustering by time in both treatment groups. Mares in the paste group showed greater change in diversity immediately follow treatment, while mares in the pellet group showed a gradual change in microbial diversity during exposure to the anthelmintic.

Introduction

The equine microbiota play key roles in nutrient acquisition, fermentation, immune homeostasis, and the overall health of the horse (Costa and Weese, 2012). Although Firmicutes are predominantly found throughout the intestinal tract, the diversity of microbiota varies between intestinal compartments (Dougal et al., 2012; Costa et al., 2015a). Fecal microbiota do not differ significantly from hindgut microbiota, making the analysis of fecal microbiota an ideal, noninvasive method for characterizing bacterial changes in the horse's hindgut (Costa et al., 2015a).

Changes in hindgut microbiota can be attributed to intestinal disease (Costa et al., 2012), diet; (Julliand et al., 2001; Daly et al., 2012; Destrez et al., 2015), and stressful events such as travel (Faubladier et al., 2013; Schoster et al., 2015). Medications including anesthesia, non-steroidal anti-inflammatory drugs (NSAID), and antimicrobials have also been shown to alter the intestinal microbiota (Schoster et al., 2015; Rodriguez et al., 2015; Costa et al., 2015b). In humans, administration of oral antibiotics such as cephalosporin, quinolone, and penicillin were shown to induce fluctuations in microbial diversity (Edlund and Nord, 2000). Antimicrobial-associated enterocolitis and diarrhea are consequences of dysbiosis in the equine gastrointestinal tract which may allow for the proliferation of opportunistic pathogens such as *Salmonella* and *Clostridium difficile* (Hird et al., 1984; Hollis and Wilkins, 2009; Barr et al., 2013). In a study of antimicrobial-associated diarrhea in horses admitted to three referral hospitals with a history of diarrhea and antimicrobial usage, intestinal colonization by *Salmonella* and *Clostridium difficile* was associated with an 18.8% mortality rate (Barr et al., 2013). Although several medications have been shown to alter the intestinal microbiota, the influence of anthelmintic products influence on the intestinal microbiota has not been studied.

Anthelmintics are routine administered to horses to aid in the control of intestinal parasites (Proudman and Matthews, 2000). Pyrantel, a common equine anthelmintic, can be administered as both pyrantel pamoate paste and prophylactic pyrantel tartrate pellets (Reinemeyer et al., 2014). While both forms of pyrantel have been shown to be effective in killing and controlling intestinal parasites, the effect of its administration on hindgut microbiota has not been studied (Reinemeyer et al., 2014). Previous research in dogs using traditional culture methods demonstrated no changes in gastrointestinal microbiota when niclosamide, dichlorophene, methylbenzene, and arecoline hydrobromide anthelmintics were administered (Gelbart et al., 1976).

An understanding of the influence of routine medications such as anthelmintics on the microbial dynamics of the gastrointestinal tract is important for optimal management of our horse population. The timing of administration could be managed more effectively to minimize stress by choosing to deworm the horse well in advance of other potentially stressful events such as travel, planned medical procedures, breeding, and competition. Additionally, deworming in the future could be coupled with probiotic treatments to stabilize any disruption of the the intestinal microbiota. In horses the most promising use of probiotics to stabilize fluctuations in microflora has come from targeting specific bacterial species and modes of action rather than general, non-specific treatments (Schoster et al., 2014). Therefore, knowing the influence anthelmintics may have on the hindgut microbiota is important to optimizing management practices.

Hypothesis

My hypothesis was that both pyrantel anthelmintics would noticeably influence the equine hindgut microflora. Additionally, the varying formulations of pyrantel anthelmintic would have different effects over time, with the pyrantel tartrate pellet creating a more gradual change in microbial diversity than the pyrantel pamoate paste.

Materials and Methods

Ten Quarter Horse mares (8.0 ± 6.0 yrs) were randomly assigned to one of two pyrantel anthelmintic treatment groups: paste or pellet. Prior to and throughout the study, all horses received a basal diet of 0.5% BW of a 12% crude protein pelleted concentrate, with water and mixed grass hay *ad libitum*. Horses were housed in two adjacent paddocks throughout the study with access to shelter and pasture at all times. Each paddock housed three horses from one treatment group and two horses from the other treatment group in order to minimize environmental differences.

