A CLINICAL ANALYSIS OF THE EFFECT OF ANTIBIOTIC ADMINISTRATION ON THE NEONATAL FOAL MICROBIOME

By

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ABSTRACT

A study was conducted to investigate the effects of antibiotic administration on the neonatal foal microbiome, complete blood count (CBC), and fibrinogen (FIB) concentration. Eleven stock-type newborn foals were studied in the clinical setting. Foals were enrolled in the trial within 24 h of birth and administered treatments according to CBC, fibrinogen, and veterinary decision. Foals were treated with an antibiotic (amikacin and ceftiofur, amikacin and penicillin, or trimethoprim/sulfadiazine; n = 7), or not treated (n = 4). Foals that did not respond to the initial antibiotic treatment based on CBC, fibrinogen, and veterinary supervision were administered subsequent treatment (n = 3). The trial began January 2018 and ended September 2018, with each foal being enrolled from birth to at least 56-d of age. Fecal samples were collected from foals at d 0, 7, 14, 21, 28, and 56. When a foal experienced approximately 1 mo without treatment or at 56 d, whichever was earliest, a corresponding dam fecal sample was collected by the consulting veterinarian or trained staff. Blood was obtained on d 0, 7, 14, 21, 28, and 56 and analyzed using an automated hemocytometer (IDEXX ProCyte Dx Hematology Analyzer, IDEXX Laboratories, Westbrook, ME) to determine complete blood count variables. Blood serum was analyzed using an automated analyzer (IDEXX Catalyst Dx Chemistry Analyzer with an Equine 15 CLIP, IDEXX Laboratories), and fibrinogen was determined via IDEXX VetAutoread (IDEXX Laboratories). Fecal samples were submitted to a commercial laboratory (Molecular Research LP, in Shallowater, TX) for DNA sequencing and

microbiome analysis. There was no effect of treatment × day interactions for hematology parameters (red blood cell concentration, $P = 0.86$; white blood cell concentration, $P = 0.69$; neutrophils, $P = 0.59$; glucose, $P = 0.43$; and fibrinogen, $P = 0.90$). There was an effect of day observed on red blood cell concentration ($P < 0.001$), white blood cell concentration ($P = 0.04$), neutrophils ($P = 0.03$), and fibrinogen ($P = 0.02$). In addition, there was an effect of treatment ($P = 0.02$) on fibrinogen. *Clostridia* and *bacteroidia* were the most abundant bacteria found in fecal samples of the foal and dam pairs. There was no effect of treatment × day interaction on *clostridia* ($P = 0.24$) or *bacteroidia* ($P = 0.35$). However, there was a main effect for day ($P < 0.001$) noted for relative abundance of each bacterium. Although present in very small amounts (< 1%), *fibrobacteria* was the only class of bacteria to result in an effect of treatment × day interaction ($P = 0.05$). Bacterial flora became more diverse and similar to the matched dam sample after the completion of any antibiotic treatment. Further research is needed to determine the role specific antibiotics play in the neonatal foal and if antibiotic use has any affects that might carry into adult life. In addition, continued microbiome studies could lead to microbiome analysis being involved in diagnostics.

Key words: amikacin, *bacteroidia*, ceftiofur, *clostridia*, *fibrobacteria*, microbiome, penicillin

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CHAPTER I

INTRODUCTION

Scientific understanding of the equine gastrointestinal tract (GIT) and microbiome has recently become a focal point in equine medicine. Researchers are discovering how the vast and varied microbes in the equine GIT can affect equine health. A forerunner in microbiome studies, Scott Weese, DVM, MSc, DACVIM, professor in the Department of Pathobiology at the University of Guelph's Ontario Veterinary College, began studying the equine microbiome in the early 2000s. Weese believes that changes in the microbiome can result in equine disease, such as colic, colitis, and laminitis (Oke, 2017).

Infectious and bacterial equine diseases, as well as disease within other species, are commonly treated with antibiotics. With the increased study of the microbiome, scientists and practitioners have begun to question the impact may antibiotics have on the microbiome of the animal and if such an impact follows the neonates treated with antibiotics into their adult lives. There is unsurmountable evidence showing the influence of antibiotics on pathogenic bacteria. However, the control over bacterial disease might be diminishing as pathogens acquire the genetic capacity to survive antibiotic treatment, mainly due to the overuse of antibiotics (Langdon et. al., 2016).

Effective diagnosis of equine disease depends on the knowledge of common pathogens, the availability, specificity and sensitivity of diagnostic tests, and the interpretation of test results. It is often time consuming (Dunkel and Wilkins, 2004), dealing with fecal samples and

retrospectively comparing clinical diagnostic tools to microbiome data, which can be fatal to the neonatal foal. Neonatal foals are at an increased risk for sepsis, septic joints, bacteremia, and a compromise of the GIT barrier (Beard, 2009). The intestinal microflora that comprises the GIT is a first line of defense for the neonate against enteric pathogens via competitive exclusion (John et al., 2015); therefore, the practitioner should have the confidence that antibiotic use in neonates, even as young as 1 d of age, is safe and effective.

CHAPTER II

REVIEW OF LITERATURE

Clinical Definition of Abnormal Health Status of the Neonatal Foal

Newborn foals present a tremendously unique challenge to the practitioner, as foals can appear normal in the morning and die by the evening with little to no explanation or symptom. Because of this phenomenon, early detection is paramount in preventing neonatal disease (Sonea, 1989). Close observation is key to this early detection. In addition, in the clinical setting, this observation can be supplemented by using diagnostic technologies that allow practitioners to monitor blood anomalies, such as immunoglobulin G (IgG), and other blood work early in the foal's life. However, in neonatal foals, any deviation from normal behavior should be considered abnormal and cause a practitioner to examine and potentially treat the animal.

Immunoglobulin G (IgG)

In the clinical setting, newborn foals were assessed during or shortly thereafter parturition in order to identify the foal's ability to stand, and more importantly, to suckle colostrum. This intake of antibody-rich milk shortly after birth provides the foal with passive protection against many equine pathogens. The practitioner can identify that the foal ingested this protection by testing for concentration of IgG (Sonea, 1989). Immunoglobulin G is easily and affordably obtained by use of a standard IgG rapid test. Foals that do not receive colostrum are prone to overwhelming infections, including septicemia, pneumonia, and other infectious diseases. The lack of colostrum ingestion would result in the

foal being labeled with an abnormal health status and would require supplementary colostrum administration. Colostrum can be milked from the dam or provided synthetically. Normal serum IgG concentration for 1-d old foals is 1000 mg/dL. In a standard IgG rapid test, failure of passive antibody protection is marked by an IgG level of less than or equal to 400 mg/dL; partial failure is marked with an IgG level between 400 and 800 mg/dl; and success is marked by a concentration over 800 mg/dL (IDEXX pamphlet).

Complete Blood Counts (CBC) and Blood Chemistry Profile

After an assessment of IgG, CBC and blood chemistry profiles further assist in identifying an abnormal equine neonate. These diagnostic profiles provide a plethora of measures, with the most notable clinical parameters being red blood cell concentration (RBC), white blood cell concentration (WBC), neutrophils (NEU), and glucose (GLU). Red blood cells, WBC, and NEU are obtained via CBC hematology, while GLU is monitored via serum biochemistry (Brown, 1989). According to Brown (1989), normal RBC for a neonatal foal should fall between 8.8×10^6 and 11×10^6 cells/L at 1 d, 8.3×10^6 and 10.7×10^6 cells/L at 3 d, and 9.6×10^6 and 16.4×10^6 cells/L at 2 wk. Normal WBC for a neonatal foal should fall between $4{,}500 \times 10^6$ and $11{,}500 \times 10^6$ cells/L at 1 d, $5{,}100 \times 10^6$ and $10{,}700 \times 10^6$ cells/L at 3 d, and $6{,}500 \times 10^6$ and $14{,}400 \times 10^6$ cells/L at 2 wk. Normal NEU for a neonatal foal should fall between $3{,}040 \times 10^6$ and $9{,}570 \times 10^6$ cells/L at 1 d, $3{,}210 \times 10^6$ and $8{,}720 \times 10^6$ cells/L at 3 d, and $3{,}990 \times 10^6$ and $11{,}520 \times 10^6$ cells/L at 2 wk. Normal GLU of a neonatal foal should be 160.2 ± 45.7 mg/dL at 1 d, 158.4 ± 5.3 mg/dL at 2 d, and 158.3 ± 8 mg/dL at 2 wk (Brown, 1989). Glucose levels are closely monitored, as both elevated and depressed blood glucose can lead to septicemia or death in the neonate (Brown, 1989).

Fibrinogen

According to Brown (1989), normal fibrinogen (FIB) in the neonatal foal should fall between 108 x 10 and 448 x 10 g/L at 1 d, 160 x 10 and 449 x 10 g/L at 3 d, and 155 x 10 and 617 x 10 g/L at 2 wk. Fibrinogen is made in the liver by hepatocyte cells (Casini et al., 2016). Because FIB is an acute-phase protein, FIB appears elevated in most forms of inflammation (Page and Schroeder, 1976). Acute-phase proteins are present in the blood of horses during episodes of colic and can be found in even higher concentrations during times of inflammation (Pihl et al., 2016).

Equine Microbiome

The microbiome of any species is a relatively new topic. Most horse enthusiasts would associate the equine microbiome with gastrointestinal tract (GIT) upset or, as in the case with neonates, diarrhea. Recent studies and advancements in technology have allowed the equine community to start asking more specific questions about the equine microbiome's potential connection to whole equine health.

A forerunner in the equine microbiome and a specialist in pathobiology, J. Scott Weese, DVM (2015), explains that the intestinal tract of the horse is composed of a complex and diverse population of bacteria, called the microbiome. The microbiome is essential for symbiosis in the horse, and disruptions in this environment can cause various types of disease. In the case with neonates, these disruptions can quickly lead to death if not monitored appropriately. Weese (2015) recognizes antibiotic use as one of the main risk factors for equine GIT upset; however, there is vague assumption this is due to beneficial bacteria becoming displaced.

Horses are classified as monogastric herbivores whose digestive system is adapted for the continuous ingestion of foodstuffs (Hutton et al., 2003). A horse's stomach is small but efficient for grain utilization and the cecum and colon utilize roughage. Compared to other domestic animals, the horse has the largest large intestine (Hutton et al., 2003). The equine is adapted to survive solely on forage, which requires the assistance of microbes for the digestion of celluloses. This digestion occurs within the cecum, farther into the digestive tract as compared to ruminants (Frandson et al., 2006). Most of the microbial activity occurs in the equine large intestine, which comprises nearly 60% of the GIT (Dicks et al., 2014). In addition to being necessary for digestion and absorption, the intestinal barrier acts as the first line of defense against antigens, food allergens, and most importantly, commensal bacteria (Tanabe et al., 2014).

Current research suggests that neonatal horses are born with a sterile GIT and are not yet hindgut fermenters (Julliand et al., 1996; Corley and Hollis, 2009). Animal scientists and veterinarians do not yet know the timeline in which the bacterial populations of the neonate develop to that of a mature horse; however, the development of bacterial populations is speculated to occur sometime between birth and weaning (Earing et al., 2012). Julliand et al., (1996) suggested that the colonization of the foal microbiome is impacted by environmental exposure and noted that the total fecal microbial population was 10^8 colony forming unit (cfu)/g in the first 24 h.

The equine microbiome is a powerful entity within the horse. Schell (2018) explained that the balance between species of bacteria, protozoa, and yeast is generally in harmony, but in states of disease, the opposite is true. The author sees an unmistakable link between the equine microbiome and equine health. The big picture is that complications occur when more harmful bacteria that are naturally and normally occurring within the digestive tract increase in number

while beneficial bacteria decreases. The solution is not an antibiotic or a probiotic, as these products often disrupt the already fragile healthy bacterial populations, and in most cases, probiotic bacteria simply cannot survive the hostile environment. Schell (2018) cultured 300 equine fecal samples in a laboratory and reported that 80% of the horses demonstrated active dysbiosis with overgrowth of lactic acid bacteria, regardless if they were being treated with a probiotic, processed foods, whole foods, or otherwise. The author equated this to an overgrowth of Coliform bacteria, including *E.coli.* When the Coliform group of bacteria is overgrown, an increase in *Salmonella* and *Clostridial* organisms commonly occurs. The fluctuations of bacterial populations aids in the understanding of why some horses develop diarrhea type conditions or endotoxemia (Schell, 2018).

An understood "standard" healthy microbiome map for the equine has yet to be established. However, preliminary evaluations are made as scientists study clinically healthy horses as various stages of life to understand microbiome fluctuations over time. Costa et al. (2015b) studied the bacterial populations of the different sections of the equine GIT and reported that fecal samples closely resembled the populations of the distal portions of the GIT. In addition, the author noted that regardless of compartment of the GIT, *Firmicutes* was the primary phylum represented in adult horse and foal fecal samples (Costa et al., 2015a). In 2017, 5 3-yr old Quarter Horses enrolled in a basic training program were sampled 4 times over an 8-m period by Martin et al. (2017). The horses did not experience any GIT upset during the trial. After fecal sample gene sequencing, *Bacteroidetes* was determined as the most abundant phylum of bacteria. As *Bacteroidetes* decreased, *Fibrobacteres* and *Spirochaetes* showed increase. However, *Firmicutes* remained constant over the trial (Martin et al., 2017).

Because specific understanding and study of the equine microbiome is not as understood as that within the human, the human microbiome sheds light on the equine microbiome. As with horses, microbial populations within humans mostly inhabit the distal gut (Johnson et al., 2017). Within the human GIT tract, *Bacteroidetes* and *Firmicutes* bacteria are often considered the most prevalent phyla, which is a common trend amongst many mammals (Johnson et al, 2017). *Bacteroidetes* are anaerobic, bile-resistant, non-spore-forming, gram-negative rods (Wexler, 2007). According the Johnson et al. (2017), the predominant *Bacteroidetes* in the human GIT are *Bacteroides, Prevotella,* and *Porphyromonas.* There are approximately 99 species of *Bacteroides* with many relatives that are known from their 16S rRNA gene sequences. *Prevotella* is most diverse according to the Greengenes database but has fewer described species as compared to *Bacteroides* (Johnson et al., 2017). *Bacteroidetes* have a unique ability to utilize polysaccharides (Backhed et al., 2005) and have a high number of carbohydrate-active enzymes (El Kaoutari et al., 2013). In addition, *Bacteroidetes* are metabolically flexible and appear suited for any variable diet (Johnson et al., 2017). According to a study on UK twins in 2016, Goodrich et al. sequenced the microbiome of 1,126 twin pairs. The authors determined that for a majority of *Bacteroidetes*, environmental factors, including diet, have the most impact on relative abundance and are not impacted by host genetics.

Firmicutes are gram-positive bacteria within the human microbiome (Koliada et al., 2017). The *Firmicutes* phyla is divided into *Bacilli* and *Clostridia* and are common soil bacteria that can be cocci or rod-shaped (Galperin, 2013). According to Koliada et al. (2017) in a study of 61 adults humans, there is a relationship between *Firmicutes* and *Bacteroidetes.* As *Firmicutes* increases, *Bacteroidetes* decreases which might lead to an increase in body mass index (Koliada et al., 2017). In a study by Costa et al. (2012), 6 healthy horses and 10 horses suffering with

colitis were subjected to fecal grabs for microbiome comparison. According to the authors, *Firmicutes* was the most abundant phyla (68%), *Bacteroidetes* was second most abundant (14%), and *Proteobacteria* comprised 10%. in healthy horses. In this study, horses with colitis had microbiome populations of 40% *Bacteroidetes* and 30% *Firmicutes.* Costa et al. (2012) determined that *Clostridia* was most prevalent in healthy horses.

Next Generation Sequencing Technology for Microbial Community Analysis

In 1953, Watson and Crick solved the three-dimensional structure of DNA (Heather and Chain, 2016). However, understanding the sequencing of DNA did not occur for many years. Between 1953 and 1972, scientists relied primarily on analytical chemistry to help them sequence microbial ribosomal RNA or the RNA bacteriophages. In 1972, Walter Fiers produced the first complete protein-coding gene sequence of the coat protein of bacteriophage MS2. Four years later, the complete human genome was completed (Heather and Chain, 2016).

The understanding of gene sequencing continued to expand, allowing scientists to identify gene sequences that had seemingly remained unchanged over time, such as the 16S rRNA gene. The 16S rRNA gene sequence is the most commonly used genetic marker, due primarily to the large size of the 16S rRNA gene sequence (Janda and Abbott, 2007).

Costa et al. (2015a) performed research on the bacterial colonization of the GIT of foals using next generation sequencing. Researchers obtained fecal samples from 11 pregnant dams and subsequently obtained fecal samples from their foals at 1 d and 9 mo. Using the V4 region of the 16S rRNA gene sequence, the researchers were able to report a richly diverse bacterial community within the foal, mainly composed of *Firmicutes.* Foals less than 30 d had decreased diversity comprising *Akkermansia* spp. *Firmicutes, Verrucomicrobia,* and 90% unclassified

bacteria that was mostly prevalent in foals aged 60 d and greater (Costa et al., 2015b). Costa et al. (2015b) also remarked that foals generally have a relatively stable microbiota at 60 d of life.

Conversely, a study performed by Fox et al. (1992) compared the sequences of three psychrophilic strains of *Bacillus globisporus* W25^T, *Bacillus psychrophilus* W16A^T, and *Bacillus psychrophilus* W5. Fox et al. (1992) identified extreme similarities between the strains that must be considered when sequencing with 16S rRNA. The scientists warned that 16S rRNA sequencing might not always identify statistically significant differences and the sequences cannot always be regarded as significant (Fox et. al., 1992). However, despite some controversy of the exactness of 16S rRNA sequencing, the genomics industry accepts the 16S rRNA sequence as the standard for analysis of genes (Zhou et al., 2015).

