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Configuration of Feed, Shelter, and Water Affects Equine Grazing Distribution and Behaviors

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Abstract

Background: Required maintenance elements such as feed, shelter, and water are not evenly distributed within pasture environments, leading horses to focus their activities around concentrated resources and creating the potential risk of overgrazing. **Aims:** To determine if 1) varying positions of required elements feed (F), shelter (S), and water (W) affected horse presence within 23 m (P23) of required elements and 2) placement of required elements had an effect on the grazing distribution and behavior of horses. **Materials and Methods:** In a completely randomized block design, six mature mares were assigned to graze three-element configurations (CONF). Individual pairs grazed one of six pasture plots for 4-7-d periods. Horse location was monitored by global positioning systems and behaviors were visually assessed and recorded daily. Linear mixed models were developed that related occurrence of behaviors or horse presence within 23m of CONF and element. An ANOVA was used to determine if the fixed effects were significant, followed by Fisher's protected LSD to compare means. **Results:** There was an effect of element on P23 ($P < 0.01$), with F being the most influential ($P < 0.05$) in that horses spent the most time within P23 for F in comparison to S and W. Horses spent more time grazing ($P < 0.05$) than other observed behaviors, regardless of CONF, followed by standing/resting, free movement, and eating grain. **Conclusion:** Moving feeding location frequently may alter grazing location, thus distributing animal concentration accordingly and decreasing the risk of overgrazing. Future studies investigating moving feed only may illuminate new methods of pasture management.

Keywords

Forage; pasture management; maintenance elements; GPS; scan-sampling

1. Introduction

Horses meet the majority of their caloric requirements through forage, traditionally offered in forms of harvested hay or fresh forage. Allowing horses access to fresh forage or pasture provides not only a source of nutrients but numerous behavioral and health benefits as well. Such benefits include reduced risk of colic, gastric ulcers, cribbing, and growth-related issues in young horses [1]. Equine grazing behavior is complex and influenced by several variables including plant composition, forage availability, social interactions, weather, and other environmental variables such as access to shade [2]. Due to the grazing of preferred plants, horses tend to damage plant integrity and create environmental concerns such as soil compaction and water pollution from run-off [3]. Pasture management techniques established for other livestock animals are still utilized for horses even though their grazing behavior is significantly different, justifying the need for establishing improved management techniques catered towards horses [1,3].

Within livestock pasture environments, maintenance elements such as water, supplemental food, shelter, and resting areas are not evenly distributed, causing the risk of overgrazing by focusing their activities around required resources [4]. Prior studies have evaluated configurations of required maintenance elements to manipulate the distribution of cattle, but to the authors' knowledge, this concept has not yet been investigated in horses. Ganskopp [4] found that altering the position of water shifted cattle activity location, as cattle remained near the water, while Bailey and Welling [5] concluded cattle can be lured with a dehydrated molasses supplement to improve uniformity of grazing underutilized rangeland. The mentioned literature investigating the impact required element placement has on grazing behavior and location of cattle may also have relevance in equine grazing management. Depending on environmental conditions, among other factors, horses require 20 to 76 liters of water daily in which drinking frequency can occur several times, increasing foot traffic around the water source [6]. Use of shelter is also dependent on environmental conditions. Literature has shown horses seek shelter during more extreme weather such as rainy, windy, hot and/or sunny days, with need varying by region [7]. Supplemental feed may also be necessary for horses depending on stage of life as well as pasture health and yield; concentrate is typically provided at a minimum of two meals daily. Horses are therefore prone to spend ample amount of time in the above areas, negatively impacting soil and forage condition. Thus, the movement of required elements may provide equine managers with an efficient technique to minimize the concentration of grazing in certain pasture areas and thus lessen potential detrimental impacts of overgrazing in these areas. The objectives of this study were to determine if 1) varying positions of required elements including feed, shelter, and water affect horse presence near required elements and 2) placement of required elements had an effect on the grazing behavior of horses. It was hypothesized that both grazing location and behaviors within a pasture would be affected by altering position of feed, shelter, and water.

2. Materials and Methods

This research was approved by the Institutional Animal Care and Use Committee of Clemson University (IACUC Protocol #: 2020-037).