Horses in the paste treatment group received one dose according to label (0.9 g /136 kg BW) of pyrantel pamoate paste. Fecal samples were collected immediately before treatment (d0) and on d 1, 2, 3, 7, 10, and 14 post-treatment. Mares in the pellet treatment group received pyrantel tartrate pellets according to label (28.3 g /113 kg BW) once daily for 14 d. Fecal samples were collected immediately before treatment (d0) and on d 1, 2, 3, 7, 10, and 14 of treatment as well as d 1, 2, 3, 7, 10, and 14 post-treatment. The d0 sample for both groups served as the control. Previously analyzed fecal microbiota in dogs, using the same laboratory methods as below, showed stable microbiota fluctuations in three samples over 10 days prior to any experimental intervention sufficiently showed treatment effects over time (Gronvold et al.,

2010a). Therefore, comparison to the single d0 sample was used in place of a pre-treatment cluster of three samples to show treatment differences in this study.

Fecal samples were pooled by treatment group and day. DNA was extracted from the pooled fecal samples via a modified bead beating method (Yu and Morrison, 2004). Concentrations of extracted DNA were then determined by PicoGreen analysis. PCR with universal primers specific to the V2-V3 region of the 16S rRNA gene sequences and subsequent denaturing gradient gel electrophoresis (DGGE) analyses were performed using IGENY phorU-2 (Igeny; Leiden, The Netherlands) and images captured with AlphaImager HP® (ProteinSimple; Santa Clara, California) (Fig. 1). Images were analyzed with BioNumerics software (Applied Maths NV; Sint-Martens-Latem, Belgium) to compare microbial diversity. Comparison dendrograms and principal component analysis (PCA) were created from BioNumerics analysis of DGGE banding patters based on the position and number of bands present using the Jaccard coefficient.

Results

The hindgut microbial profiles of the paste treatment group (Figs. 2A, 2B) showed differences from the d0 control. The d0 control sample taken prior to administration of anthelmintic was the least closely related microbial profile to any other sample. The greatest shift in microbial diversity in the hindgut occurred immediately following administration of the anthelmintic paste. Additionally, d1, d2, and d3 following the paste treatment clustered together in a more closely related group. Samples continued by time to be more closely related to that cluster with d7 being more similar to d1-d3 than to the original d0 sample, and with d10 and d14 being most similar to each other and then the d7 sample. The greatest finding from these data is

the stark difference between the d0 sample and the rest of the samples, again suggesting that the greatest difference in microbial diversity occurred immediately following administration of the pyrantel pamoate paste.

The microbiota profiles of the pellet treatment group (Figs. 2C, 2D) also displayed clustering during the course of sample collection. In this treatment, the d0 control sample was not markedly different from the rest of the treatment samples. A cluster was observed with d7 P, d10 P, and d14 P samples. These three samples are the farthest away from the samples when treatment was administered, but are not as similar to the d0 control as the treated day samples. Also of note, the pair of d14 and d1 P that are set off from all other samples are from consecutive days coming at the longest point of sustained treatment. These two samples only showed similarity to each other and no other samples in the dendrogram. A clear line can be drawn to separate all samples from treatment days from the post treatment days in the PCA (Fig. 2D) and again the only points appearing in the bottom right quadrant are d7 P, d10 P, and d14 P, just like the oral paste group.