Antibiotics

Antibiotics have been deemed one of the most successful forms of chemotherapy in medical history (Aminov, 2010). Research conducted by Bassett et al. (1980) and Nelson et al. (2010) discovered traces of tetracycline in skeletal human remains from Sudanese Nubia, dating back to 350 to 550 AD. This discovery uncovered the understanding of early humans about the effects of antibiotic compounds that could be obtained through diet. Through time, the scientific understanding of microbes and antibiotics continued to advance from the primitive ideology of the Sudanese Nubians. In 1909, Paul Ehrlich cured syphilis, or *Treponema pallidium,* with synthesized organoarsenic derivatives of Atoxyl in rabbits (Ehrlich and Hata, 1910). Notably, and arguably the most well-known discovery in antibiotic history, is the discovery of penicillin by Alexander Fleming in 1928. Toward the end of the Second World War, lyophilized penicillin was made available to veterinarians, mainly for the treatment of bovine mastitis (Gustafsson and Bowen, 1997). The research and study performed by Ehrlich and Fleming became the

cornerstones of drug research strategies that resulted into thousands of drugs that could be used in the clinical setting in the modern age (Aminov, 2010).

Modern antibiotics are divided into three different types of antibiotics, depending on the ability to impact gram-positive, gram-negative, or a mix of the two (broad spectrum; Brunning, 2014). The nomenclature "gram" is derived from the Gram test, where gram-positive bacteria retain the Gram test dye and turn a violet or purple color. In the Gram test, gram-negative bacteria do not retain the dye, turning red or pink. In general, gram-negative bacteria are more resistant to antibodies over gram-positive bacteria due to the differences in cell wall permeability (Brunning, 2014).

In addition, antibiotics can be labeled as bactericidal (bacteria killing) or bacteriostatic (growth slowing; Bush, 2012). Bactericidal agents include the antibiotic classes: penicillin, aminopenicillin, cephalosporins, fluoroquinolones, and aminoglycosides. Bacteriostatic agents include antibiotic classes: tetracyclines, florinenicol, macrolides, sulfonamides, and spectinomycin (Papich, 2016). Aminoglycosides are active against aerobic gram-positive bacteria and some gram-negative bacteria. Cephalosporins are grouped into three "generations" based on their spectrum of antibacterial activity. The first generation cephalosporins are highly effective against gram-positive bacteria; second generation cephalosporins are similar to or slightly less effective than first generation cephalosporins regarding efficacy against gram-positive bacteria; however, the decrease in efficacy is against *S. aureus* and *S. intermedius.* Second generation cephalosporins are effective against gram-negative bacteria that the first generation is not effective against. Third generation cephalosporins are the most effective against gram-negative bacteria (Papich, 2016). The activity of penicillin includes aerobic and anaerobic gram-positive bacteria and has little activity against staphylococci and many gram-negative

bacteria. Aminopenicillin is similar to penicillin in its activity; however, it is more efficient against gram-negative bacteria. Sulfonamide antibiotics are bacteriostatic and broad spectrum, acting not only on gram-positive and gram-negative bacteria, but also some protozoa, such as coccidia (Papich, 2016). Tetracyclines are bacteriostatic and broad spectrum. Tetracycline has some activity against gram-positive aerobes and some gram-negative bacteria, such as the common pneumonia pathogen (Papich, 2016).

A significant factor to consider with antibiotics is resistance. In general, the level of resistant infections correlates to antibiotic consumption (Goossens et al., 2005). Complying with drug use instructions as prescribed by physicians is important; however, at times, compliance can be difficult to maintain in cases where numerous, long-term antibiotics might be used. In addition, physicians may contribute to resistance by empirically prescribing antibiotics (Aminov, 2010). In the agriculture industry, the prophylactic use of antibiotics is common, given primarily to the fact that antibiotics are more effective when used early in diagnosis, and especially through the prevention of predicable disease during various stages of an animal's life (Gustafsson and Bowen, 1997).

Use of Antibiotics in Neonatal Horses

In 2009, Corley and Hollis explained an antibiotic treatment will only be successful if therapy is implemented early, the correct antibiotic is chosen by the practitioner, and the appropriate dosages are given. The manifestation of certain bacteria occur in varying geographic areas and the practitioner should be aware of microbial threats in their region, as well as the spectrum of activity of the treatments provided, to ensure that an appropriate treatment selection had been made. Corley and Hollis (2009), like current practicing practitioners, were primarily

concerned with septicemia, often caused by *Escherichia coli* (*E. coli*), *Actinobacillus, Streptococcus,* and *Enterococcus.*

Neonatal foals are not treated in the same manner as adult horses. Because foals are not yet hindgut fermenters, antibiotics that cause colitis in mature horses do not have the same effect on foals. Due to size, medications that are cost-prohibitive in mature horses can be used in foals. Also related to size, toxicity levels differ in foals (Corley and Hollis, 2009).

Cephalosporins- Ceftiofur

Cephalosporins inhibit synthesis of the bacteria cell wall, which leads to cell death. Cephalosporins are divided into first, second, third, and fourth-generation medications, depending on the spectrum of activity. Ceftiofur sodium, a third-generation cephalosporin, is a gram-negative antibiotic, particularly effective against *Enterobacteriacaea*. Ceftiofur has some activity against gram-positive cocci. In horses, ceftiofur is extra-labeled for infections caused by gram-negative bacteria, like *E. coli, Klebsiella pneumoniae,* and *Salmonella* (Papich, 2016). Ceftiofur is rapidly metabolized to desfuroylceftiofur, making ceftiofur less active against *Staphylococci* and *Streptococci, in vitro.* Ceftiofur is recommended to be administered at 4.4 mg/kg intramuscularly (IM) every 12 h, 4.4 mg/kg intravenously (IV) every 6 to 12 h, 5 mg/kg IV every 6 h, and 10 mg/kg IV every 6 h (Corley and Hollis, 2009).

In a study of 373 horses with naturally acquired pneumonia, the efficacy of ceftiofur was tested (McClure et al., 2011). Ceftiofur sodium was administered intravenously (IV) two times, 4 d apart at a dosage of 2.2 to 4.4 mg/kg. The authors discovered that ceftiofur was 66.9% effective in treating pneumonia (McClure et al., 2011).

Penicillin

Penicillin is a beta-lactam antibiotic. Penicillin binds penicillin-binding proteins to weaken or cause lysis of microbe cell walls. Penicillin has a time-dependent action and the bactericidal effect is observed when the drug concentrations are kept above minimum inhibitory concentration. Penicillin activity is limited to gram-positive bacteria, anaerobic bacteria, and some highly susceptible gram-negative bacteria, such as *Pasteurella* spp. *Enterobacteriaceae* and beta-lactamase-producing *Staphylococcus* spp. are penicillin resistant. Clinically, penicillin is indicated for treatment of gram-positive cocci that cause respiratory infections, urinary tract infections, and abscesses. Streptococci is usually susceptible to penicillin treatment, as are many gram-positive bacilli and anaerobic bacteria (Papich, 2016). Penicillin, when injected IV to horses, is given at 22,000 to 44,000 IU/kg every 6 to 8 h, administered slowly, as to decrease the occurrence of abdominal discomfort and upset (Corley and Hollis, 2009).

Penicillin G is one of the most commonly used penicillins in the veterinary field (Harvey and Hunter, 2002). A study was conducted on 7 healthy horses to evaluate plasma concentration of penicillin administration over a 24-h period (Younkin et al., 2018). The 7 horses were monitored throughout the 24-h period and were administered penicillin G IV at 22,000 IU/kg of body weight at time 0, 6, and 12 h of the trial. The authors retrieved venous blood samples at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 h. Younkin et al. (2018) determined that penicillin should be effective against many equine isolates of *Streptococcus equi* and *Streptococcus zooepidemicus* when given IV, resulting is optimal microbe death.

Aminoglycosides- Amikacin

Aminoglycosides are known for their activity against gram-negative and some gram-positive bacteria. They have synergistic action with penicillin against *streptococci* and

enterococci. The therapeutic dose in equine neonates is considerably higher than adult equines because of the higher volume of distribution (Corley and Hollis, 2009). Aminoglycosides inhibit bacteria protein synthesis by binding to the 30S ribosome. Aminoglycosides are broad-spectrum antibiotics, but are not effective against *streptococci*, unless used with penicillin, and is commonly used to treat serious or life-threatening infections (Papich, 2016). Amikacin is the aminoglycoside of choice for neonatal foals, mainly due to reduced nephrotoxicity and low frequency of resistance to organisms that commonly plague foals. Amikacin is recommended to be dosed at 20 to 25 mg/kg every 24 h. Amikacin is particularly effective as a local therapy for septic joints (Corley and Hollis, 2009).

A trial was conducted on 6 mature, sound horses to compare concentrations of amikacin in normal and inflamed joints (Taintor, Schumacher, and DeGraves, 2006). Five hundred mg amikacin was injected into the horses' contralateral antebrachiocarpal joint after draining of approximately 1.5 mL synovial fluid. Synovial fluid was subsequently collected from each of the previously injected joints at 24, 48, 72, and 96 h. The same procedure was followed for the same 6 horses with inflamed joints, which was achieved by injecting the contralateral antibrachio-carpal joint with 50 mg endotoxin after a 4 wk clean-out period (Taintor, Schumacher, and De Graves, 2006). The authors determined that 500 mg amikacin was appropriate for treating inflamed joints.

Trimethoprim-sulfadiazine

Trimethoprim and sulfonamides are often combined to increase the action of the 2 drugs, and the combination was first commercially sold in 1974 (Torok, 2006). Trimethoprim/ sulfonamide is a successful antibiotic due to a synergistic effect in inhibiting folic acid metabolism in bacteria. Trimethoprim/sulfonamides are obtained through a licensed veterinarian.

Sulfonamides competitively inhibit dihydrofolate synthesis, while trimethoprim inhibits dihydrofolate reductase. Clinically, the combination of trimethoprim and sulfonamides is efficient for treating bacterial infections associated with respiratory disease, skin infection, wounds, and joint infections. Trimethoprim/sulfonamide is commonly administered orally to neonatal foals at a dose of 24 mg/kg twice a day for 10 d (Papich, 2016). According to Corley and Hollis (2009) trimethoprim/sulfonamide is an effective and reasonable choice to treat neonatal foals, due to the impact on blood culture isolates. In addition, the authors recommend trimethoprim/sulfonamide for the treatment of foals due to affordability.

In one study, 6 healthy horses were given repeated treatments of trimethoprim/ sulfadiazine IV (2.5 mg/kg trimethoprim and 12.5 mg/kg sulfadiazine) and oral paste (5 mg/kg trimethoprim and 25 mg/kg sulfadiazine; Gustafsson et al., 2002). The authors tested the antibiotic's effect on the microbiome and pharmacokinetic parameters. Gustafsson et al. (2012) determined that trimethoprim/sulfadiazine did not have any significant changes on the microbiome and there was no clinical evidence of GIT upset.

Combinations and Interactions

In the clinical setting, medications, including antibiotics, are commonly used with other medications to treat difficult cases or to ensure a specific result. Amikacin, an aminoglycoside, is often administered alongside beta-lactam antibiotics, such as penicillin, or cephalosporins, like ceftiofur. In a Japanese study by Takigami in 1982, 20 human patients with severe bacterial infections (infections that had not responded to previous treatments) were treated with a combination therapy of amikacin (200mg intramuscularly, IM, once a day) and penicillin (twice a day drip infusions). Takigami (1982) determined that the combination of amikacin and penicillin resulted in a 75% clinical efficacy rate of excellent or good results to treat severe

bacterial infections. In a study in 1985 by Bouvet et al., 71 rabbits were used to test the efficacy of 7 treatments. One of the treatment groups was a combination of penicillin (75,000 IU) and amikacin (7.5 mg/kg). According to Bouvet et al. (1985), treatment with penicillin plus amikacin was more effective than when using penicillin alone. According to Furr and Mogg (2003), the combination of a penicillin and an aminoglycoside provides broad spectrum coverage and bactericidal action. Furr and Mogg (2003) explain that numerous drug combinations are clinically acceptable, including penicillin or cephalosporin with an aminoglycoside. Due to penicillin-mediated damage to the cell walls of bacteria, there is a synergistic relationship between the penicillin class and aminoglycosides, causing an increased uptake of the aminoglycoside by the bacteria (Moellering, 1983).

Statement of the Problem and Justification

Given the current concern for antibiotic stewardship, any antibiotic use in animals might face scrutiny in both the veterinary community and in the eyes of the general public. Veterinarians hold a unique position ensuring responsible antibiotic usage while also meeting the needs of their animal patients, particularly neonates. Neonatal foals present a unique challenge to the veterinarian, as their health can change in an instant, and, at times, the foal holds significant value for the owner, and their survival is critical. Veterinarians are charged with treating valuable neonates at an early age, especially at birth to the first few days of life. With the growing understanding of the microbiome of many species, including the horse, veterinarians should have confidence to treat neonatal foals with appropriate treatments of antibiotics, without fear of devastating impacts to the animal's future. The objectives of the current study are to: 1) identify the impact of commonly used antibiotics on the neonatal foal microbiome, and, 2) identify if the microbiome begins to resemble a healthy foal after antibiotics are completed.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Animals

In January to September 2018, 11 stock-type foals were used in a clinical study to evaluate the effect of commonly used antibiotics on the neonatal equine microbiome, red blood cell (RBC) concentration, white blood cell (WBC) concentration, neutrophils (NEU), glucose (GLU), and fibrinogen (FIB). Dams that were the mothers of the foals, located at a veterinary clinic near Canyon, TX (n = 11), received a diet of alfalfa hay twice daily and water ad libitum. Dams were housed individually in 7.5 × 11 m foaling stalls at least 2 wk prior to parturition and until 28-d post-partum.

Foals were enrolled into the trial within 24 h of birth and assigned a treatment based on clinical indicators, such as ability to stand and nurse, indication of "dummy foal" syndrome, and the foal's clone status, in addition to results of the foal's first complete blood count (CBC), bloody chemistry, and FIB test, and ultimately, veterinary decision. The foals were administered no treatment (n = 4), or amikacin and ceftiofur, amikacin and penicillin, or trimethoprim/ sulfadiazine (treated group; n = 7), based on previously stated clinical indicators. Treated foals that did not respond to the initial treatment based on CBC, FIB, and clinical observation were administered a subsequent treatment (n = 3). Only foals that were born by natural delivery and considered clinically normal by the consulting veterinarian were enrolled. Post-partum

management included administration of a multivalent vaccine (Encephalomyelitis-Rhinopneumonitis-Influenza-West Nile Virus Vaccine Eastern and Western, Killed Virus, Tetanus Toxoid, Vetera Gold^{XP}, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and a naval dip (Chlorhexidine, VetOne, Boise, ID).

Foals enrolled in the trial received standard care in case of illness (failure of passive transfer, contracted tendons, or diarrhea) according to the consulting veterinarian. Foals with an immunoglobulin G (IgG) blood concentration less than 400 mg/dL (according to the IgG rapid test, IDEXX SNAP* Foal IgG Test, IDEXX Laboratories, Westbrook, ME) received 400 mL of donor-colostrum. Foals exhibiting contracted tendons were administered a single dose of oxytetracycline (Bio-Mycin 200, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) at 200 mg/mL, and legs were wrapped with cotton bandages until the tendons relaxed, prior to d 2. The consulting veterinarian monitored health of foals with diarrhea via CBC, serum chemistry, and fibrinogen analyses and treated with daily intestinal absorbent product (Bio-Sponge, Platinum Performance Inc., Buellton, CA) until bowel movements became firm, as indicated by the consulting veterinarian. Mineral oil was applied to the foals' tail and perianal area as needed to prevent scalding.

Any dams with retained placentas were treated with standard procedure of oxytocin (Oxytocin, VetOne, Boise, ID) injections at 20 IU/dose, and a weighted palpation glove tied to the placenta. Dams were also administered flunixin meglumine (Prevail, VetOne, Boise, ID) as an anti-inflammatory while the placenta was retained. All animals enrolled in the trial were under observation by consulting veterinarian and hospital personnel.

Treatments

Foals were assigned a treatment according to clinical diagnosis and need. The foals were enrolled into one of two groups, a treatment group or a non-treatment group. Foals were initially treated with one of the following treatments: 10 mg/kg amikacin intravenously (IV) and 20,000 units/kg IV penicillin once a day (SID), 10 mg/kg amikacin IV and 5 mg/kg ceftiofur IV SID, or 11 mL of trimethoprim/sulfadiazine 333 mg/67 mg/mL orally SID. All medications were prescribed and administered by a licensed veterinarian. The duration of each treatment was in 7-d increments and was dictated by bloodwork performed on d 0, 7, 14, 21, 28, and 56. The day the foal is born is classified as d 0. All foals began treatment during the following 24 h. Foals that were not treated with antibiotics were subjected to the same physical handling by hospital personnel at the same rate and frequency as foals assigned to any of the treatments. Foaling dates and times differed for each dam.

Sample Collection and Laboratory Analysis

Blood

Blood was collected by the attending veterinarian or assistants from foals via jugular venipuncture on d 0, 7, 14, 21, 28, and 56 using a 3 mL syringe (Covidien, Mansfield, MA), and dispensed into a 1 mL EDTA tube (IDEXX VetCollect Tubes, Westbrook, ME). Upon collection tubes were inverted 4 to 6 times to thoroughly mix EDTA to prevent unwanted blood clotting. Blood samples were then transported immediately at ambient temperature to the on-site veterinary laboratory for d 0 IgG. Blood was obtained on d 0, 7, 14, 21, 28 and 56 and analyzed using an automated hemocytometer (IDEXX ProCyte Dx Hematology Analyzer, IDEXX Laboratories) to determine CBC variables. Blood serum was analyzed using an automated

analyzer (IDEXX Catalyst Dx Chemistry Analyzer with an Equine 15 CLIP, IDEXX Laboratories), and FIB was determined via IDEXX VetAutoread (IDEXX Laboratories).

Feces

Fecal samples were collected from the rectum via grab sample using a sterile glove at d 0, 7, 14, 21, 28, and 56 and placed in a 50-mL conical tube. A dam fecal sample was also collected on d 56 via grab-sample from fresh feces on the ground or by palpation and placed into a 50-mL conical tube (UltraCruz Centrifuge tube, Santa Cruz Biotechnology, Santa Cruz, CA). All samples were immediately frozen at -20 C° at the veterinary clinic until submitted for analysis.