2.1. Animals and Environment

This research study was conducted at the Clemson University Equine Center in Pendleton, SC. All horses were university owned and included five mature American Quarter Horses and one Warmblood mare (12.7 ± 2.9 yr, 500 ± 12.4 kg). Horses underwent grazing at a stocking rate of 0.47 horses per ha [3]. Horses grazed six pasture plots approximately 0.95 ha in size that were mowed to a sward height of approximately 20 cm prior to grazing **(Figure 1)**. The pasture stand had not been renovated in over ten years prior to the current trial with no fertilization or seeding, and thus forage composition reflects that of past establishment. The soil in all pasture plots consisted of Cecil sandy loam with approximately 80% at a slope of 2-6% and the remaining at 6-10%. Horses had the majority of a free line of sight to horses grazing in other pasture plots with less than 10% of a single plot not visible by the remaining plots. Climate measurements were also acquired from the National Weather Service throughout the course of the trial, with an average temperature of 17.2°C, range of -0.56°C – 28.9°C, and average precipitation of 3.3 ± 1.27 mm per day.

2.2. Experimental Design

Horses were paired and assigned to graze three element configurations (CONF) of feed (F), shelter (S), and water (W) within two pastures divided into three plots each. This resulted in six adjoining pasture plots (0.95 ha each) grazed in a completely randomized block design. The six pasture plots utilized in this study were randomly assigned to one of the CONF such that each CONF was replicated in two plots [**Figure 1**; randomization applied via (RAND=), Microsoft® Excel Version 16.67]. Plots were defined using electric 38 mm polytape (Pasture Management Systems®, Inc., Mt. Pleasant, NC). A pair of horses was randomly assigned to one of the CONF and grazed within that pasture plot for 7 d [pasture location for each pair was randomly assigned via (RAND=), Microsoft® Excel Version 16.67]. Three pasture plots were grazed simultaneously, each by a different pair of horses within one 7-d period. To ensure pasture forage availability and CONF replication, the trial consisted of four 7-d periods, subsequently referred to as Periods 1-4, and four preceding 72-hr washout phases, totaling 40 d. For instance, CONF1-B, CONF2-A, and CONF3-B were grazed in Periods 1 and 3 each by a different pair of horses; the other three CONF were rested in those periods. In Periods 2 and 4, CONF1-A, CONF2-B, and CONF3-A were grazed each by a different pair of horses while the other three CONF were rested. All pairs of horses grazed four CONF and no pair grazed the same CONF twice. Each period was followed by a 72-hr washout in which horses were placed in individual outdoor stalls with no pasture access. During washout periods, horses were fed *ad libitum* long-stem forage along with a concentrate hay balancer fed to manufacturer's recommendation twice daily at 0715 and 1615 (0.23 kg of Nutrena® Empower® Topline Balancer, Cargill Incorporated©, Minneapolis, MN). While in the pasture plots, horses were also fed concentrate hay balancer (0.23 kg) twice daily at 0715 and 1615. Shelters were portable man-made structures with canvas tops, in which horses had a one-week adjustment period to pre-trial. Water was provided *ad libitum* in portable 100-gallon stock tanks.

2.3. Pasture Sampling and Analysis

Prior to the start of each Period, forage composition and quality were determined through collection of ten samples from each pasture plot. Pasture composition was visually assessed using the double DAFOR scale in which the relative abundance of forage and weed species within a 0.5-m^2 quadrat were measured [8,9]. Forage species that covered >75% of the area assessed were assigned "dominant" (D); "abundant" (A) to species that covered 50-75%; "frequent" (F) to species that covered <50% but were well distributed in the area; "occasional" (O) species were those found a few times; and "rare" (R) are species that only occurred one or two times in the given area. Post-composition analysis, forage within the 0.5-m^2 quadrat were collected via hand-clippings to ground level and subsequently dried at 55°C for 48 hr in a forced-air oven [10]. Dry samples were ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Ground samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) content. Neutral detergent fiber and ADF concentrations were determined using an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NW) and corrected for ash concentration. Sodium sulfite and α-amylase (Sigma no. A3306: Sigma Chemical CO., St. Louis, MO) according to Van Soest *et al.* [11] were included for NDF analysis.

