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Cross-Sectional Analysis of Salivary Cortisol and Cyathostome Infestation in Horses

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CROSS-SECTIONAL ANALYSIS OF SALIVARY CORTISOL AND CYATHOSTOME INFESTATION IN HORSES

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Submitted in partial fulfillment of the requirements for graduation with Honors

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Abstract

With an increase in anthelmintic resistance and decreased efficacy of many commercial
dewormers, understanding factors that contribute to parasite infestations in horses is integral to
their management. The goal of this study was to look at the potential relationship between
parasites and stress response by evaluating salivary cortisol levels and cyathostome egg shedding
levels. Using a sample size of n = 200 horses from the state of Ohio, fecal and saliva samples
were collected from each horse. Fecal egg counts were performed for each horse with a modified
Stoll method, and saliva samples were tested with an enzyme-linked immunosorbent assay
(ELISA). Questionnaires were generated to gain information about each horse and its
management. A total of 23 variables were tested against dichotomized fecal egg count levels
using Chi-Square Tests of Independence or Fisher’s Exact Tests for significance. Variables with
p < 0.30 were analyzed for association with fecal egg count level with a stepwise multiple
logistic regression model. The three variables included in the final logistic regression model were
age (p = 0.0002), cortisol level (p = 0.036), pasture mowing frequency (p = 0.025), and turnout
(p = 0.0573). These p-values are adjusted for the other variables within the model. Location at
the time of sampling (p = 0.818) was also forced into the model to account for a naturally lower
cortisol level for those horses who were outside. This study analyzed factors contributing to fecal
egg shedding levels, and determined managerial practices that can reduce cyathostome levels in
horses.

1. Introduction

Parasite infestation is a routine condition that plagues horses. Horses are susceptible to a
wide variety of parasites depending on a litany of factors [1-2]. Several factors, including age,
management practices such as deworming protocol, and amount of turnout have been shown to
affect parasite egg shedding levels [3], [4]. The most common types of parasites that horses can acquire include tapeworms, small strongyles (also known as cyathostomes), and ascarids [5]. High levels of parasite infestation can be qualitatively determined by assessing physical characteristics of the horse including hair coat quality and the presence or absence of a “pot-bellied” appearance [6]. Parasite infestation for small strongyles, large strongyles and ascarids can be quantitatively measured by doing a Fecal Egg Count (FEC) and determining the number of parasite eggs per gram of feces (EPG). A common method utilized to calculate EPG is through the use of a McMaster’s slide or through the use of the Stoll Method [7]. Both of these approaches uses a microscope and various methods of obtaining a representative amount of parasite eggs onto a slide or cover slip and then manually counting visible eggs. There is a rising concern over the development of resistance to dewormers by cyathostomes in particular [5], and the need to find alternate methods for prevention of parasite infestation is growing.

Innately weak or compromised immunity has the potential to lead to susceptibility of a horse to parasites. While this relationship has been shown in humans [8], this association has not been well-described in horses. In stressful situations, a complex web of hormones are released through the hypothalamic-pituitary-adrenocortical axis. The hypothalamus secretes corticotropin-releasing hormone, which stimulates the production of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Glucocorticoids and catecholamines are also involved in the stress response [9]. Stress triggers a response from the adrenal gland as well, producing the hormone cortisol [9]. The release of cortisol triggers many reactions within the body, one of which is a suppression of the immune system [10]. This situational suppression of the immune system, combined with the opportunistic nature of parasite, is what could hypothetically lead to increased infestation during times of stress [10,11]. Cortisol levels can be
estimated with equal accuracy from either blood or saliva samples and detected quantitatively via a salivary enzyme immunoassay (ELISA) test [12]. This study chose to use saliva samples in an attempt to avoid causing distress to the horse during the collection process.