Fecal samples were submitted to a commercial laboratory (Molecular Research LP, Shallowater, TX) for DNA sequencing and microbiome analysis. The commercial laboratory isolated genomic DNA from fecal samples using industry standard Inhibitor DNA Removal Technology to isolate microbial genomic DNA (PowerSoil DNA Isolation Kit, MO BIO Laboratories, Quiagen, Hilden, Germany) and followed the manufacturer's instructions. Approximately 200 mg of fecal sample was added to a beads beating tube (PowerBead, MO BIO Laboratories, Quiagen, Hilden, Germany) for cell lysis. Amplicon sequencing using next generation technology (bTEFAP) was originally described by Dowd et al. (2008) and has been utilized in describing a wide range of microbiome populations. The 16S universal Eubacterial primers 515F GTGYCAGCMGCCGCGGTAA and 806R GGACTACNVGGGTWTCTAAT were used to evaluate the microbial ecology of each sample on the MiSeq with methods using bTEFAP DNA analysis. The samples underwent a single-step 30 cycle PCR using HotStarTaq Pls Master Mix Kit (Qiagen, Valencia, CA). After PCR, amplicon products from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads and sequenced using the Illumina MiSeq chemistry.

Statistical Analysis

Due to small sample size, comparisons were not made between antibiotic groups. Analysis was conducted between foals treated with any antibiotic (n = 7) and untreated foals (n = 4). Statistical comparisons were conducted using ANOVA. The Mixed Procedure in SAS was used with a repeated effect of day to analyze blood parameters and bacterial classes. Kenward-Rogers degree of freedom approximation was used within the repeated measure. The covariance structure used, Autoregressive (1), was determined by comparing the Akaike's information criterion for each type of covariance structure and selecting the lowest Akaike's information criterion, which was true for the blood parameters and prominent bacteria. Statistical significant was established when $P < 0.05$.

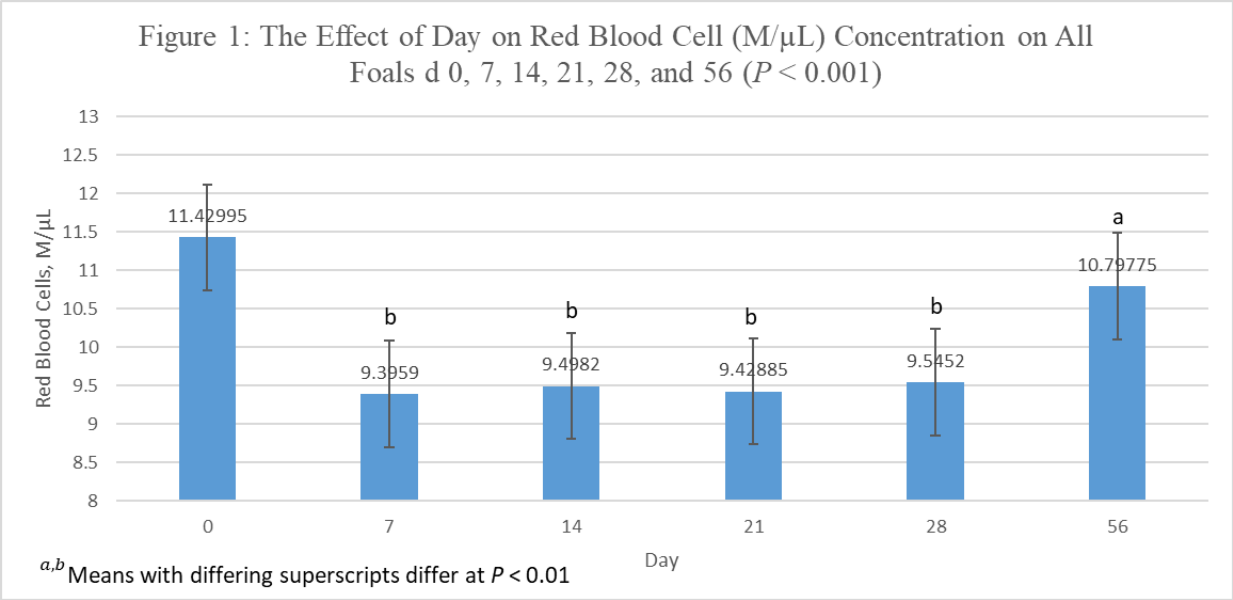
CHAPTER IV

RESULTS AND DISCUSSION

There were 11 foals located at a veterinary hospital near Canyon, TX that were treated with the previously outlined antibiotic protocols. Foals with contracted tendons (n = 3; all were treated with one of the three antibiotic treatments) were treated with previously mentioned regimes. One foal's dam did not produce adequate milk and the foal was sustained on milk replacer (Foal First Milk Replacer Powder, Progressive Nutrition, Hopkins, MN), until transferred onto a milk dam. Two foals did not have submission of the dam's fecal sample. One foal passed away between d 28 and 56.

Complete Blood Count

Individual hematology values are shown in Appendix Tables A-1, A-2, A-3, A-4, A-5, A-6, A-7, and A-8. All blood parameters for all foals were within normal range according to Brown (1989). There was no effect of the interaction of treatment × day ($P = 0.86$) on foal red blood cell (RBC) concentration. There was a main effect of day ($P < 0.001$), as depicted in Figure 1, but no effect of treatment ($P = 0.51$) on foal RBC concentration. Foals had greater ($P < 0.01$) mean RBC concentration at d 0 and 56 as compared to d 7, 14, 21, and 28 (Figure 1). In a study of 23 draft foals, RBC was shown to decrease gradually from birth to approximately 4 wk (Aoki and Ishii, 2011). Red blood cells can be decreased after birth during the absorption of colostrum proteins on the first day of life, because of decreased RBC production, and the short lifespan of RBC in the uterus (Aoki and Ishii, 2011).

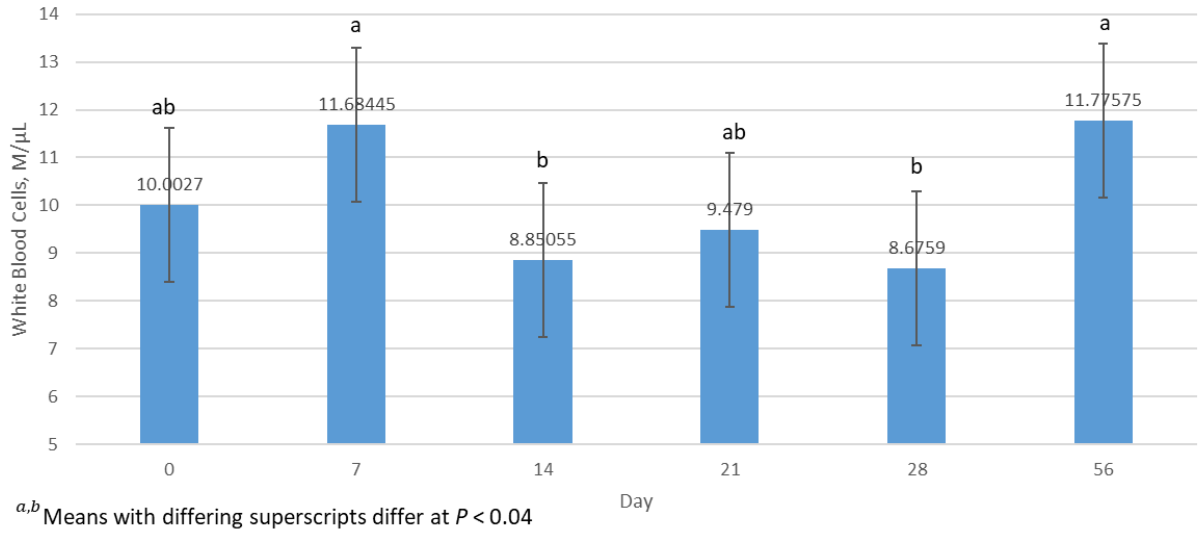


There was no effect of the interaction of treatment × day (P = 0.69) on foal white blood cell (WBC) concentration. There was a main effect of day (P = 0.04), as depicted in Figure 2, but no effect of treatment (P = 0.81) on foal WBC concentration. Foals had greater (P < 0.04) WBC concentration on d 7 and 56 as compared to d 14 and 28. Days 0 and 21 had statistically similar means to all other days (Figure 2). According to Aoki and Ishii (2011), WBC increase after parturition, supporting the results found in this study.

There was no effect of the interaction of treatment × day (P = 0.66) on foal neutrophil (NEU) concentration. There was a main effect of day (P = 0.03), as depicted in Figure 3, but no effect of treatment (P = 0.66) on foal NEU concentration. Mean NEU was similar at d 0 and 7 and decreased (P = 0.03) at d 14, 21, and 28, before increasing to a statistically similar value at d 56 (Figure 3). This observation is supported by Aoki and Ishii (2011), where NEU were found to be increased at 1 wk and decreased thereafter.

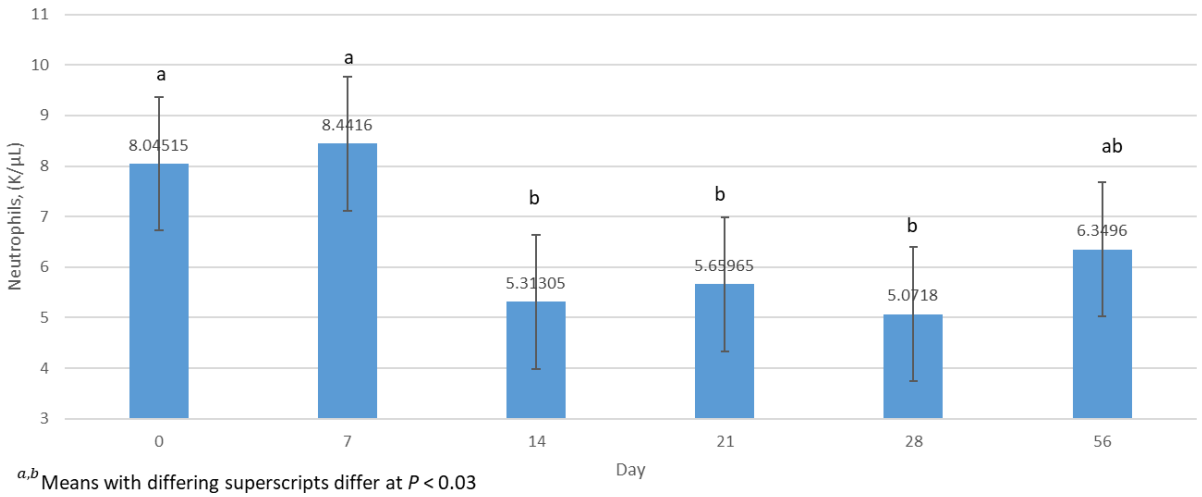
Glucose (GLU) concentration was not affected by treatment (P = 0.55), day (P = 0.58), or treatment × day (P = 0.43). Glucose was measured along with CBC to ensure proper nursing of the foal occurred and to monitor for hypoglycemia. Abnormal neonatal foals are at a greater risk

Figure 2: The Effect of Day on White Blood Cell (M/µL) Concentration on All Foals d 0, 7, 14, 21, 28, and 56 ($P = 0.04$)



^{a,b}Means with differing superscripts differ at $P < 0.04$

Figure 3: The Effect of Day on Neutrophils (K/µL) on All Foals d 0, 7, 14, 21, 28, and 56 ($P = 0.03$)

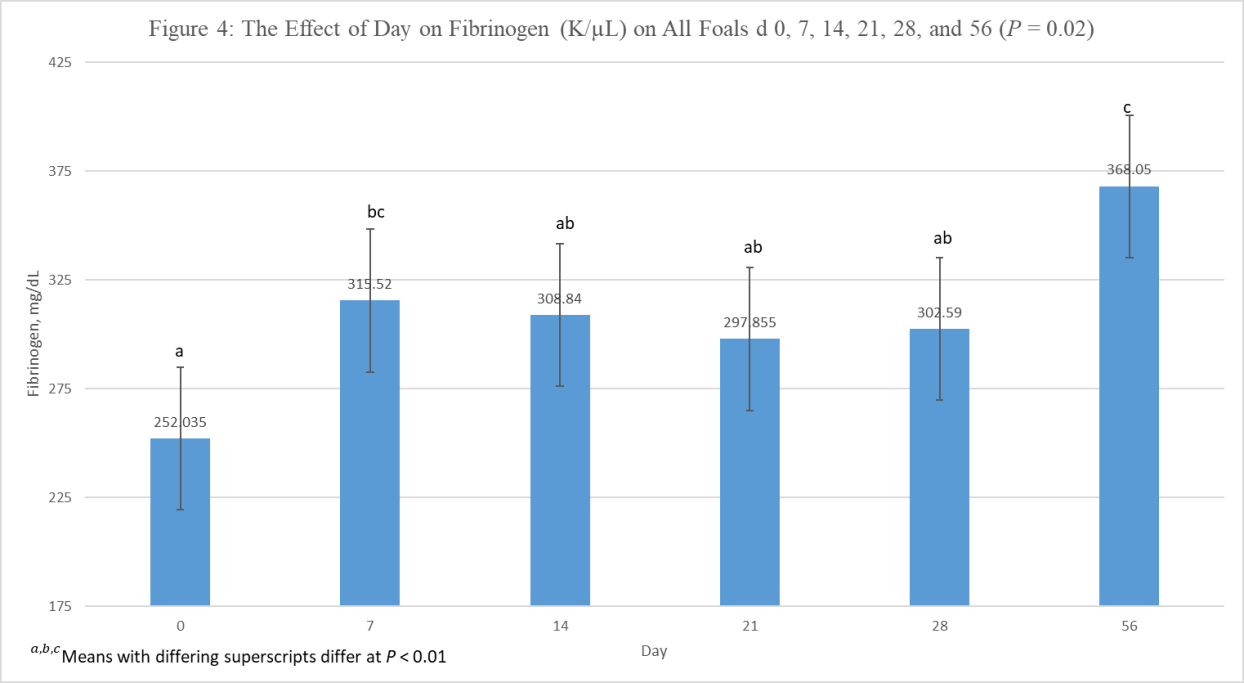


^{a,b}Means with differing superscripts differ at $P < 0.03$

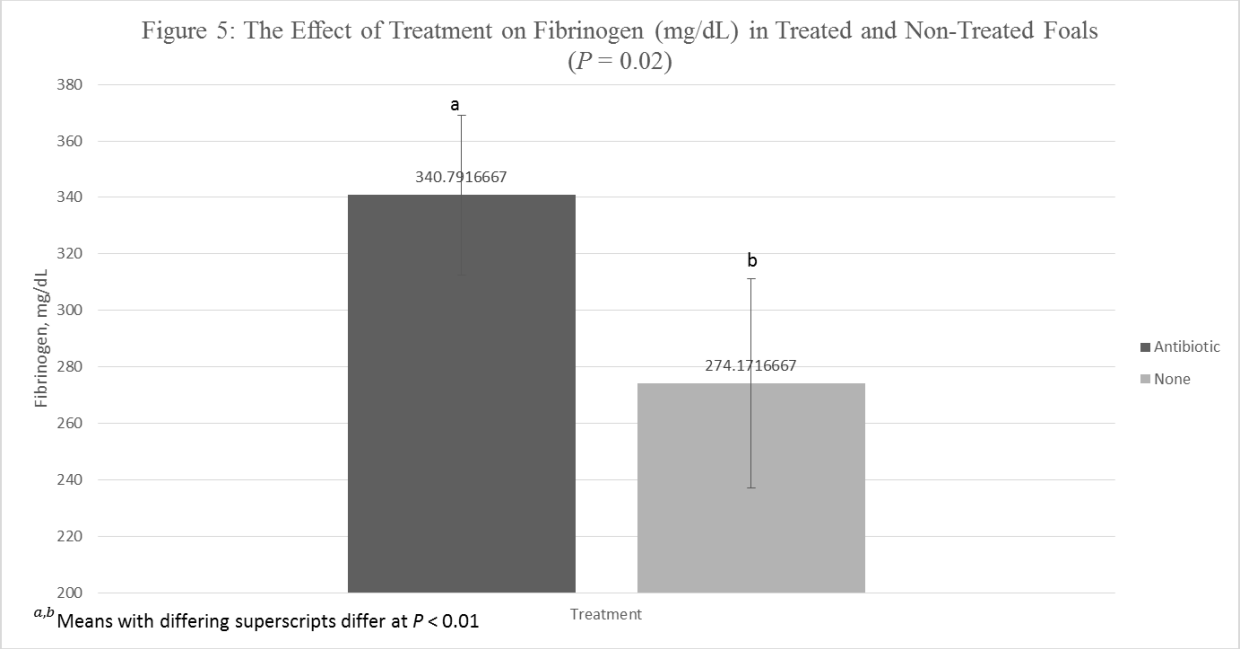
for hypoglycemia. Foals are born with low fat and glycogen stores; therefore, are prone to hypoglycemia (Hollis et al., 2008). In addition, foals are prone to septicemia, which might contribute to hypoglycemia and compound medical decline (Hollis et al., 2008). Glucose is part of the dam's milk and would not be impacted by treatment regimens unless treatment caused a decrease in eating due to lethargy, which led to secondary complications. In a study performed by Hollis et al. (2008), GLU was monitored in 896 foals at various times during their stay at a

veterinary hospital. In critically ill foals, hypoglycemia was associated with sepsis (Hollis et al., 2008).

There was no effect of the interaction of treatment × day (P = 0.90) on foal fibrinogen (FIB). There was a main effect of day (P = 0.02), as depicted in Figure 4, and a main effect of treatment (P = 0.02), as depicted in Figure 5, on FIB. Foal FIB at d 0 was lower (P < 0.01) as compared to d 7 and 56. Foal FIB was statistically similar at d 14, 21, and 28. Foal FIB values at d 56 were greater (P < 0.01) as compared to d 0, 14, 21, and 28 (Figure 4).