2.4. Behavior Sampling

During the grazing periods, horses were fitted with a Global Positioning System (GPS) unit (Trak-4 GPS Tracker, Pryor, OK) mounted onto individual identification collars [1]. Horses carried collars for a one-week adjustment period prior to the study [4]. GPS units remained mounted on the upper neck of horses for all 7-d grazing periods, logging location measurements every 10 min, thus producing an expected 4128 recorded positions per horse. The GPS response variable included frequency of horses present within 23 m (P23) in relation to elements. The 23-m distance was utilized due to being the halfway point between elements [4].

Horses were live observed for three, 2-hr timepoints (0700-0900; 1200-1400; 1700-1900) per day throughout all 7 d of each period. Horses were conditioned to their designated pasture plot 12 h before the first observation of each Period began. Activity was recorded using the scan sampling method [12], where a 5-s scan of the horses was made every 5 min and the activity of each individual was recorded. Two individuals from the same set of observers throughout the trial were randomly assigned to each three, 2-h timepoint to both observe and concur all horse behavior within all pasture plots. Horse behavior was classified as either grazing (actively consuming pasture forage) or non-grazing activity, otherwise recorded as free movement, drinking, standing/resting, social interaction, biting at flies/insects, lying down/rolling, eating grain, or licking salt block.

2.5. Statistical Analyses

A linear model was developed that related forage composition to the fixed effects of plots and period; and interactions. Another linear model was developed that related forage quality to fixed effects pasture plots and period; and interactions. A linear mixed model that related horse presence within 23 m to the fixed effect of element; the random effect of configuration, horse, day, and period; and interactions. A final linear mixed model was developed that related the frequency of behaviors to the fixed effect of activity; the random effects of period, configuration, time, day, and horse; and interactions.

An analysis of variance (ANOVA) was used to determine if the fixed effects were significant. If the fixed effects were found to be significant, then Fisher's Protected Least Significant Difference was used to compare the means. All statistical analyses were completed using JMP version 15 (2019 SAS Institute Inc.). Data are presented as least square mean (LSM) ± standard error mean (SEM) and P-values less than 0.05 were considered evidence of statistical significance. Examination of residuals plots combined with tests (Shapiro-Wilk and Levene) were used to assess ANOVA assumptions concerning normality and stable variance. ANOVA independence assumptions were addressed by including all possible factors (that could possibly lead to clustering and correlation of observations) in the linear mixed models.

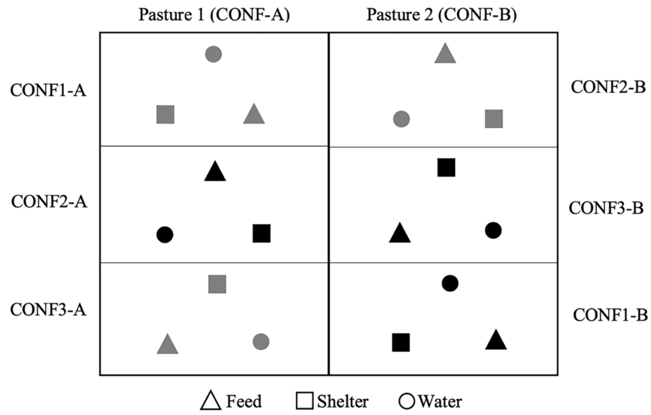

Figure 1: Schematic diagram of pastures 1 and 2, denoted by CONF-A or -B, were divided into three plots (0.95 ha each) to make six adjoining pasture plots. Three element CONF were grazed by two horses each simultaneously within each Period (a total of 6 horses grazed per Period). CONF1-B, CONF2-A, and CONF3-B were grazed in Periods 1 and 3 (black) while the remaining CONF were grazed in Periods 2 and 4 (grey). Each Period lasted for 7 grazing days, in the months of October and November.