The objective of this thesis is to study the relationship between cortisol levels and cyathostome infestation in different groups of horses, while accounting for factors that can affect parasite levels (age, workload, diet, turnout, etc). It can be hypothesized that since cortisol suppresses the immune system, those horses with higher cortisol levels will also have higher levels of parasite infestation, considering the immunosuppression will render them more susceptible to the parasites. This association has been studied in several other animal models including various types of fish, primates and also in lambs [13-16]. The majority of these studies looked at environment factors and their effect on the relationship between parasites and cortisol. In several mammalian models, mainly different species of primates, a positive correlation was found between parasite infestation and cortisol levels [17-19], while in fish models the exact opposite was discovered [20]. This relationship, however, is not well-represented in scientific literature in the equine model. Cyathostome levels were determined using the Stoll Method for FEC and cortisol levels based from saliva samples were analyzed using an enzyme immunoassay test (ELISA).

The expectation of this study was to find a positive association between cyathostome parasite infestation and salivary cortisol levels, and by proxy, stress levels in horses. If this association can be proven, it will lead to greater understanding of how stress affects the equine athlete and what factors play the biggest roles in contributing to cyathostome infestation. Through this knowledge, the treatment and prevention of parasites can become more directed and effective. Additionally, further studies could be conducted from this cross-sectional basis to
delve deeper into each individual associated factor and its relationship to parasite infestation or stress levels.

2. Materials and Methods

2.1 Horses

Horses (n=200) from across the state of Ohio were used for this study (Figure 1). The equine participants ranged in age, sex, breed, workload, diet and management. Horses participating in this study had not been dewormed for approximately three months prior to their collection date in order to ensure there was no residual effects of a dewormer in their system.

2.2 Questionnaire Collection

A questionnaire was generated with the approval from both the Institutional Review Board (IRB) and the Institutional Animal Care and Use Committee (IACUC) at Otterbein University. The questionnaire was completed individually by the horses’ owners for each equine participant. The questionnaire gathered basic information about the horse, such as age and breed, as well as questions related to the care of the horse, maintenance of the facility, and barn parasite management protocol. The questions aimed to capture as much relevant information about the horse for variables that could influence either parasite or salivary cortisol levels of the horse (Appendix A). The horses were scored for body condition by the researchers using the Henneke system at the time of collection. Workload for each horse was determined using the National Research Council (NRC) Definitions of Workload [21], and can be seen in Appendix B. Questionnaires were completed just prior to the researchers collection date, at the time of collection, or shortly thereafter. If the questionnaires were not filled out at or near the immediate
time frame of the collection, the information was recorded to reflect the management, care and status of the horse at the time of the sample collection.

2.3 Fecal Egg Counts

Parasite levels were determined using a modification of the Stoll Method [7]. Fecal samples were collected from each equine participant as freshly as possible, but within 12 hours. To ensure accurate samples, owners were asked to not clean stalls the morning of collection. Horses living primarily or completely outside were brought into an arena or cross-tie area until a fecal sample could be collected. Horse numbers 67, 68, 69, and 79 did not provide fecal samples and were subsequently removed from the study.

A plastic sandwich bag was filled with the fecal sample, sealed and labeled with the horse’s name, barn, assigned number and the date. Samples were then stored at 4°C until the time of testing. All 200 fecal samples were tested within one week of being collected, using the modified Stoll method. Once ready for testing, contents of the fecal sample were homogenized for up to two minutes in the bag to make the sample more uniform. Then, ten grams of the sample were weighed out into a cup and returned to storage at 4°C. Ninety milliliters of distilled water were placed in a graduated cylinder, and then added to the fecal sample cup. The sample slurry was then stirred to create a more uniform mixture, and a 1 milliliter (mL) sample was taken from the middle of the cup and placed into a 15 mL conical tube. A sucrose solution (specific gravity 1.28) was added to the conical tube to a total volume of fourteen milliliters. The tubes were centrifuged at 200*g for 10 minutes. After centrifugation, a coverslip was placed on the top of the conical tube, and the tube then sat idle for approximately 30 minutes to allow the parasite eggs to float to the top. After the allotted time, the coverslip was then placed on a
slide and viewed under a microscope. Both strongyle and ascarid eggs were counted. One horse had ascarid eggs present in their fecal egg count, however as previously mentioned, this study focused on strongyle eggs only. To determine the number of eggs per gram of feces, the number of strongyle eggs were counted and then multiplied by ten. This test has a sensitivity of ten eggs per gram.