Antibiotic treated foals had greater (P < 0.01) mean FIB as compared to non-treated foals (Figure 5). Fibrinogen is a key biomarker in treatment diagnostics in horses, especially foals. Santos et al. (2014) explained that FIB concentration may increase and decrease due to inflammation and infection, and clinically, a decrease in FIB concentrations can be an indication of successful therapy, especially when assessed with WBC. Because FIB is a trauma indicator, as a foal becomes more active and therefore increases the possibility for minor injury or excitement, FIB might fluctuate. The FIB concentrations in this study differ slightly from studies performed

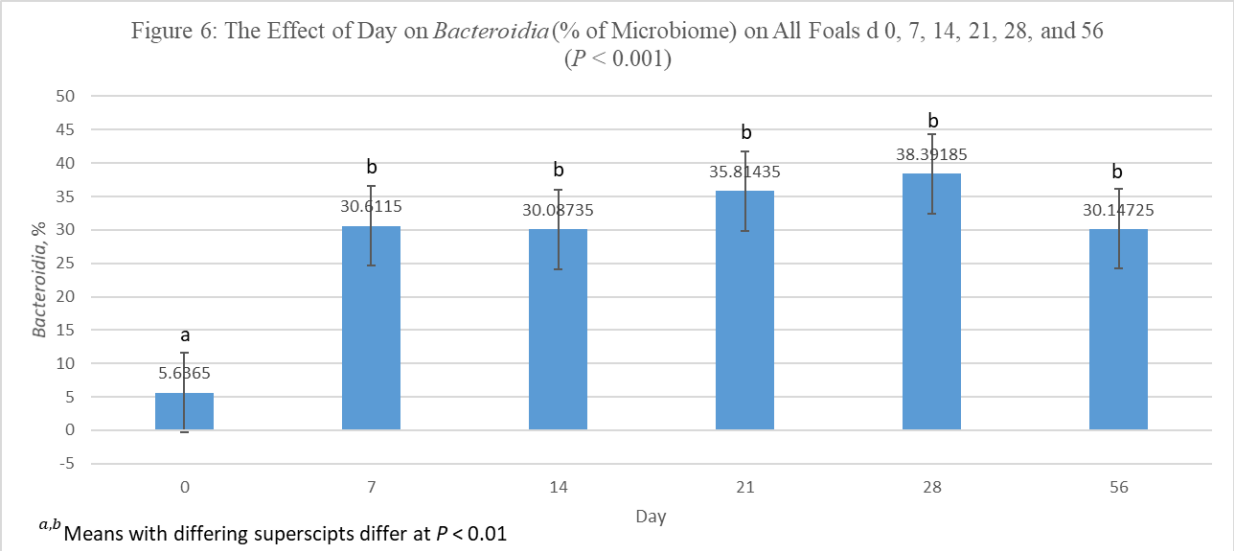


by Santos et al. (2014), Axon and Palmer (2008), and Harvey et al. (1984). These authors identified FIB to be low at birth with a gradual increase until 4 mo. The gradual increase is attributed to the normalization of the foal's immune system and increased contact with pathogens (Santos et al., 2014).

Fecal Bacteria Microbiome

Eighty-one fecal samples were analyzed for bacterial DNA. Seven samples were submitted from foal 7288, Cosmic, and 0506, 6 samples from foals 1261, 8327, 9345,1410, and 4115, 5 samples from foal 1263 and foal Dash, and 10 samples were collected from foal 1495. Fecal samples used for this study coordinated to blood samples obtained on d 0, 7, 14, 21, 28, and 56. A single fecal sample was obtained from each dam when the foal was 56 d of age.

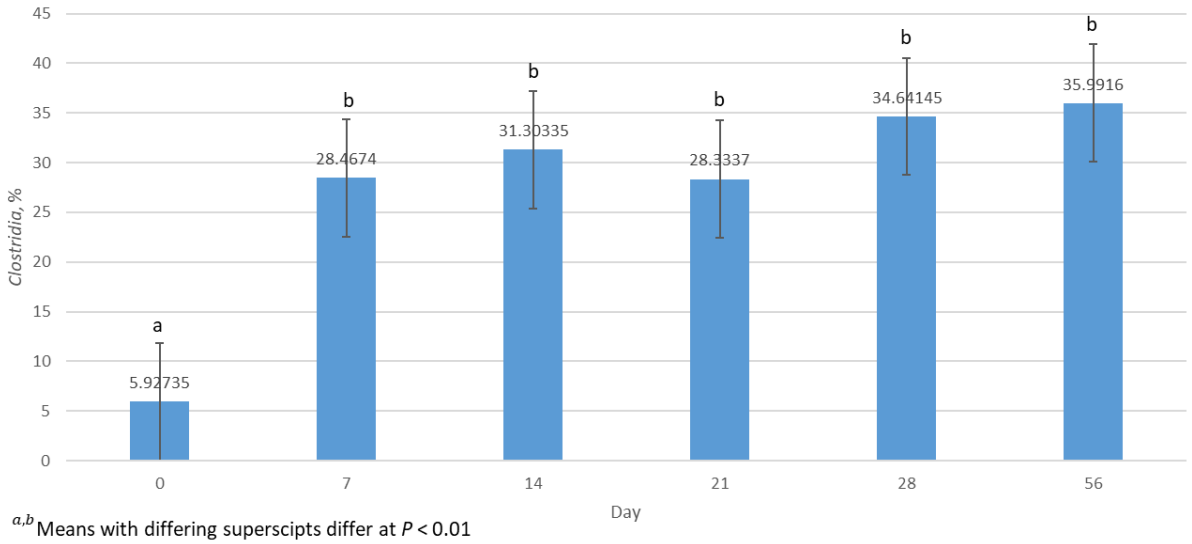
An effect of the treatment × day interaction was not observed for *bacteroidia* ($P = 0.35$) or *clostridia* ($P = 0.24$). However, there was an effect of day (*Bacteroidia*, $P < 0.001$; *Clostridia*, $P < 0.001$; Figures 6 and 7). Mean *bacteroidia* ($P < 0.01$) was lowest on d 0 as compared to d 7, 14, 21, 28, and 56 (Figure 6). The same mean pattern was observed for *clostridia* ($P < 0.01$), as



seen in Figure 7. The most abundant classes of bacteria were analyzed by a fecal microbiome laboratory (Molecular Research, LP, Shallowater, TX). Bacteria classes that comprised less than 0.2% of the microbiome were excluded. *Clostridia* and *bacteroidia* were most abundant amongst the foal and dam pairs.

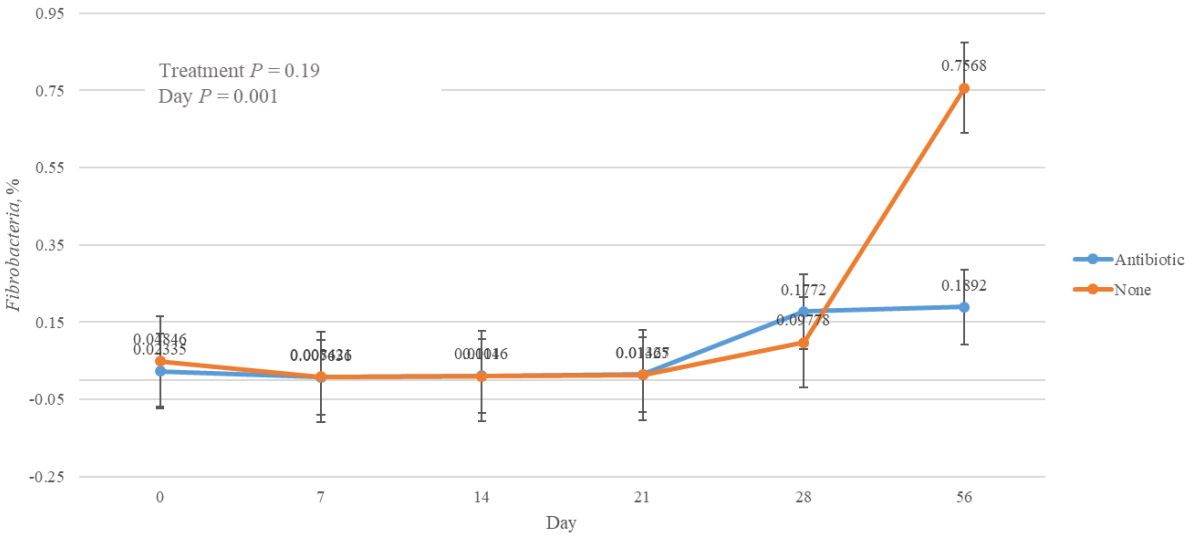
In a study by Costa and Weese (2012), 6 normal and 10 diarrheic horses' microbiomes were sequenced. The authors found that there was a greater abundance of *Clostridiales* in normal horses, which might be an indicator of the overlooked role of commensal *clostridia* in the GIT (Costa and Weese, 2012). In a study by Jevit (2016), *bacteroidetes* were identified as part of the core biome. *Bacteroidetes* are gram-negative and commonly found in the GIT of animals (Jevit, 2016). Although present in very small amounts (< 1.0%), *fibrobacteria* was the only class of bacteria to have an effect of treatment × day interaction ($P = 0.05$; Figure 8). Jevit (2016) also identified *fibrobacteria* as being a small portion of the core biome. *Fibrobacteraceae* take up one of the smallest groups of the equine microbiome (Dougal et al., 2013).

Figure 7: The Effect of Day on *Clostridia* (% of Microbiome) on All Foals on d 0, 7, 14, 21, 28, and 56 ($P < 0.001$)



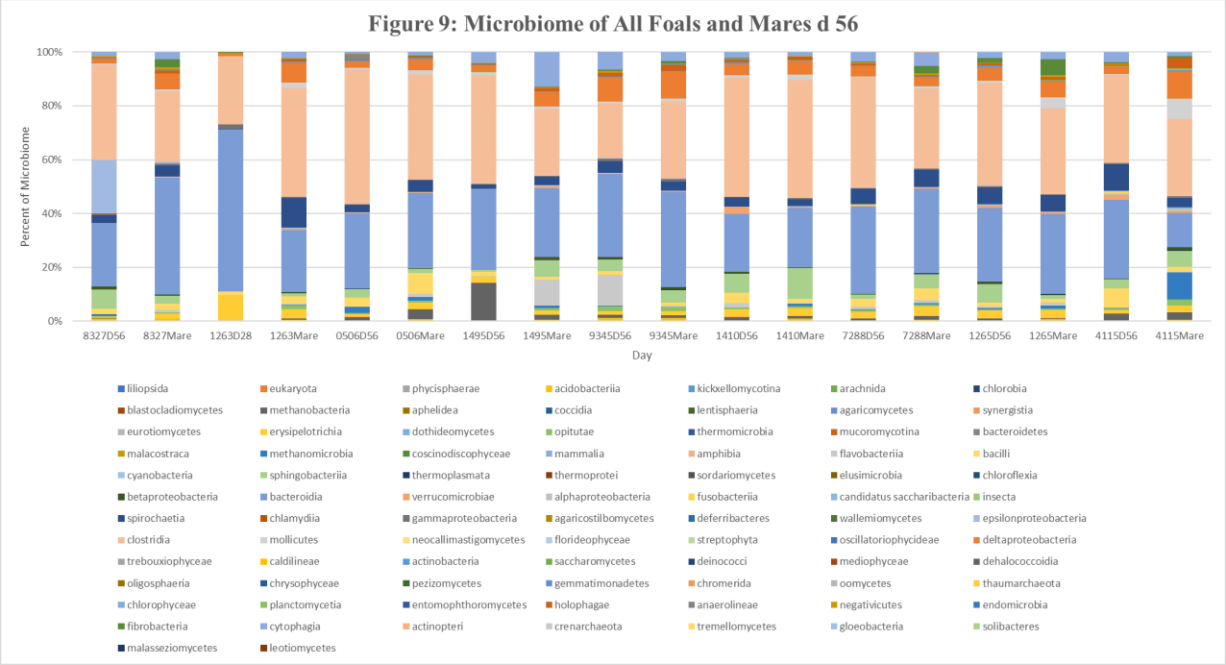
^{a,b}Means with differing superscipts differ at $P < 0.01$

Figure 8: The Effect of the Interaction of Treatment × Day on *Fibrobacteria* (% of Microbiome) on d 0, 7, 14, 21, 28, and 56 in Treated and Non-Treated Foals ($P = 0.05$)



Individual foal microbiome figures are shown in Appendix Figures A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14, A-15, and A-16. The microbiome of all foals and mares on d 56 can be seen in Figure 9. In this study, *clostridia* and *bacteroidia* were the primary classes of bacteria found amongst the foals and mares, regardless of treatment administered to the foals, on d 56. These findings are supported by Costa et al. (2012, 2015a),

who identified *Bacteroidetes (bacteroidia)* and *Firmicutes (clostridia)* as the primary phyla within the equine microbiome.



CHAPTER V

CONCLUSIONS AND IMPLICATIONS

Blood variables and microbiome analysis did not reveal effects of treatment or treatment × day interaction. However, despite the limited sample size, this study illustrates the changes that the neonatal foal microbiome experiences in the first 56 d of life. Foals experience many changes in their first wk of life. Understanding the enteric microbiome can help the practitioner treat foals more effectively by aiding in the selection of microbial treatment as technology and research continues, especially with societal concerns for resistance. As each foal aged, the microbiome became more like the dam. Further research is needed to identify more specific impacts of antibiotics on the microbiome of neonatal foals and if there are any long-term effects from antibiotic use early in life.

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APPENDIX TABLES

Table A-1. Individual red blood cells (M/µL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	10.92	7.81	8.42	8.84	8.47	8.90
1263	13.69	10.25	9.57	---	11.06	---
Dash	9.65	8.67	8.97	9.26	9.20	---
506	12.25	9.97	9.31	8.39	8.12	8.66
1495	9.03	8.10	8.10	7.61	6.66	7.98
Cosmic	8.95	9.17	10.07	10.77	11.35	10.69
9345	12.60	10.11	9.90	10.75	10.54	13.57
			Untreated			
7288	12.57	10.55	10.99	11.37	10.60	10.26
1261	11.26	8.95	8.42	7.44	7.86	9.35
4115	10.84	8.96	9.28	9.49	9.31	12.67
1410	12.25	10.09	10.53	9.37	11.22	12.70

Table A-2. Individual white blood cells (K/µL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	17.11	13.95	7.24	10.65	8.97	7.75
1263	2.13	12.62	9.80	---	6.82	---
Dash	5.76	8.07	4.00	6.09	4.52	---
506	14.39	14.71	5.57	6.79	8.04	10.93
1495	10.67	10.95	7.81	12.39	9.84	14.32
Cosmic	10.99	11.63	9.93	10.74	12.56	10.43
9345	16.94	14.46	11.36	11.09	7.38	22.45
			Untreated			
7288	10.25	13.05	12.04	11.21	11.43	10.92
1261	8.23	10.72	9.20	6.65	6.77	7.68
4115	10.67	10.63	10.37	10.48	9.14	15.77
1410	8.30	9.71	7.36	8.90	8.85	11.90

Table A-3. Individual neutrophils (K/µL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	14.82	10.82	4.10	7.00	5.83	4.27
1263	0.91	8.56	6.51	---	4.20	---
Dash	4.09	4.71	0.84	2.52	0.81	---
506	11.66	12.63	3.16	4.44	5.54	7.51
1495	9.11	9.26	5.47	8.91	6.48	9.16
Cosmic	9.13	8.42	6.45	6.80	9.15	5.91
9345	13.20	9.27	5.10	4.78	2.35	13.29
			Untreated			
7288	8.42	9.34	7.89	6.87	7.32	5.53
1261	6.29	6.62	5.12	3.29	3.17	3.66
4115	8.82	8.01	7.14	7.35	5.84	8.25
1410	6.49	7.18	4.28	4.47	4.61	5.40

Table A-4. Individual glucose (mg/dL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	173	138	136	158	159	136
1263	106	174	157	---	147	---
Dash	---	164	---	168	---	---
506	146	134	136	148	122	138
1495	123	102	112	163	155	142
Cosmic	211	---	191	---	---	---
9345	181	158	155	167	163	136
			Untreated			
7288	109	172	161	152	153	137
1261	110	161	150	178	148	161
4115	149	175	161	159	136	161
1410	181	147	181	159	177	153

Table A-5. Individual fibrinogen (mg/dL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	243	387	315	313	293	324
1263	312	290	232	253	276	---
Dash	335	518	440	306	383	---
506	183	355	396	281	268	395
1495	269	316	547	467	419	331
Cosmic	337	341	274	292	352	411
9345	171	252	305	445	343	529
			Untreated			
7288	254	241	275	293	300	319
1261	263	276	212	235	284	328
4115	195	265	249	249	235	310
1410	208	337	301	259	268	385

Table A-6. Individual hematocrit (%) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	47.70	31.50	31.80	30.80	38.40	29.80
1263	58.70	39.80	35.20	---	45.60	---
Dash	35.00	30.20	31.00	31.90	31.60	---
506	50.70	36.00	33.80	29.10	26.90	32.10
1495	34.00	29.30	29.30	27.80	23.30	26.40
Cosmic	32.70	32.80	34.30	36.00	36.90	41.40
9345	50.90	36.60	34.80	37.60	35.00	56.60
			Untreated			
7288	50.90	38.50	39.00	39.00	35.10	29.60
1261	45.20	33.10	29.80	24.30	25.30	36.80
4115	44.60	33.10	34.30	33.70	31.20	52.50
1410	45.60	34.20	37.00	30.40	36.40	47.50

Table A-7. Individual lymphocytes (K/µL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