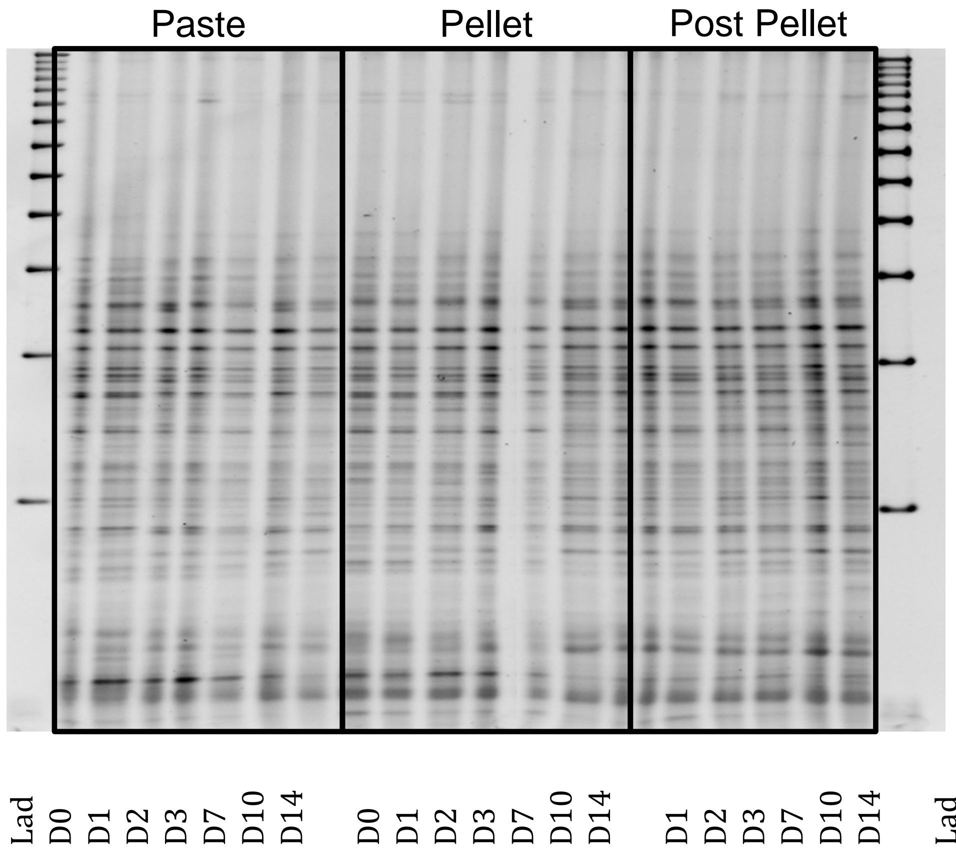
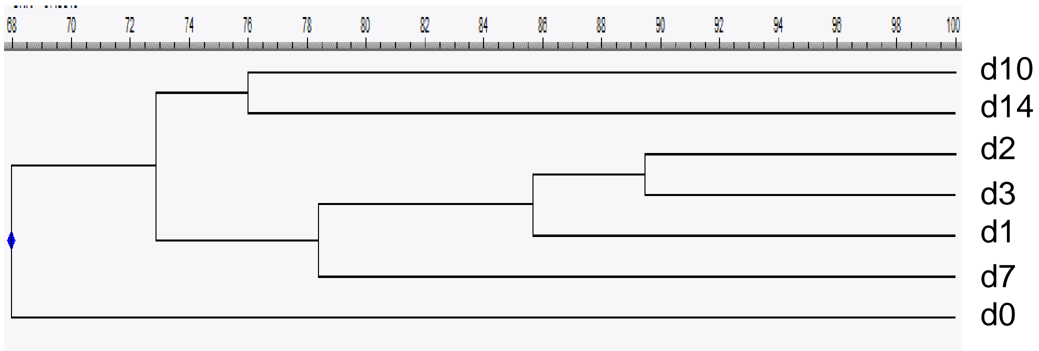
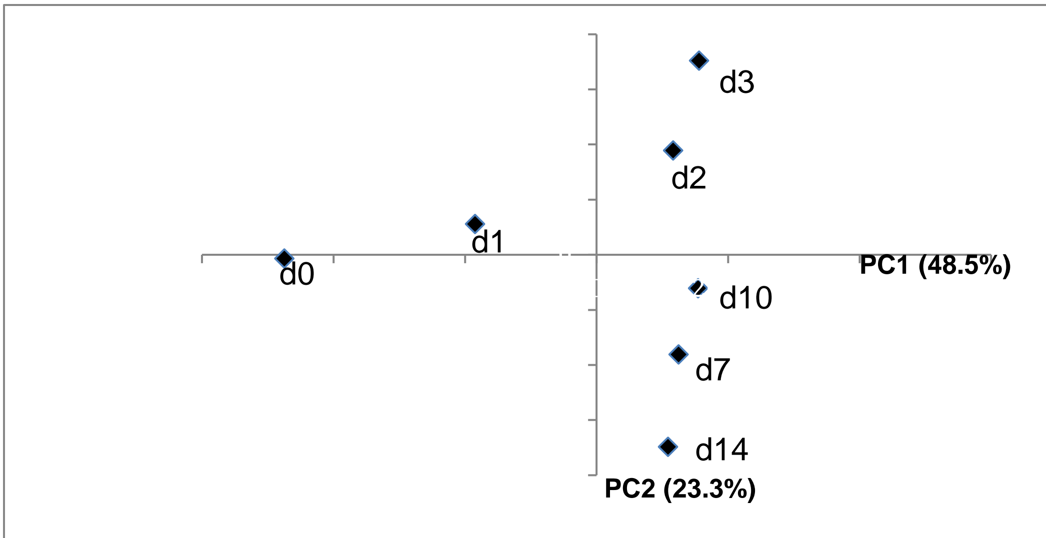