2.4 Cortisol Testing

Cortisol levels was determined using a salivary cortisol enzyme immunoassay. Saliva samples were taken from the equine participants using an oral swab from Salimetrics (Salimetrics, LLC, State College, PA). Samples were collected midday (between 11:00 AM and 1:00 PM from each horse, more than an hour after their last meal. Debris was removed from the horse’s mouth, if present, prior to sample collection. Little to no physical restraint was used to hold the horse’s head steady during the saliva sample collection process. The swabs were held under the tongue of each horse for approximately sixty to ninety seconds. The researchers wore a new pair of gloves for each participant to avoid contamination between samples. Date and time of collection were recorded. Horse numbers 111 and 112 did not produce any saliva after centrifugation, and were not included in the study. Numbers 111 and 112 were reassigned to other horses later in the collection process. Samples were refrigerated (within thirty minutes of collection) and then frozen at -20°C (within four hours of collection) for long-term storage until ready for assay. In preparation for freezing, samples were centrifuged at 1800 x g for 15 minutes. Once ready to be analyzed, the samples were thawed, vortexed and centrifuged again at 1500 x g for 15 minutes before being assayed. All samples were tested using an ELISA method. Reagents from Salimetrics, LLC were prepared per the company’s instructions [22]. Each Salimetrics plate contained ninety-six wells and tested thirty-eight samples in duplicate per plate.
Twenty-five microliters (uL) of controls, standards or samples were pipetted into their respective wells. Two wells were designated the negative control wells, which contained only assay diluent. Twenty-five uL of assay diluent were then added to each well. A diluted conjugate was also added to each well. The plate was rotated at 500 revolutions per minute for five minutes, and then incubated at room temperature for approximately 55 minutes. In this step, all of the plates were rotated mechanically on a plate rotator. The plate were then washed with 1X wash buffer four times and blotted dry. All of the plates were washed by hand, via multichannel pipette, with 1X wash buffer. Next, 200 uL of tetramethylbenzidine (TMB) solution was added to each well, mixed at 500 revolutions per minute for five minutes and incubated in the dark at room temperature for 25 minutes. Lastly, 50 uL of stop solution was added to each well and mixed at 500 revolutions per minute for three minutes. The plate was then read at 450 nanometers (nm) within ten minutes of adding the stop buffer. In order to reduce the background noise of the cortisol data, the plates were also read at 560 nm. This was also completed within ten minutes of adding the stop buffer.

Results of the plate reading were determined by computing the optical density (OD) for the duplicate wells, subtracting the average OD for the non-specific binding wells from the average OD of the standard, control and unknown wells. Finally, the percent bound was calculated by dividing the average OD by the average OD for the zero (B/B0) for each control, standard and unknown. Cortisol values were calculated based off of these averages.

2.5 Statistical Analysis

Exactly 200 horses were used order to provide statistically relevant data for multiple logistic regression [23]. The results were statistically analyzed and compared using a logistic regression that inspected different groups of the equine subjects. The association between the
parasite infestation levels from the fecal egg counts and the salivary cortisol levels from the salivary enzyme immunoassay test were analyzed using a multinomial logistic regression that accounted for factors associated with FEC including, but not limited to, age, sex, workload, diet and turn-out.