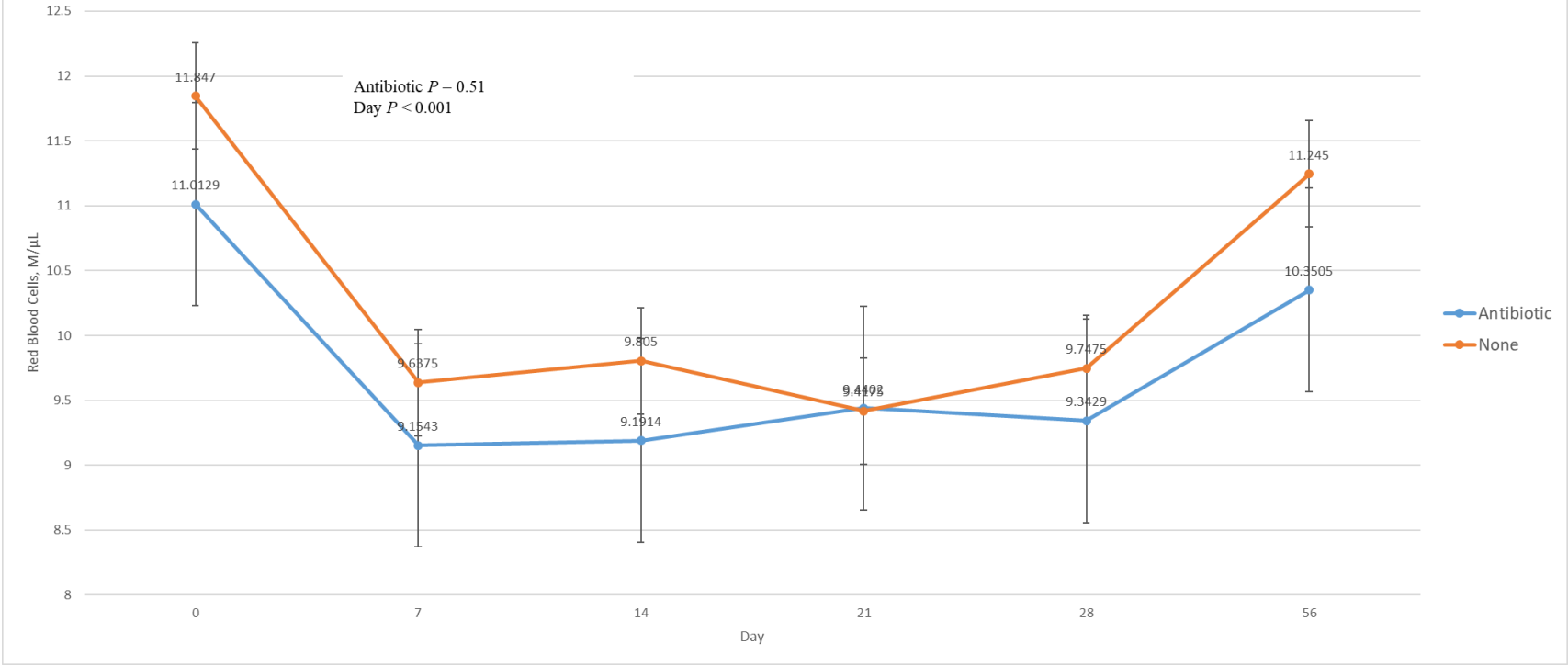
	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	1.93	2.35	2.60	2.93	2.46	2.62
1263	0.82	3.17	2.59	---	2.15	---
Dash	1.45	2.60	2.72	3.05	3.11	---
506	2.01	1.66	1.96	1.90	2.06	2.61
1495	1.25	1.18	1.81	2.69	2.76	4.42
Cosmic	1.56	2.65	2.95	3.34	2.91	3.89
9345	3.46	4.40	5.10	5.59	4.40	7.98
			Untreated			
7288	1.45	3.03	3.33	3.55	3.36	3.95
1261	1.80	3.51	3.46	2.86	2.82	3.42
4115	1.68	2.09	2.45	2.52	2.39	6.15
1410	1.60	2.08	2.61	3.29	3.65	5.82

Table A-8. Individual eosinophils (K/µL) in neonatal foals on d 0, 7, 14, 21, 28, and 56.

	Day					
Foal	0	7	14	21	28	56
			Treated			
8327	0.01	0.04	0.06	0.09	0.11	0.15
1263	0.00	0.03	0.02	---	0.01	---
Dash	0.00	0.02	0.03	0.04	0.02	---
506	0.01	0.04	0.02	0.04	0.05	0.02
1495	0.02	0.03	0.06	0.05	0.16	0.06
Cosmic	0.01	0.02	0.03	0.03	0.01	0.02
9345	0.00	0.06	0.05	0.05	0.03	0.09
			Untreated			
7288	0.01	0.05	0.05	0.07	0.09	0.10
1261	0.00	0.03	0.02	0.08	0.34	0.03
4115	0.01	0.02	0.06	0.12	0.45	0.16
1410	0.00	0.02	0.01	0.01	0.02	0.05

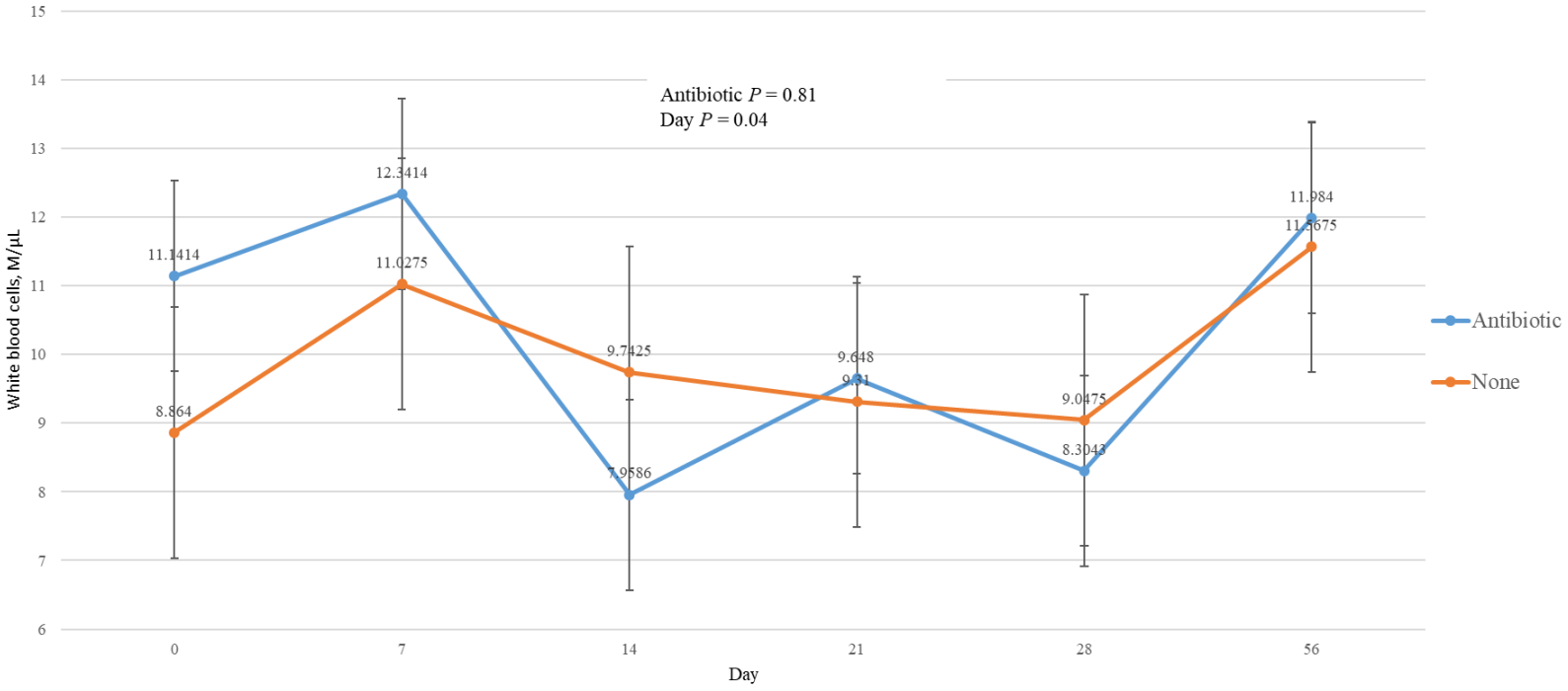
APPENDIX FIGURES

Figure A-1: The Effect of the Interaction of Treatment × Day on Red Blood Cells (M/µL) on d 0, 7, 14, 21, 28, and 56 in Treated and Non-Treated Foals ($P = 0.86$)



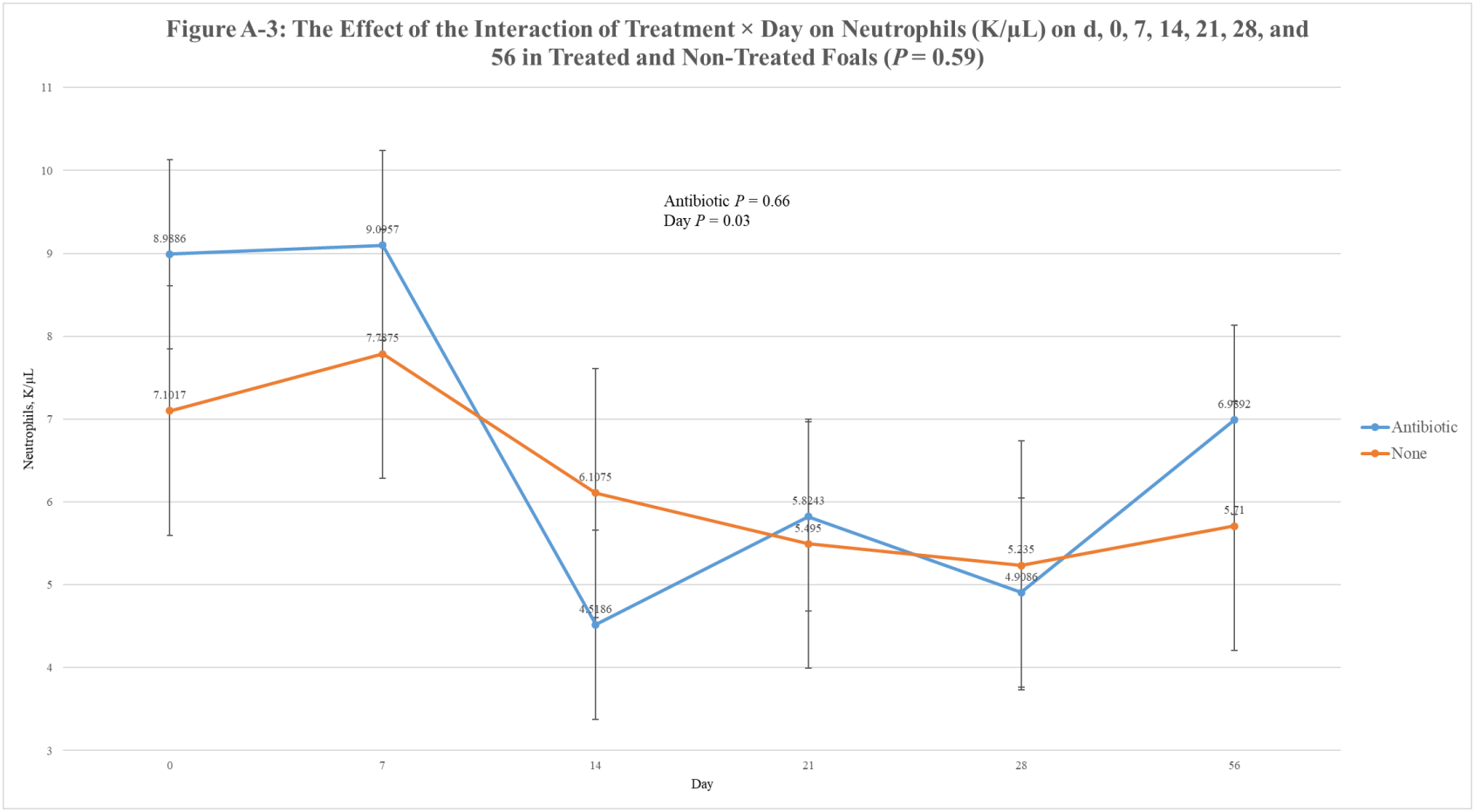
* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.

Figure A-2: The Effect of the Interaction of Treatment × Day on White Blood Cells (M/µL) on d 0, 7, 14, 21, 28, and 56 in Treated and Non-Treated Foals ($P = 0.69$)



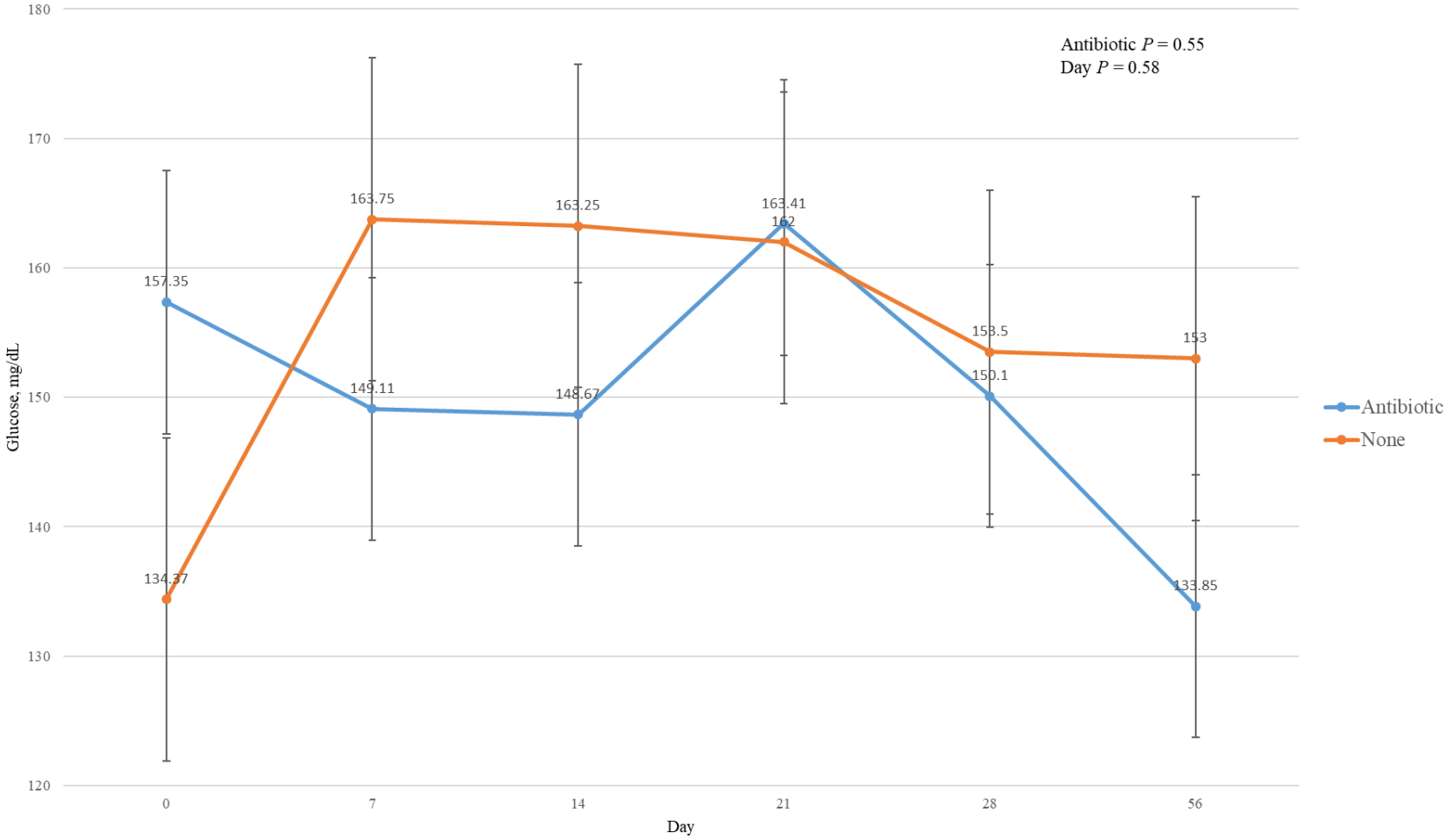
* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.

Figure A-3: The Effect of the Interaction of Treatment × Day on Neutrophils (K/µL) on d, 0, 7, 14, 21, 28, and 56 in Treated and Non-Treated Foals ($P = 0.59$)



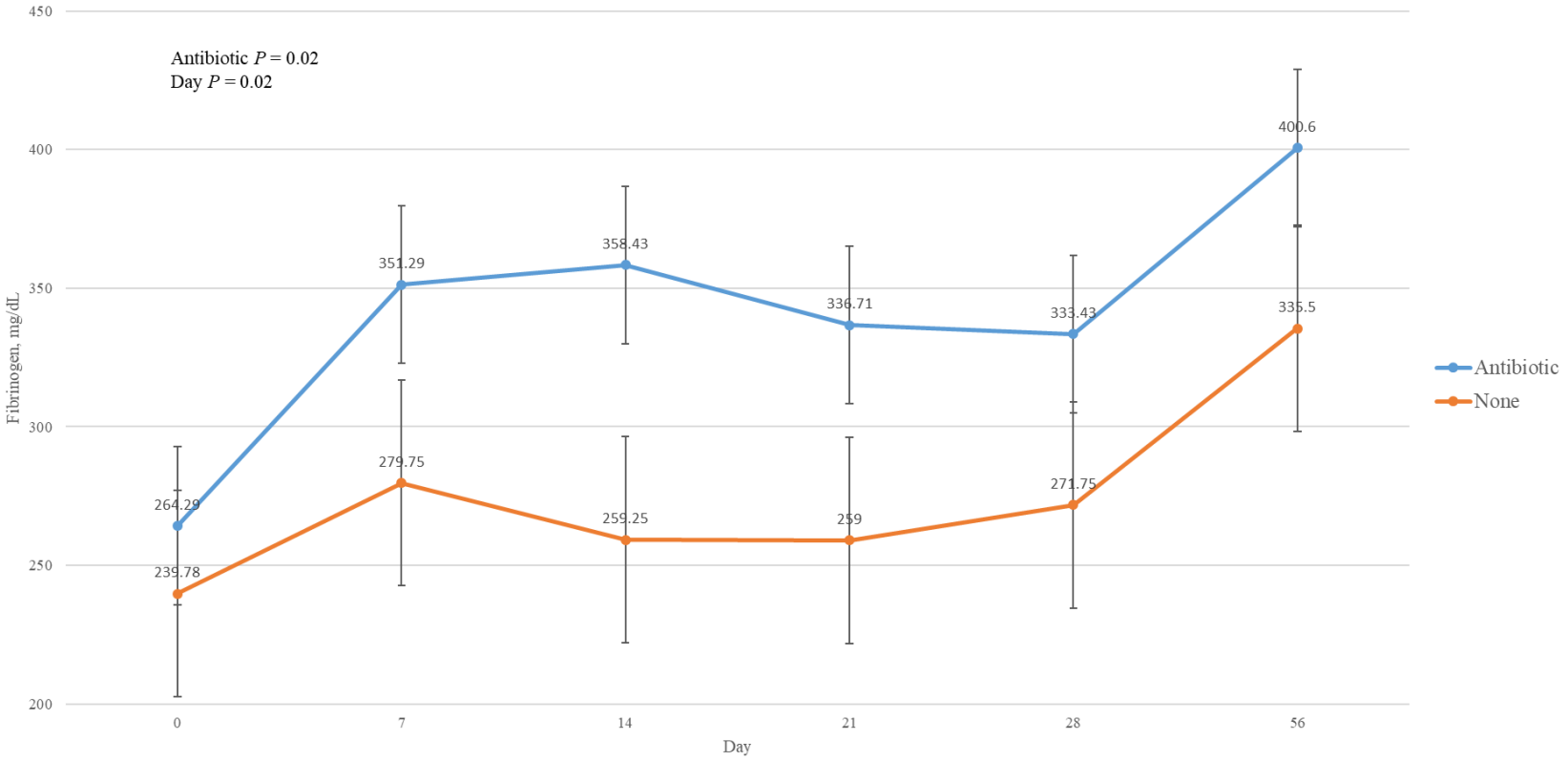
* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.