Figure 1: DGGE image of pooled fecal samples PCR product. Each lane represents the bacterial DNA present in the pooled group's fecal sample on each day. Differences between banding patterns were determined and qualitatively analyzed based on band position and number.

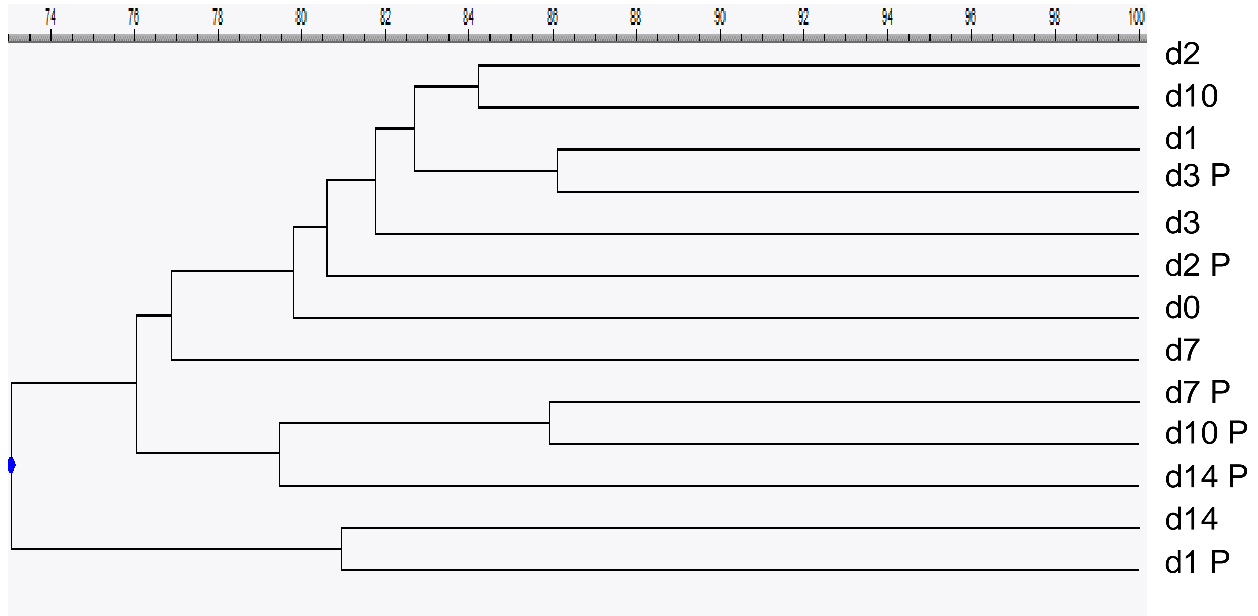
2A



2B



2C



2D

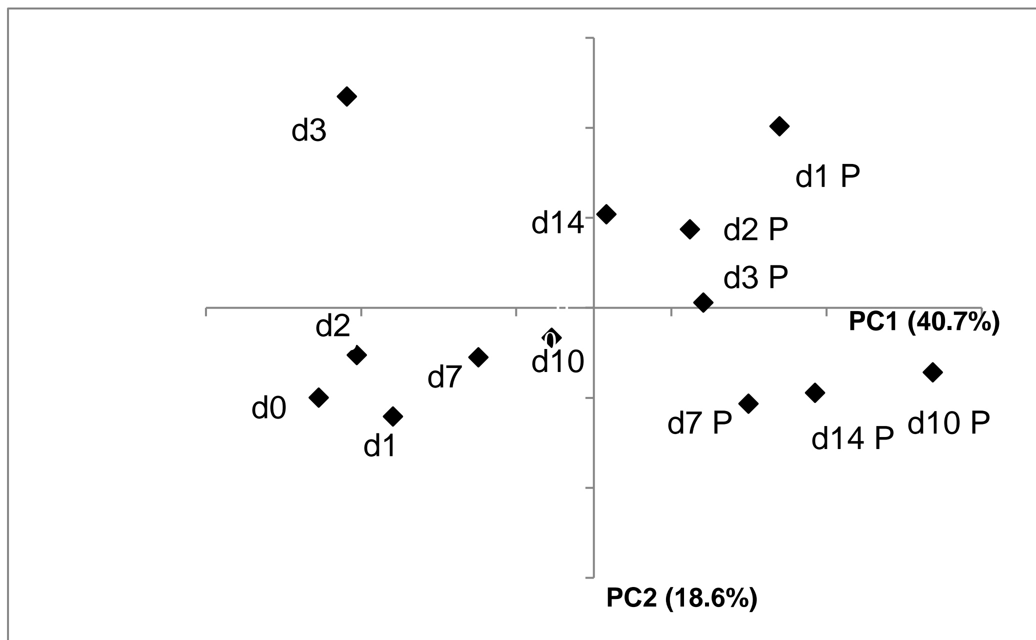


Figure 2: PCR-DGGE Fecal Microbiota Analysis after Pyrantel Treatments. Paste dendrogram (Panel A) shows d0 bacterial diversity least similar to any day after pyrantel pamoate treatment. Paste PCA (Panel B) graphically describes the similarity of the bacterial diversity, again showing d0 set apart from any of the days after pyrantel paste treatment. Pellet dendrogram (Panel C) shows relatedness of bacterial diversity during pyrantel tartrate treatment fluctuating, with a cluster of similar bacterial diversity occurring at 7, 10, and 14 days post-pellet treatment. Pellet PCA (Panel D) also shows bacterial diversity on days 7, 10, and 14 post-pellet treatment as most related to themselves, yet dissimilar to the original d0 bacterial diversity.

Discussion

While previously no changes were observed in the microbiota of dogs treated with anthelmintics (Gelbart et al., 1976), the present study in horses showed changes in the microbial diversity of fecal samples after treatment with anthelmintics. Shifts in the microbial profiles of horses given the Pyrantel paste were more distinct compared to changes in the microbial profiles of horses given the Pyrantel pellet in both the dendrogram (Figure 2A) and the principal component analysis (PCA; Figure 2B). This type of difference seems indicative of a more