In order to determine statistical relevancy, Statistical Analysis Software (SAS version 9.3) was used. The dependent (response) variable was EPG, and every other variable was tested against EPG using a Chi-Square Test of Independence or Fisher’s Exact Test. Horses with a fecal egg count of 50 EPG or higher were considered a ‘high’ shedder for the purposes of this study. Due to a break in the data set at 50 EPG, a high shedder was defined as a horse shedding 50 EPG or greater. Variables with a significance level of $P<0.30$ were offered to the final model. Independent variables were tested against one another using a Chi-Square Test of Independence to determine if there was overlapping variability. The final variables were then entered offered to the multiple logistic regression model, and run using a stepwise selection. Variables were also tested manually in the multiple logistic regression model in order to confirm the results generated by SAS, as well as evaluating the c-statistic and the Hosmer-Lemeshow Goodness-of-Fit Test to confirm the reliability of the model.

The model was confirmed as being sound by analyzing model convergence status, testing Global Null Hypotheses, Type 3 Analysis of Effects, and Hosmer and Lemeshow Goodness-of-Fit Tests. In all steps of the stepwise multiple logistic regression, model convergence was met. Additionally, when testing the Global Null Hypothesis, the likelihood ratio, score and Wald all agreed, supporting a trustworthy model [23].
3. Results

3.1 Questionnaires

A total of 207 questionnaires were collected; however, a total of 200 horses were used in the final study and statistical analysis due to availability of fecal or saliva samples. Horses participating in the study were located across the state of Ohio (Figure 1). Some data collected from the questionnaire in open-ended form were formatted into categorical responses for the purpose of Chi-Square testing.

3.2 Fecal Egg Counts

A FEC cutoff of 50 EPG resulted in a total of 70 high shedders and 130 low shedders. The data set ranged from 0 to 1810 EPG, with a mean of 117 EPG, a standard deviation of 247 EPG, and a median of 0 EPG.

3.3 Cortisol Testing

Due to a lack of consistent and relevant information on baseline cortisol levels in the equine model, we chose to define cortisol within the sample set after evaluating the distribution of the data. High cortisol was defined as being above the third quartile, or greater than 0.142 ug/dL, up to the maximum of 0.774 ug/dL. Medium cortisol was defined as being between the first and third quartiles, or between 0.045 ug/dL and 0.142 ug/dL. Low cortisol was defined as being below the first quartile, or less than 0.045 ug/dL.
3.4 Statistical Analysis

A total of 23 variables were evaluated by Chi-Square Test of Independence or Fisher’s Exact Test against fecal egg count level ($p < 0.30$). Ten variables met the cutoff level to be offered to the multiple logistic model, and are shown in Figure 2. These variables include cortisol level ($p = 0.076$), age ($p = 0.008$), sex ($p = 0.285$), if the horse cribs (a stereotypic behavior) or not ($p = 0.256$), if the horse is turned out on pasture or an alternate form of turnout such as a dry lot, or not at all ($p = 0.074$), frequency of manure removal from the stall ($p = 0.018$), frequency of pasture mowing ($p = 0.030$), if rotational grazing is a management practice used ($p = 0.087$), type of deworming schedule ($p = 0.157$), and the location of the horse at the time of saliva sample collection ($p = 0.185$).

These ten variables were offered to the multiple logistic regression model and evaluated using the stepwise method to determine which variables were valuable in predicting fecal egg count levels. Location at the time of sample collection was forced into the model to account for some horses being sampled while outdoors versus inside a stall [24]. The final model included horse age, cortisol level (high, medium or low), pasture mowing frequency (annually, quarterly, monthly, weekly or not at all), and turnout type (pasture, dry lot, split between pasture and dry lot, stalled with no turnout, or mainly stalled with minimal pasture) being the variables predictive of fecal egg count levels. The results of the Type 3 Analysis are summarized in Figure 3. Because the $p$-values were low, the Type 3 Analysis also suggests a trustworthy model [23]. Lastly, the Hosmer and Lemeshow Goodness-of-Fit tests revealed an accurate model. The $c$-statistic was also evaluated when adding variables to the model. The area under the curve for the multiple logistic regression was $c = 0.762$, indicating an acceptable model [25].
Ultimately, the results of this model are summarized with the odds ratio estimates (Figure 4). As age increases one year, the estimated odds of a high fecal egg count (>50 EPG) decreases 11%. The estimated odds of high fecal egg count are 66% less in horses with a high cortisol level as compared to a low cortisol level. Horses with a medium cortisol level are 1.178 times more likely to have a high fecal egg count than horses with a low cortisol level. Barns that mowed their pastures at intervals less frequently than once per week (quarterly, biannually, annually or not at all) increased the estimated odds of a horse with a high fecal egg count by 4.649 to 9.180 times. Horses turned out on pasture in any capacity also had an increased risk of having a high fecal egg count over horses turned out in dry lots, or not turned out at all. Horses outside at the time of sample collection also had an increased risk of having a high fecal egg count.