Figure A-4: The Effect of the Interaction of Treatment × Day on Glucose (mg/dL) on d 0, 7, 14, 21, 28, and 56 in Treated and Non-Treated Foals ($P = 0.43$)



* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.

Figure A-5: The Effect of the Interaction of Treatment × Day on Fibrinogen (mg/dL) d 0, 7, 14, 21, 28, and 56 in Treated and Non-Treated Foals ($P = 0.90$)



* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.

Figure A-6: Microbiome of Untreated Foal (1410) on d 0, 7, 14, 21, 28, and 56, and Mare Microbiome d 56

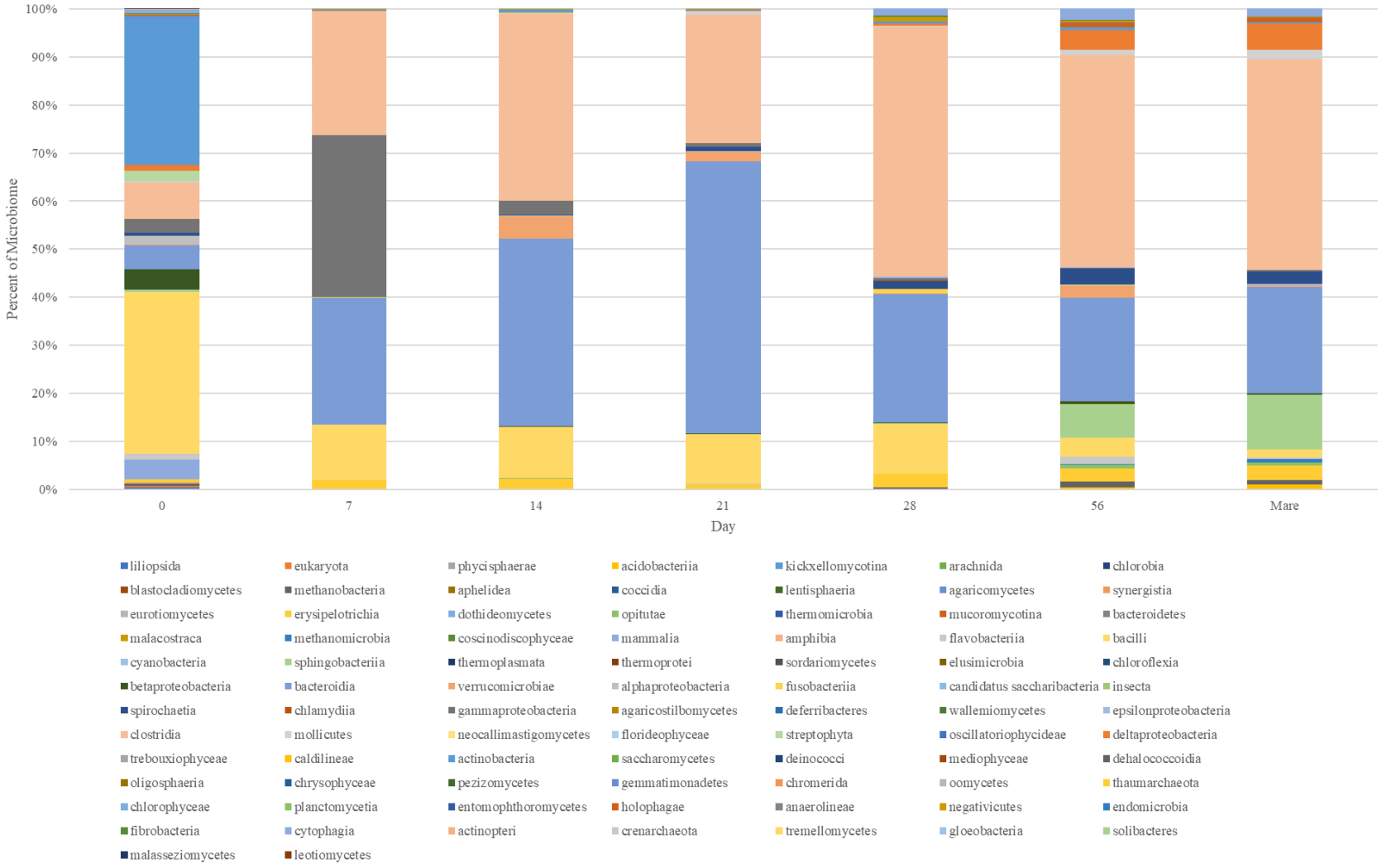


Figure A-7: Microbiome of Untreated Foal (7288) on d 0, 7, 14, 21, 28, and 56, and Mare Microbiome d 56

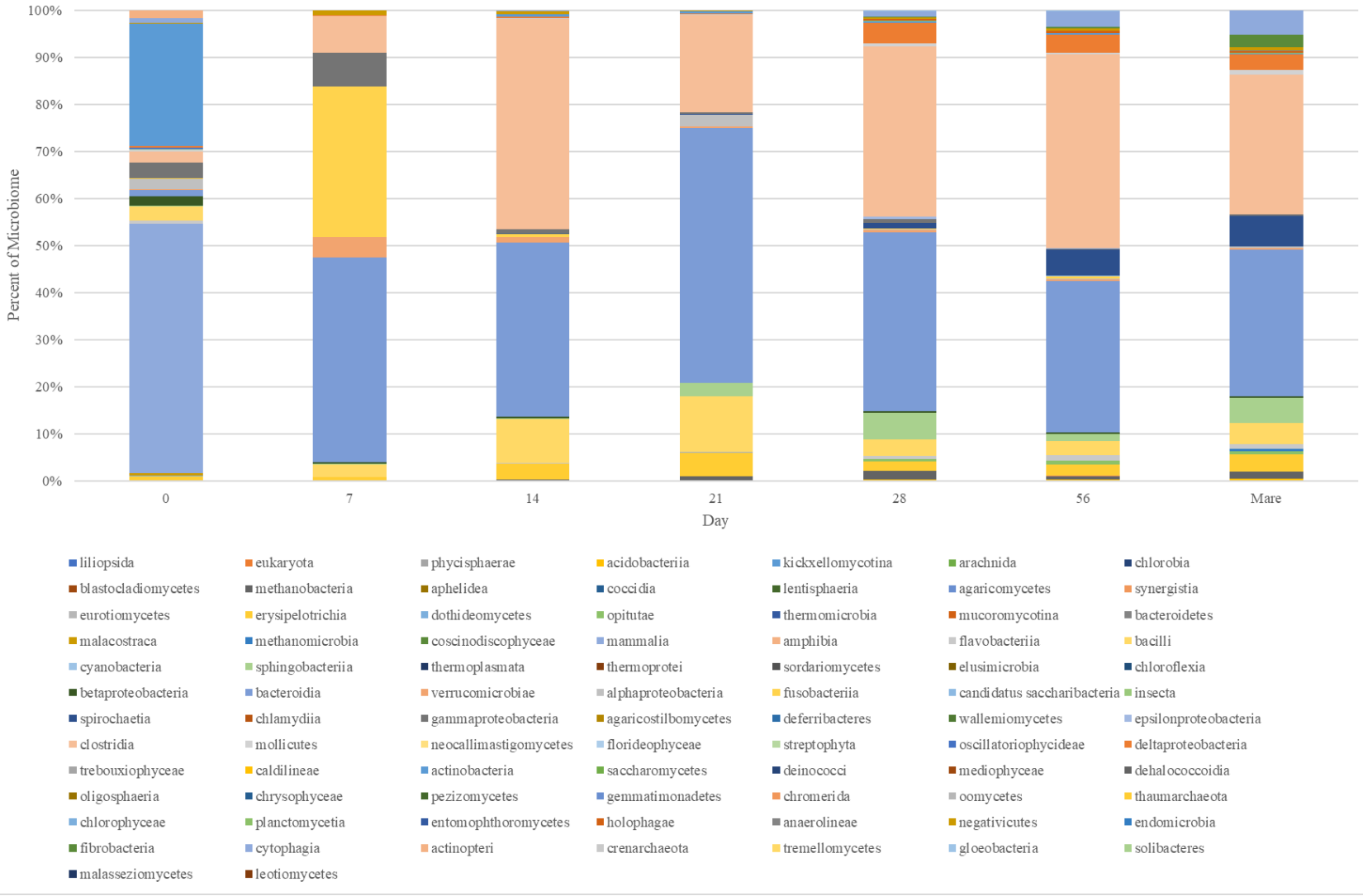
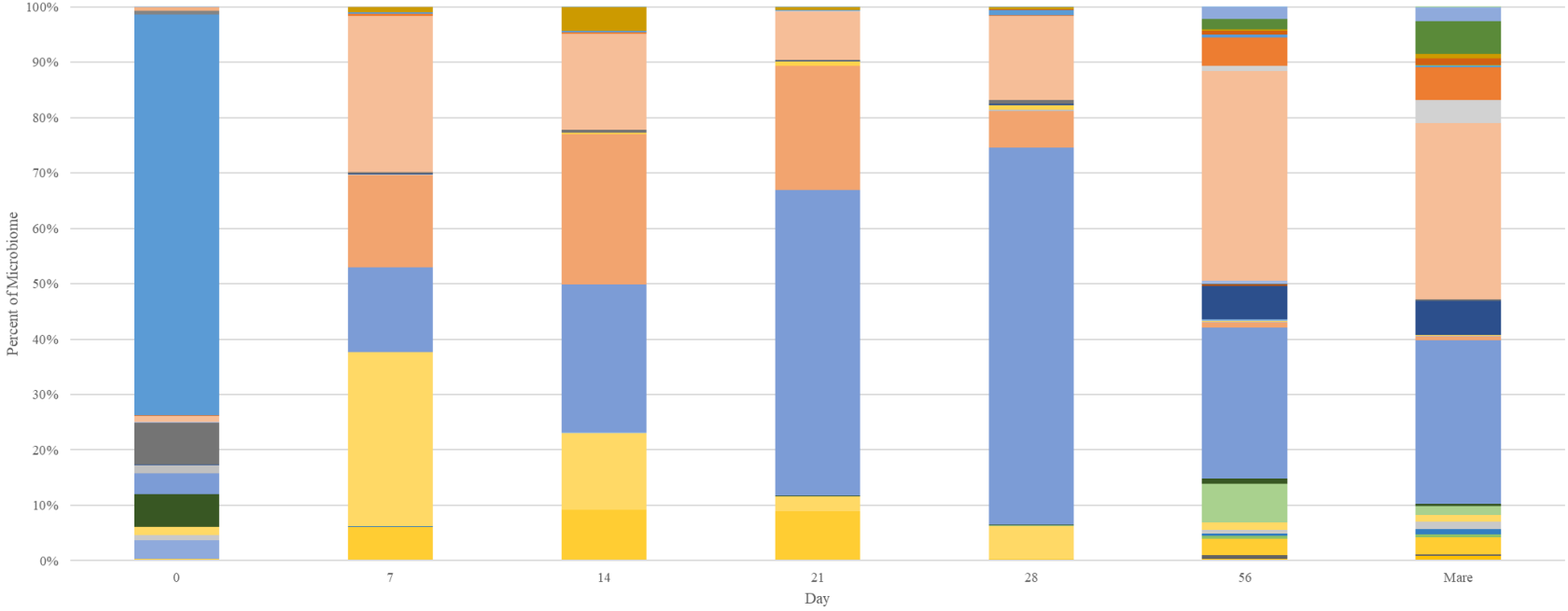


Figure A-8: Microbiome of Untreated Foal (1265) on d 0, 7, 14, 21, 28, and 56, and Mare Microbiome d 56



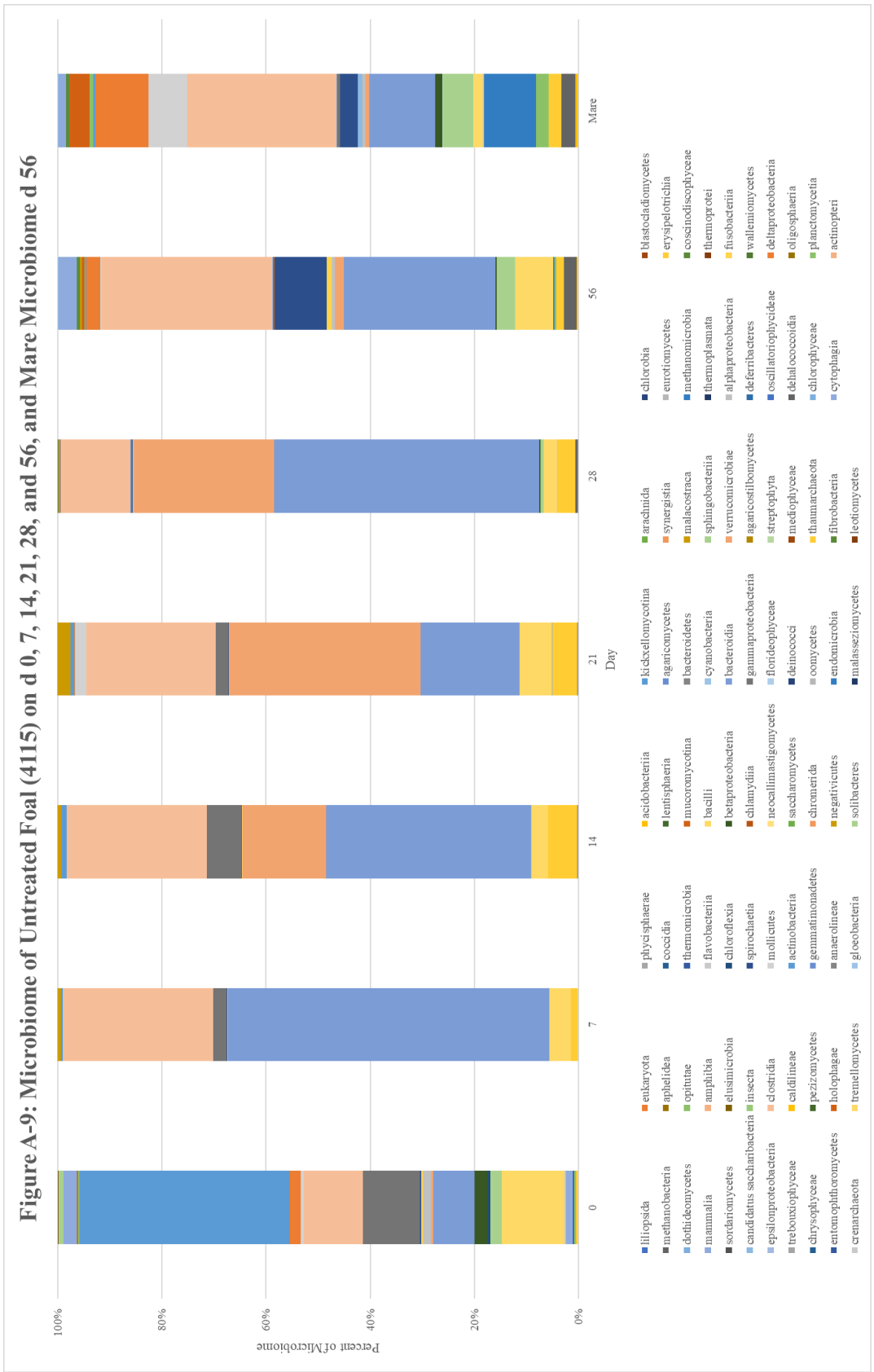


Figure A-9: Microbiome of Untreated Foal (4115) on d 0, 7, 14, 21, 28, and 56, and Mare Microbiome d 56
Percent of Microbiome
100%
80%
60%
40%
20%
0%
0
7
14
21
28
56
Mare
Day
liliopsida
eukaryota
phycisphaerae
acidobacteria
kickxellomycotina
arachnida
chlorobia
blastocladiomycetes
methanobacteria
aphelidea
coccidia
lentisphaeria
agaricomycetes
synergistia
eurotiomycetes
erysipelotrichia
dothideomycetes
opiitae
thermomicrobia
mucoromycotina
bacteroidetes
malacostraca
methanomicrobia
coscinodiscophyceae
mammalia
amphibia
flavobacteriia
bacilli
cyanobacteria
sphingobacteriia
thermoplasmata
thermoprotei
sordariomycetes
elusimicrobia
chloroflexia
betaproteobacteria
bacteroidia
verrucomicrobiae
alphaproteobacteria
fusobacteriia
candidatus saccharibacteria
insecta
spirochaetia
chlamydiia
gammaproteobacteria
agaricostilbomycetes
deferribacteres
wallemiomycetes
epsilonproteobacteria
clostridia
mollicutes
neocallimastigomycetes
florideophyceae
streptophyta
oscillatoriophycideae
deltaproteobacteria
trebouxiophyceae
caldilineae
actinobacteria
saccharomycetes
deinococci
mediophyceae
dehalococcoidia
oligosphaeria
chrysophyceae
pezizomycetes
gemmatimonadetes
chromerida
oomycetes
thaumarchaeota
chlorophyceae
planctomycetia
entomophthoromycetes
holophagae
anaerolineae
negativicutes
endomicrobia
fibrobacteria
cytophagia
actinopteri
crenarchaeota
tremellomycetes
gloeobacteria
solibacteres
malasseziomycetes
leotiomycetes