abrupt change in microbiota diversity than the pellet. Furthermore, it is interesting that after treatment the microbial profiles do not return to a state more similar to the d0 sample collected prior to treatment. Rather, the cluster of d7, d10, and d14 are the least related samples to d0 for the oral paste group. Therefore, this suggests that a new equilibrium of microbiota, or at least an equilibrium not similar to the original results from anthelmintic treatment. Further study with observation of fecal samples for an additional 14 d post treatment would help to confirm this trend.

Dendrogram and PCA analysis suggest similar microbial profiles on d 7P, 10P and 14P in horses given the Pyrantel pellet (Figures 2B and 2C). This cluster is again not as similar to the d0 pre-treatment sample as the samples while the horses were treated. The drift in microbial profile similarity occurs more slowly, suggesting that continual treatment with a pelleted anthelmintic can cause a sustained change over time to the hindgut microbiota. Again, the equilibrium that appears to be established seven days after discontinuing the pellet treatment is different from the control equilibrium of the d0 sample.

While the present study provides evidence for changes in hindgut microbial populations after the administration of Pyrantel anthelmintics in horses, PCR-DGGE could only detect shifts

in microbial diversity rather than identify specific bacterial populations. Further studies utilizing DNA sequencing to identify specific bacterial species are needed to better characterize the changes occurring after anthelmintic administration.

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