4. Discussion

Through the analysis of multiple statistics, including the multiple linear regression, type 3 analysis, global null hypothesis, odds ratios and the Hosmer and Lemeshow Goodness-of-Fit Test, we found four factors to be associated with increased risk of a high (>50 EPG) fecal egg count. These factors include cortisol level, pasture mowing frequency, turnout type, and age.

The notion that cortisol has an impact on fecal egg count levels indicates a potentially new approach to cyathostome management for horses. Salivary cortisol testing could be an indicator for deworming practices. Additionally, for future study, by reversing this study, and making cortisol levels the new dependent variable, factors that influence salivary cortisol levels could be determined. If these potential factors are management based, or can be influenced by an owner or barn manager, the salivary cortisol levels could be reduced, thereby also reducing
the risk of a high fecal egg count. The implication is that by managing stress and cortisol levels, there may be an ability to influence and manage fecal egg count levels, without ever having to use a dewormer. It appears that management practices have the ability to influence fecal egg counts [1, 3-4]. Further study could be done regarding what factors influence cortisol, and determine what, if any, of these potential factors can be managed. Combined with management practices already known to lower the risk of fecal egg shedding levels, this has the potential to be another method that could alleviate and curb cyathostome infestation without ever having to resort to a dewormer.

We recognize several potential limitations of the study, including the open-endedness of the questionnaire that the owners filled out and the reliance on the owners to fill out those survey. If this project were repeated, the questionnaire could be filled out by the researchers at the time of sample collection in the presence of the owner in order to reduce the subjectivity of the questionnaire. Additionally, because some horses were outside at the time of saliva sample collection, location at the time of sample collection had to be factored into the model, despite it not fitting in the model.

There may also be an association between age and cortisol. When looking at the odds ratio for high cortisol levels (Figure 4), these horses are actually at a decreased risk of a high fecal egg count. However, horses with medium cortisol levels are at an increased risk of high fecal egg counts. When we analyzed the group of horses with high cortisol more closely using an Analysis of Variance (ANOVA), they had a higher mean age, suggesting that there is a relationship between age and cortisol levels. Horses with high salivary cortisol levels (n = 50) had a mean age of 15, while horses with low cortisol levels (n = 49) had a slightly lower mean
age of 12. However, when an interaction between age and cortisol was included in the model, it did not improve the quality of the model.

The finding that more frequent mowing of the pasture leads to decreased FEC may be due to the disruption of the cyathostome life cycle preventing infestation from occurring [6]. Our findings with regard to turnout being associated with higher FEC and increased age being associated with lower FEC correspond with findings described in previous literature [1], [3]. We also acknowledge the variability within fecal egg count testing, and while we used the Stoll method, which has a sensitivity of 10 EPG, multiple fecal egg counts could be run in order to establish consistency of shedding levels for each sample.

For future repetitions of this study, it may be advantageous to take multiple saliva samples in order to account for the diurnal and circadian rhythm of cortisol levels in horses [24].