Figure A-10: Microbiome of Treated Foal (8327; Amikacin/Ceftiofur d 0 to 7) on d 0, 7, 14, 21, 28, and 56, and Mare Microbiome d 56

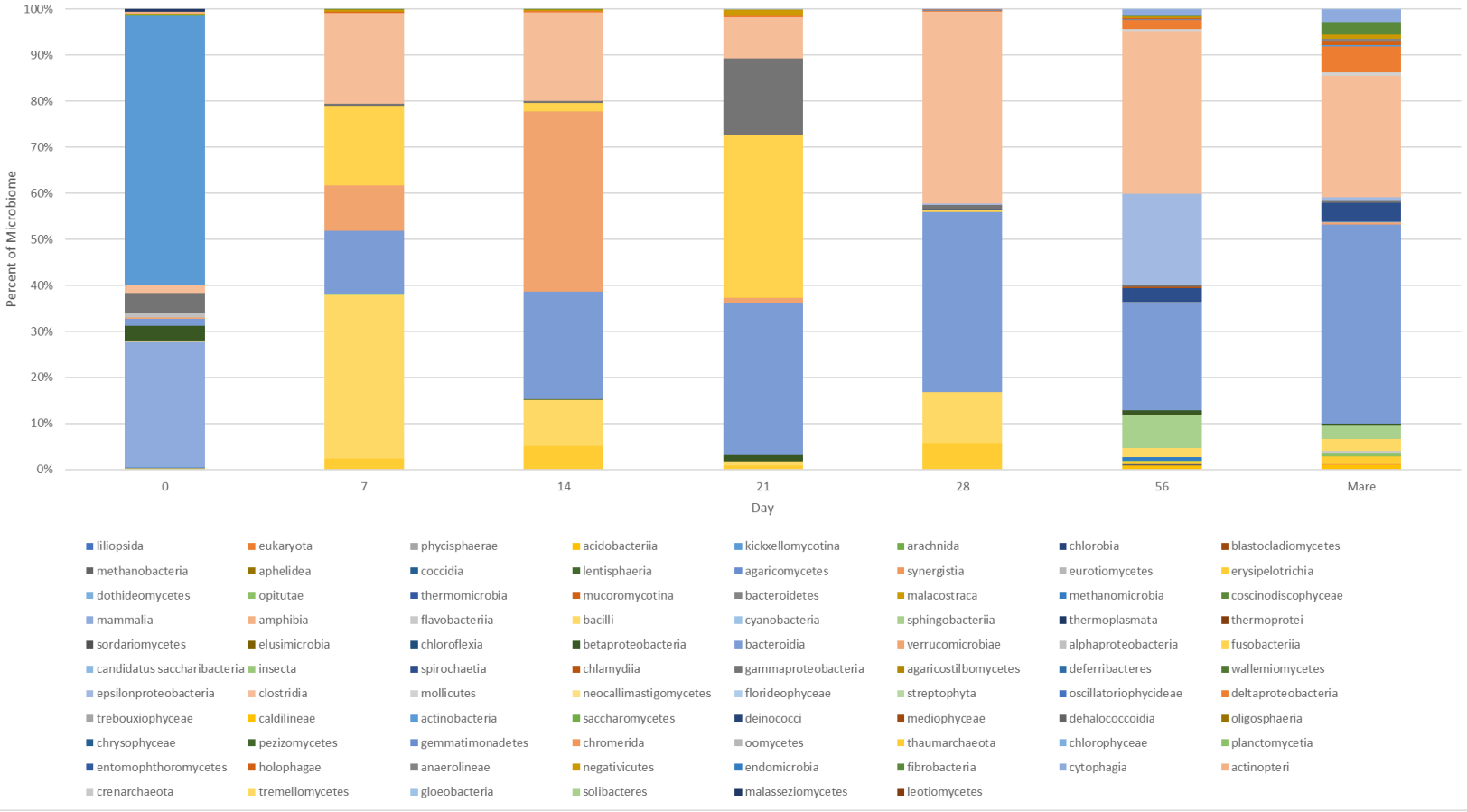


Figure A-11: Microbiome of Treated Foal (1263; Amikacin/Penicillin d 0 to 14) on d 0, 7, 14, 21, and 28, and Mare Microbiome d 56

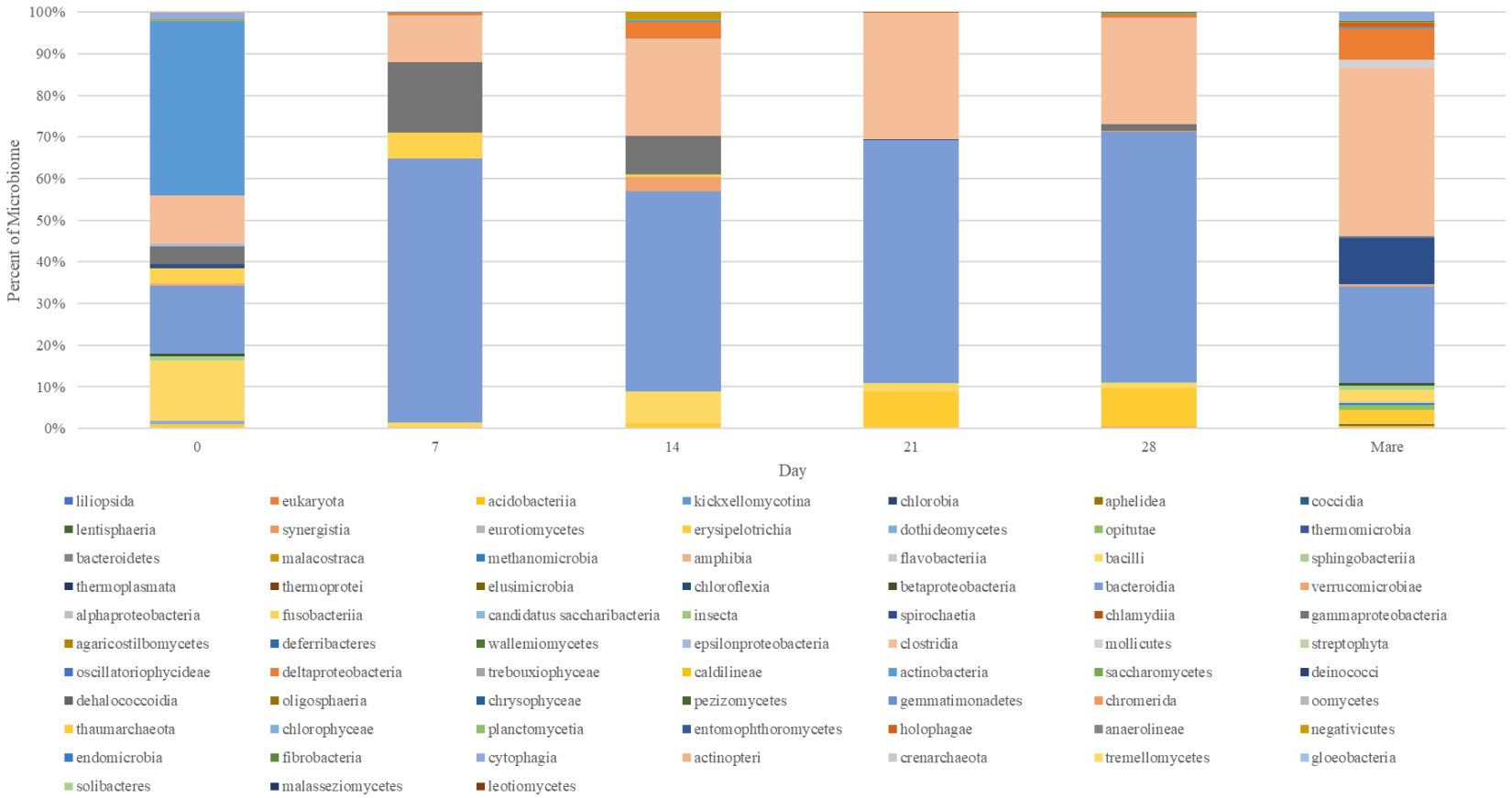


Figure A-12: Microbiome of Treated Foal (0506; Amikacin/Ceftiofur d 0 to 7) on d 0, 7, 14, 21, 28, and 56 and Mare Microbiome d 56

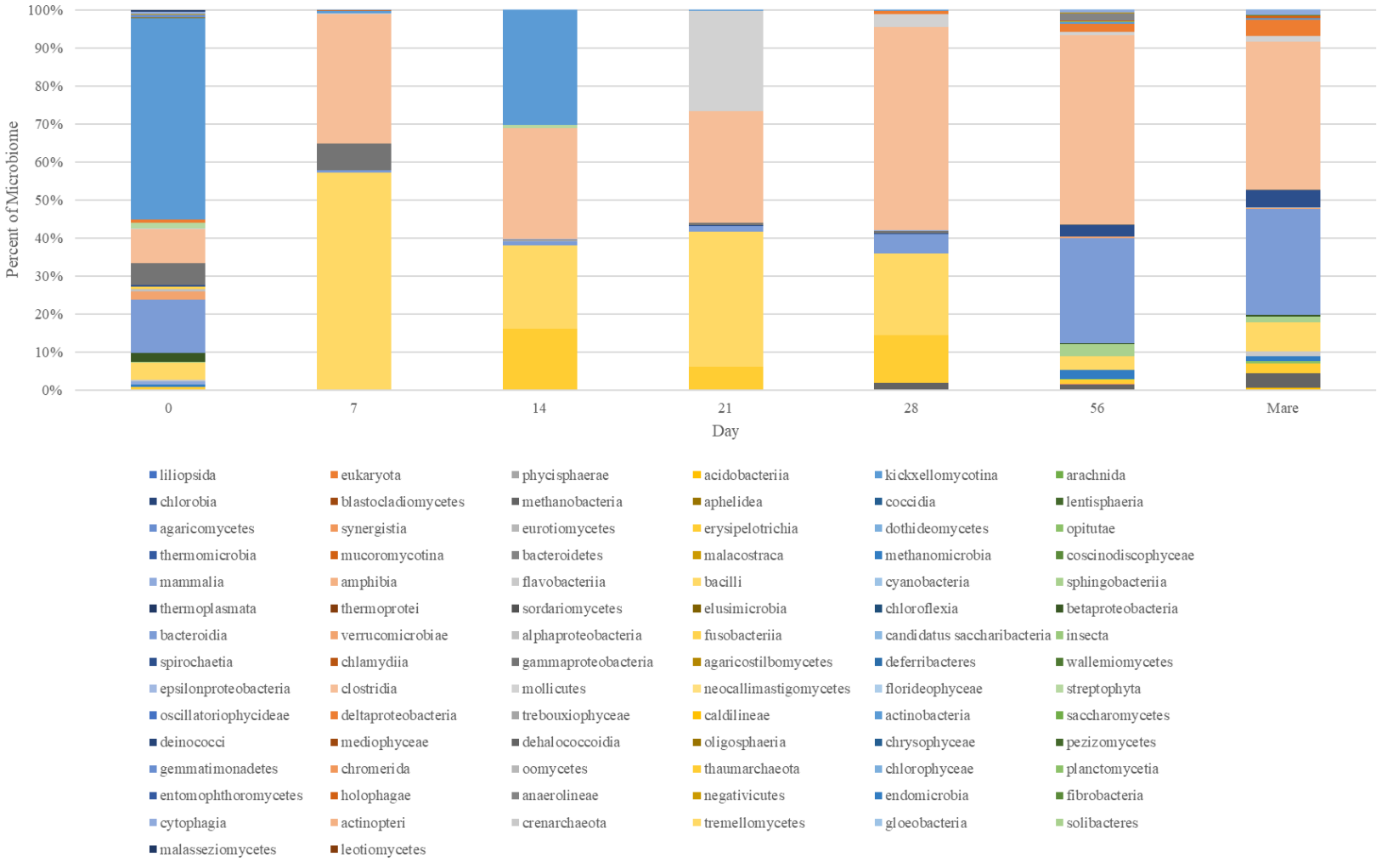


Figure A-13: Microbiome of Treated Foal (1495; Amikacin/Ceftiofur d 0 to 14; trimethoprim/sulfadiazine d 14 to 21) on d 0, 7, 14, 21, 28, and 56 and Mare Microbiome d 56

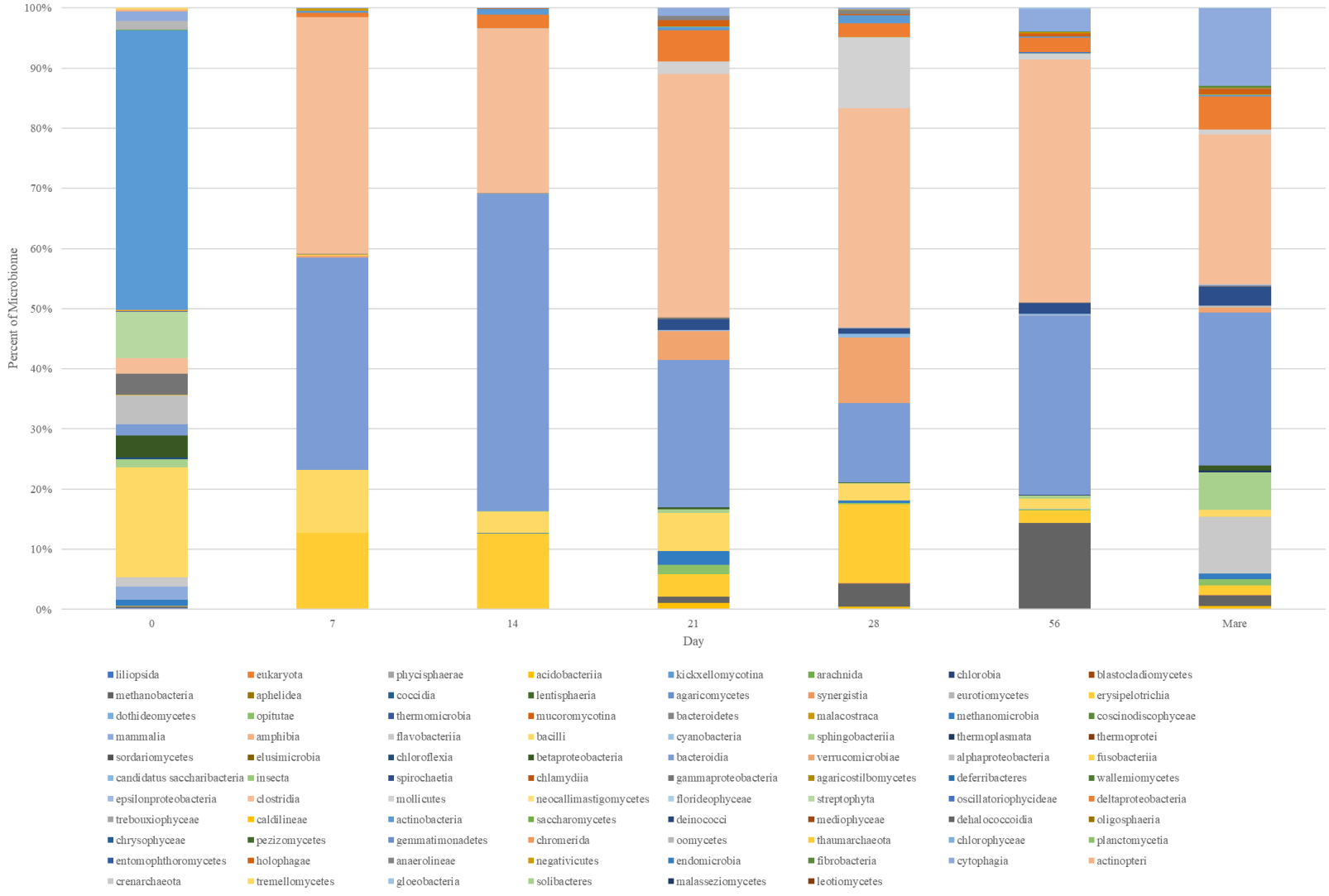


Figure A-14: Microbiome of Treated Foal (9345; Amikacin/Ceftiofur d 0 to 7; trimethoprim/sulfadiazine d 21 to 56) on d 0, 7, 14, 21, 28, and 56 and Mare Microbiome d 56