The overall implication is that by managing stress and cortisol levels, there may be an ability to influence and manage fecal egg count levels, without ever having to use a dewormer. It appears that management practices have the ability to influence fecal egg counts [1, 3-4]. Further study could be done regarding what factors influence cortisol, and determine what, if any, of these potential factors can be managed. Combined with management practices already known to lower the risk of fecal egg shedding levels, this has the potential to be another method that could alleviate and curb cyathostome infestation without ever having to resort to a dewormer.
Figure 1 - Map of barn locations

Made with Mapcustomizer.com
Table 1: List of variables tested against FEC and their significance by Chi Square Test of Independence or by Fisher’s Exact Test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance (P)</th>
<th>Test Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol level</td>
<td>0.0755</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Breed type</td>
<td>0.9205</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>Age</td>
<td>0.0075</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>Sex</td>
<td>0.2849</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>Vices</td>
<td>0.3157</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Cribber</td>
<td>0.2558</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Health conditions</td>
<td>0.6411</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Medications</td>
<td>0.9319</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Workload</td>
<td>0.9586</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Discipline group</td>
<td>0.6122</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Concentrate amount</td>
<td>0.5491</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Turnout</td>
<td>0.0738</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Stocking density</td>
<td>0.6214</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>Amount of turnout</td>
<td>0.5638</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Turnout time of day</td>
<td>0.4744</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>Manure removal - stall</td>
<td>0.0179</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>Manure removal - paddock</td>
<td>0.5925</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Pasture mow frequency</td>
<td>0.0297</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Pasture harrow frequency</td>
<td>0.7286</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Rotational grazing</td>
<td>0.0866</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Deworming schedule</td>
<td>0.1567</td>
<td>Fisher’s Exact Test</td>
</tr>
<tr>
<td>New arrival practices</td>
<td>0.3257</td>
<td>Chi Square</td>
</tr>
<tr>
<td>Location during saliva swab</td>
<td>0.1847</td>
<td>Chi Square</td>
</tr>
</tbody>
</table>
Figure 3: Type 3 Analysis of Effects

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; Chi Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>13.8887</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cortisol Level</td>
<td>2</td>
<td>6.6581</td>
<td>0.0358</td>
</tr>
<tr>
<td>Mowing Frequency</td>
<td>5</td>
<td>12.8702</td>
<td>0.0246</td>
</tr>
<tr>
<td>Turnout Type</td>
<td>4</td>
<td>9.1563</td>
<td>0.0573</td>
</tr>
<tr>
<td>Location during Sample Collection</td>
<td>1</td>
<td>0.0530</td>
<td>0.8179</td>
</tr>
</tbody>
</table>

Figure 4: Odds Ratio Estimates

<table>
<thead>
<tr>
<th>Effect</th>
<th>Point Estimate</th>
<th>95% Wald Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.883</td>
<td>0.828 0.943</td>
</tr>
<tr>
<td>Cortisol: High vs. Low</td>
<td>0.344</td>
<td>0.110 1.074</td>
</tr>
<tr>
<td>Cortisol: Medium vs. Low</td>
<td>1.178</td>
<td>0.511 2.716</td>
</tr>
<tr>
<td>Mow: Annual vs. Weekly</td>
<td>7.626</td>
<td>1.618 35.936</td>
</tr>
<tr>
<td>Mow: Biannual vs. Weekly</td>
<td>7.504</td>
<td>1.684 33.434</td>
</tr>
<tr>
<td>Mow: Monthly vs. Weekly</td>
<td>4.649</td>
<td>1.344 16.073</td>
</tr>
<tr>
<td>Mow: None vs. Weekly</td>
<td>7.435</td>
<td>1.996 27.693</td>
</tr>
<tr>
<td>Turnout: Dry Lot vs. Pasture</td>
<td>0.222</td>
<td>0.066 0.749</td>
</tr>
<tr>
<td>Turnout: Dry Lot/Pasture vs Pasture only</td>
<td>1.085</td>
<td>0.371 3.176</td>
</tr>
<tr>
<td>Turnout: Stalled/Minimal Pasture vs. Pasture</td>
<td>1.406</td>
<td>0.251 7.891</td>
</tr>
<tr>
<td>Turnout: Stalled vs. Pasture</td>
<td>0.261</td>
<td>0.063 1.077</td>
</tr>
<tr>
<td>Location: Outside vs. Stalled</td>
<td>1.172</td>
<td>0.303 4.541</td>
</tr>
</tbody>
</table>
Appendix A - Questionnaire used to collect information from each equine participant

**Questionnaire:**
Owner Name: ___________________________
Owner Address: _______________________________________________________________________
Owner Phone: _____________________
Owner Email: ____________________________________________________________

**Basic Information:**
1. Horse Name: ______________________________
2. Horse Breed: ______________________________
3. DOB (mm/dd/yyyy): ____/____/____
4. Sex: ___Mare           ___Gelding           ___Stallion
5. Please list any vices this horse has:
   ______________________________________________________________________________
6. Please list any health conditions this horse has currently:
   ______________________________________________________________________________
7. Workload and Type/discipline(s) of work
   (please reference the attached NRC Workload guidelines):
   Check one:
   ___ Maintenance           ___ Light           ___Moderate           ___Heavy           ___ Very Heavy
   Discipline(s):______________________________________________________________

**Feeding:**
8. Diet/type/brand of feed:
   ______________________________________________________________________________
9. Frequency of feedings/Amount per feeding (weight):
   ______________________________________________________________________________
   Time of feedings:_______________________________

**Turnout:**
10. Turnout type (check one): ___Pasture           ___Dry Lot           ___ Stalled/no turnout
11. Size of turnout area: ______________
12. Hours per day of turnout: ______________
13. Turnout time of day: _______________

14. Number of horses turned out together with this horse: _______

Barn Information/Maintenance:

15. Address of Barn:_____________________________________________________________

16. Barn manager: ________________________________

   Contact information: ________________________________

17. Number of horses on property: ____________

18. Frequency of manure removal from stall: ______________

   Frequency of manure removal from pasture/dry lot: ______________

19. Pasture mowing frequency: ______________

20. Pasture harrowing time/frequency: ______________

21: Rotational grazing: ___Yes ___No

22. Type of deworming schedule (biannual, based off of fecal egg counts, rotational, etc.):

   ______________________________________________________________________

23. Date of last deworming (mm/dd/yyyy): ____/_____/____

24. Brand of dewormer last used: ______________

25. Parasite control practices for new arrivals at barn:

   ______________________________________________________________________

26. Do you wish your name/the name of your horse/the name of your facility/the facility your
horse is kept to remain anonymous and confidential? ___Yes ___No

For researchers' use only:

Body Condition Score: ____________ Date: ____________
Appendix B - The National Research Council (NRC) Definitions of Workload

A Horse in Maintenance/Rest/Spelling: A horse that is not being lunged, worked or ridden

A Horse in Light Work/Young Horse:
- 1-3 hours of work per week
- 40% walk, 50% trot, 10% canter
- Examples of horses in light work: horses used for recreational (trail or pleasure) riding, horses beginning to be trained or broken, or show horses given only occasional work

A Horse in Moderate Work:
- 3-5 hours of work per week
- 30% walk, 55% trot, 10% canter, 5% low jumping, cutting, reining or other skill work
- Examples of horses in moderate work: horses used for recreational (trail) riding, beginning training/breaking, show horses, dressage, campdraft, polo or polocrosse, stock work, cutting horses, showjumpers and low level eventers

A Horse in Heavy Work:
- 4-5 hours of work per week
- 20% walk, 50% trot, 15% canter, 15% gallop, jumping or other skill work
- Examples of horses in heavy work: stock horses, polo, higher level dressage & show jumping, medium level eventing, race training

A Horse in Very Heavy Work:
- 1 hour per week of speed work or 6-12 hours per week of slow work
- Examples of horses in very heavy work: racehorses, elite 3-day eventers or endurance horses
References