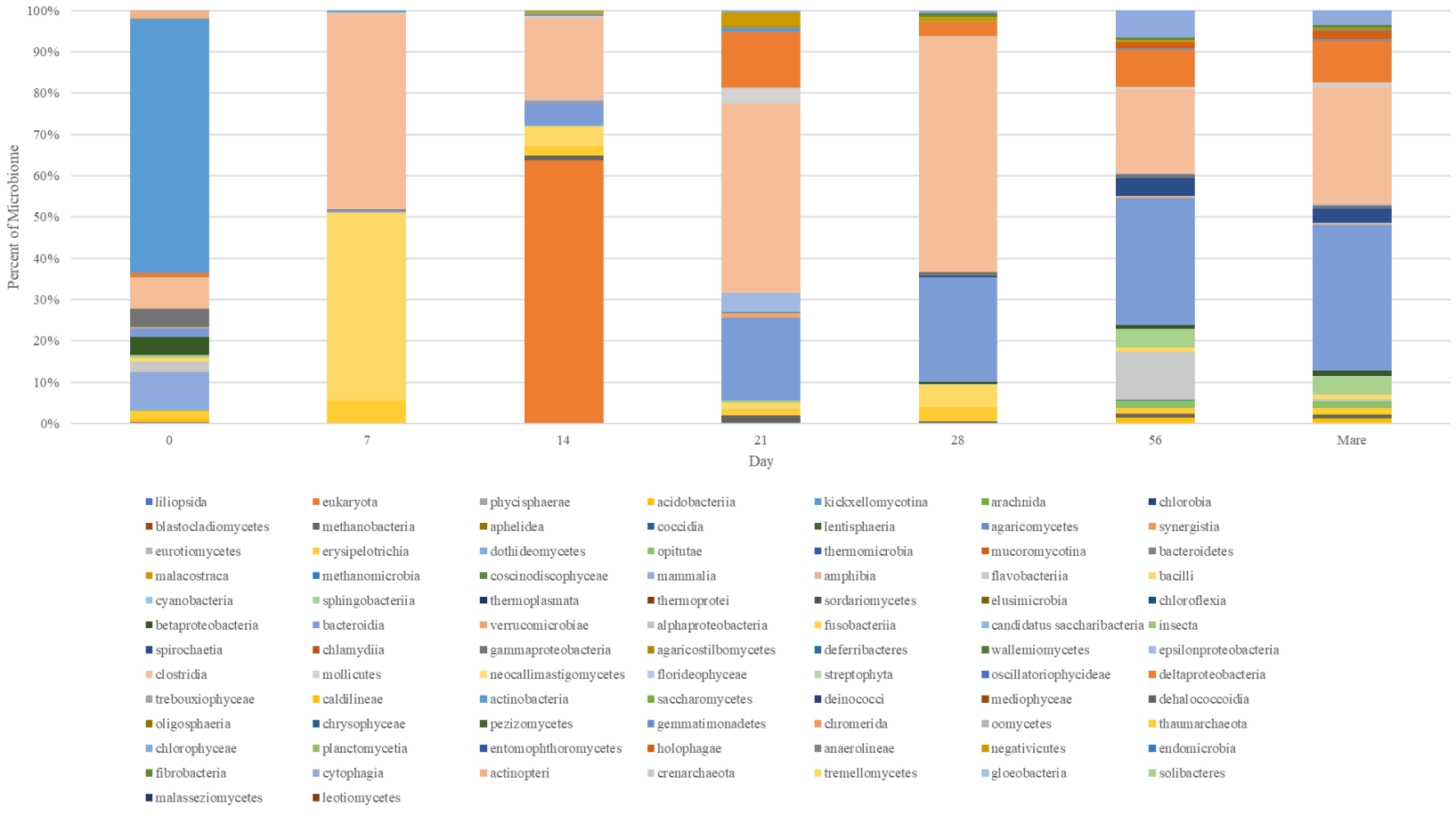


Figure A-15: Microbiome of Treated Foal (Cosmic; trimethoprim/sulfadiazine d 28 to 35) on d 0, 7, 14, 21, 28, and 56

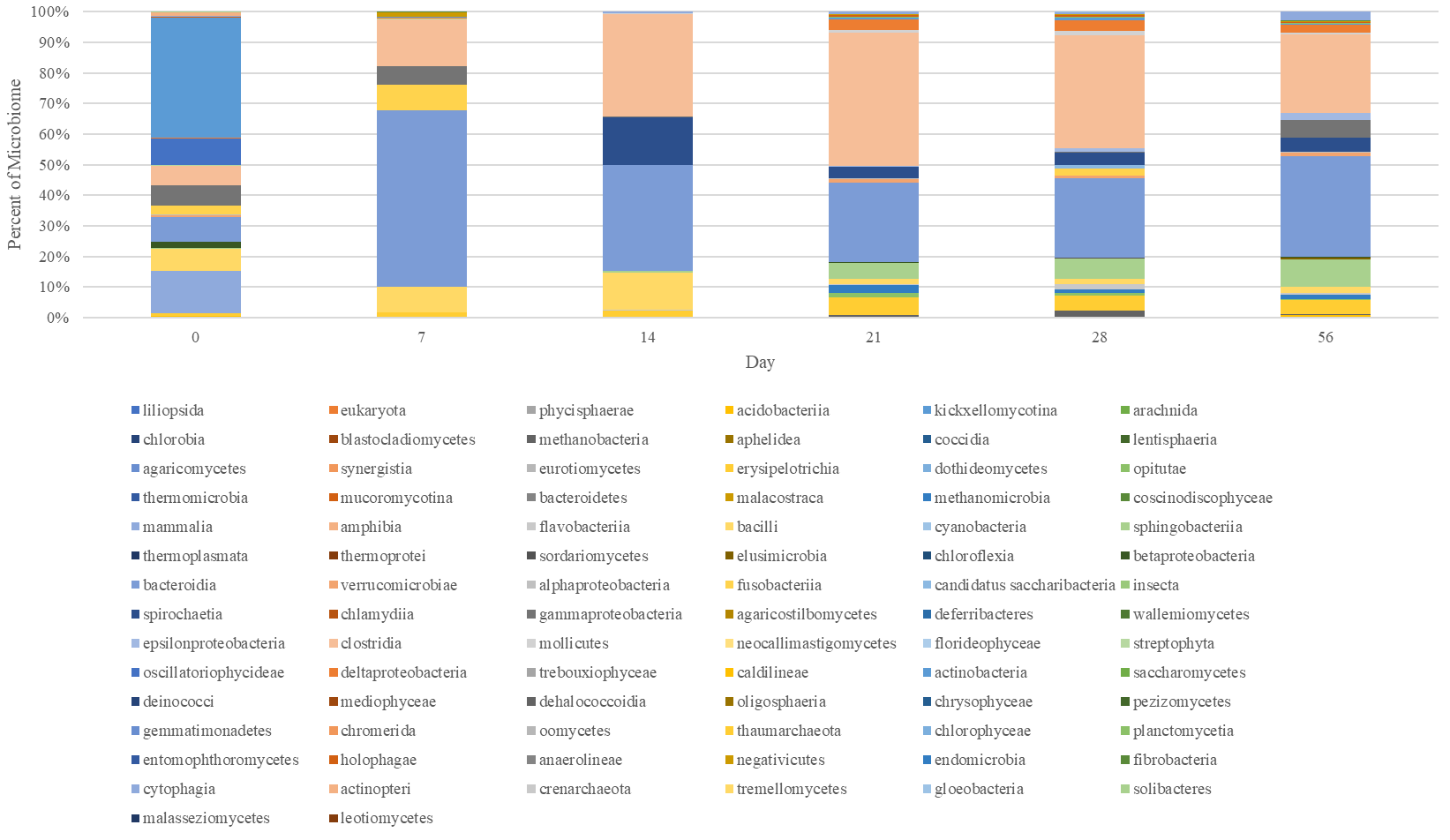


Figure A-16: Microbiome of Treated Foal (Dash; trimethoprim/sulfadiazine d 7 to 28) on d 0, 7, 14, 21, and 28

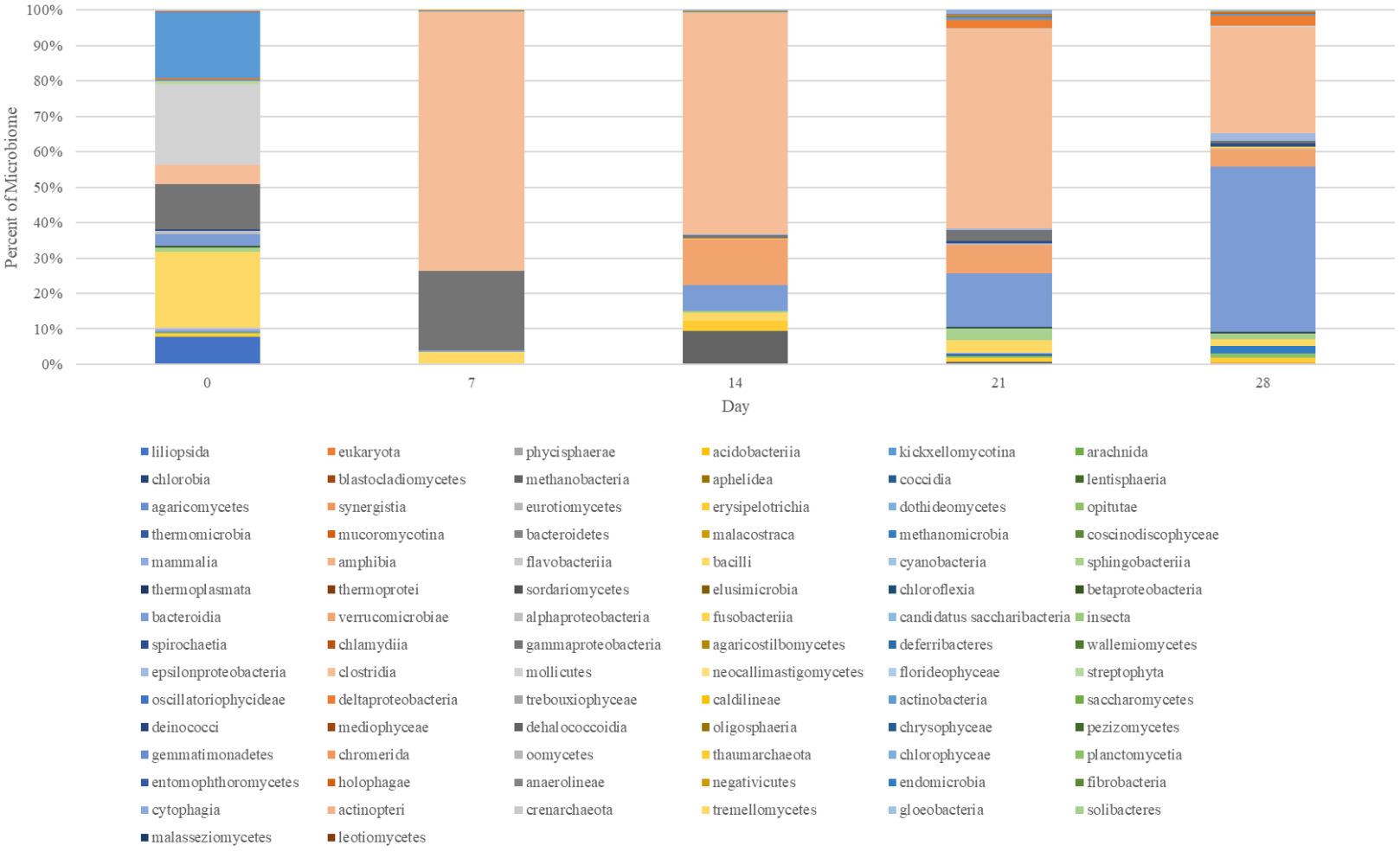
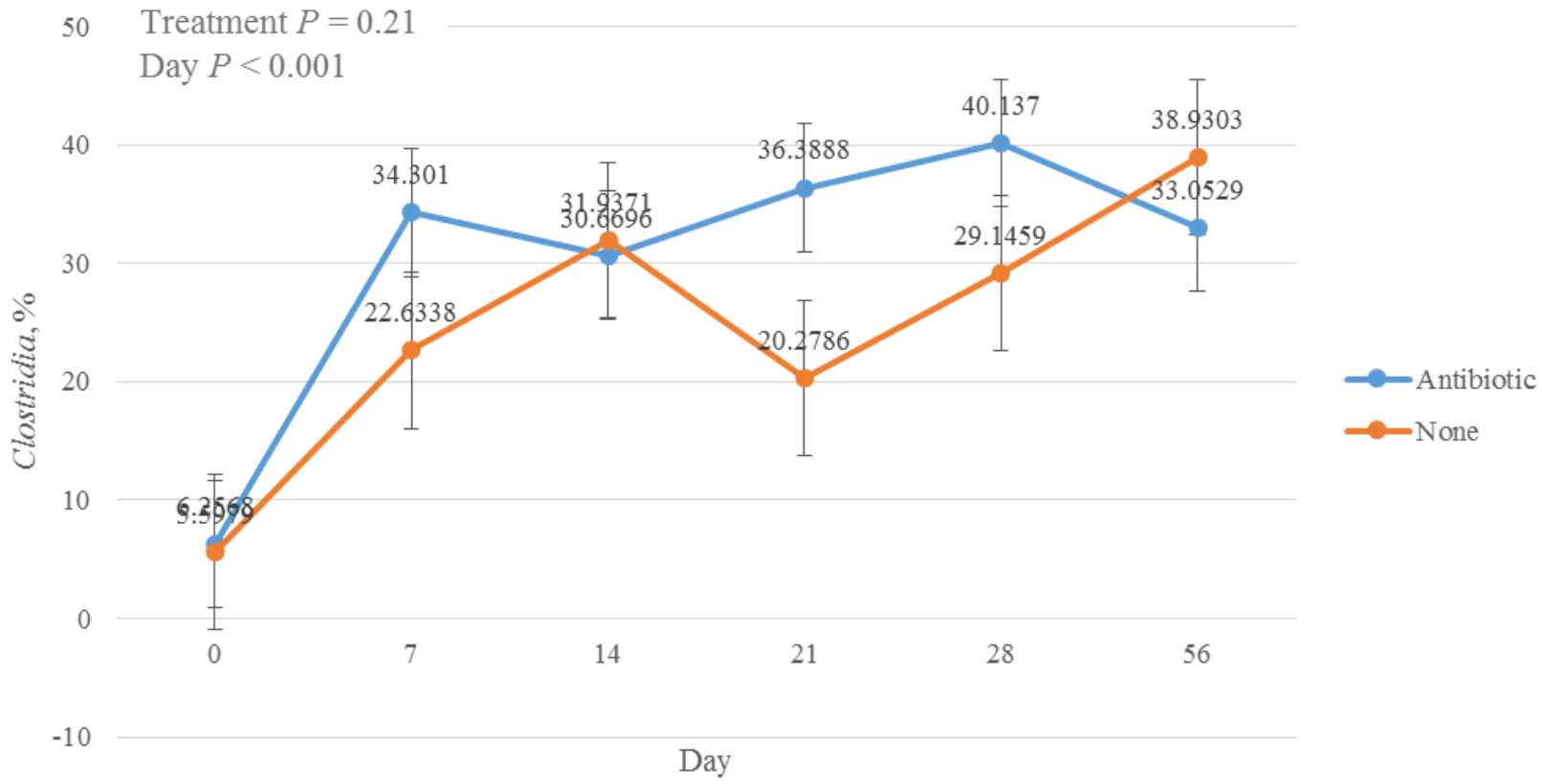
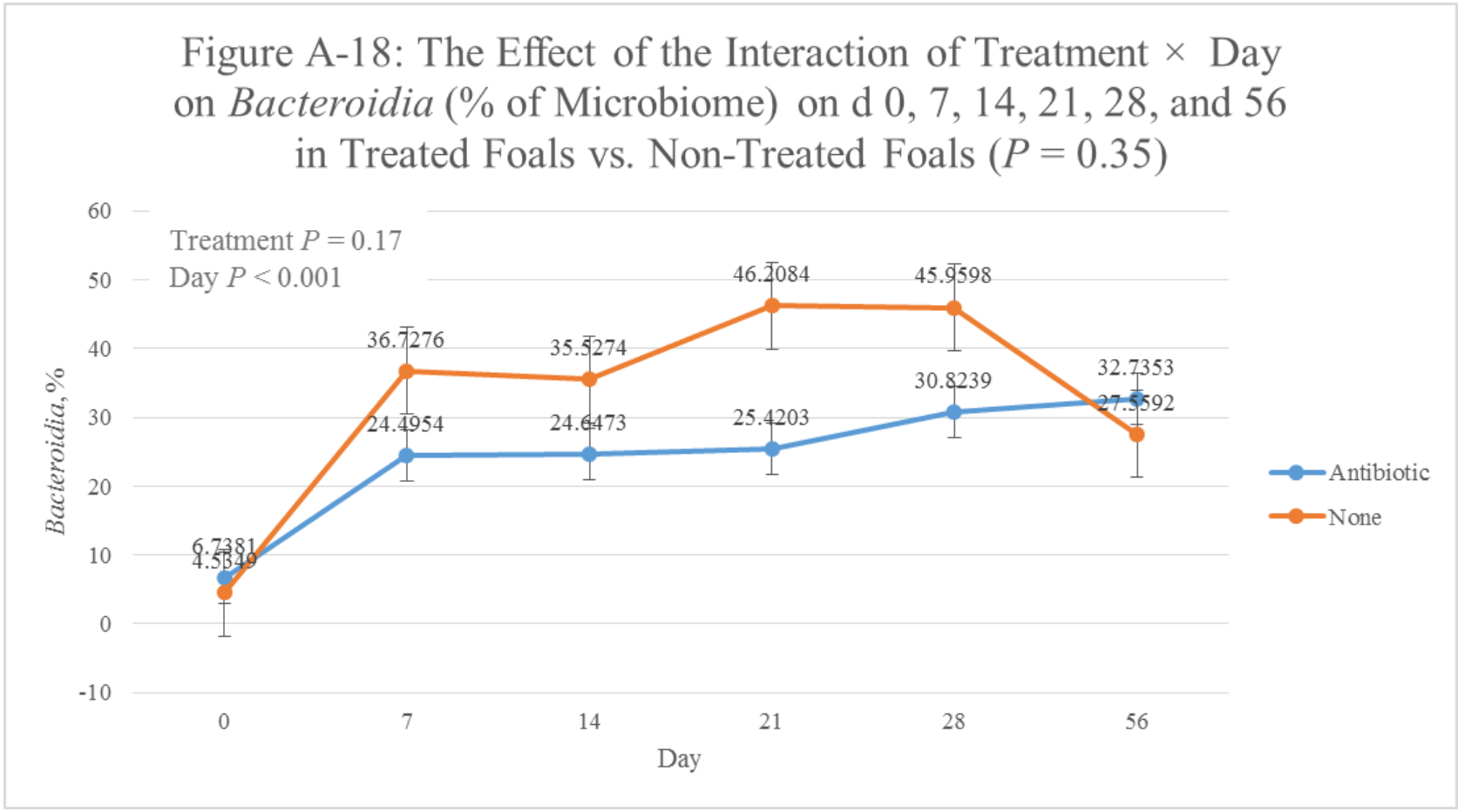


Figure A-17: The Effect of the Interaction of Treatment × Day on *Clostridia* (% of Microbiome) on d 0, 7, 14, 21, 28, and 56 in Treated Foals vs. Non-Treated Foals ($P = 0.24$)



* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.



* Although treatment × day is presented in this chart, note there is no significant treatment × day interaction.