3. Results

3.1. Pasture

When evaluating composition, a total of five plant species were found within each of the six pasture plots, including Bermudagrass (*Cynodan dactylon*), broad leaf weed, Crabgrass (*Digitaria sanguinalis*), Tall Fescue (*Schedonorus pheonix*), White Clover (*Trifolium repens*), and dead material or bare ground categorized as 'Other' (**Figure 2**; **Figure 3**). All species were found during each period and pasture plot with the exception of Crabgrass in Period 2 CONF2-B and CONF3-A and Tall Fescue in Period 2 CONF1-A. Some differences in species abundance were seen between periods and CONF within pasture plots. An increase in both Bermudagrass ($P = 0.04$) and Tall Fescue ($P = 0.03$) abundance was observed from Period 1 to 3 in CONF1-B. White Clover also increased between Period 1 to 3 in CONF2-A ($P = 0.004$), whereas the amount of broad leaf weed ($P = 0.004$), Crabgrass ($P = 0.04$), and 'Other' ($P = 0.003$), decreased. No forage composition differences were observed in CONF3-B between Periods 1 and 3. Within Periods 2 and 4, Tall Fescue occurrence in CONF1-A increased, but decreased in CONF3-A. Also, in CONF3-A, there was an increase in White Clover between Period 2 and 4. A difference in 'Other' also occurred in CONF2-B, decreasing from Period 2 to 4.

Mean NDF and ADF values varied among forages across periods within pasture plots (**Table 1**). Between Periods 1 and 3, ADF values increased from plot to plot, i.e., 41.3% to 45.2% ($P = 0.001$) in CONF1-B, 41.4% to 46.5% ($P < 0.0001$) in CONF3-B and 36.4% to 42.9% ($P = 0.002$) in CONF2-A (Period 1 to 3, respectively; SEM of 0.63). Neutral detergent fiber remained mostly consistent with only a single decrease in CONF3-A from Period 2 to 4 (89.6% to 67.6%; SEM of 6.9; $P = 0.04$).

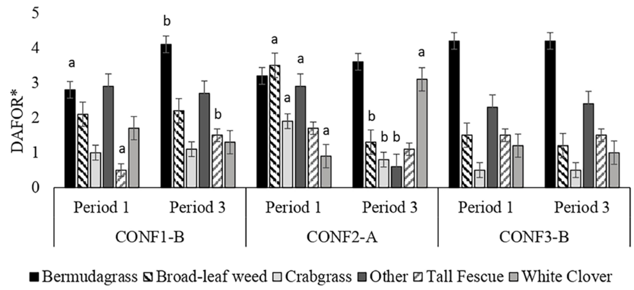

Figure 2: Comparison within element CONF (CONF1-B; CONF2-A; CONF3-B) across Periods (1 and 3); showing differences in forage composition, via the double DAFOR scale; D=5, A= 4, F=3, O=2, and R=1. Data are presented as LSM with SEM error bars.

*Standard error of all LSM were 0.56.

[ab]Identical forage species across Periods 1 and 3 within one replicate of each of the three plot CONF not connected by the same letter are significantly different ($P < 0.05$). An ANOVA was used to determine if the fixed effects were significant; LSD used to compare the means.

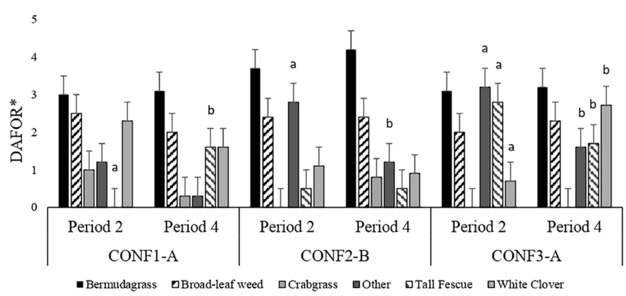

Figure 3: Comparison within element CONF (CONF1-A; CONF2-B; CONF3-A) across Periods (2 and 4); showing differences in forage composition, via the double DAFOR scale; D=5, A= 4, F=3, O=2, and R=1. Data are presented as LSM with SEM error bars. Data are presented as LSM with SEM error bars.

*Standard error of all LSM were 0.56.

[ab]Identical forage species across Periods 2 and 4 within one replicate of each of the three plot CONF not connected by the same letter are significantly different ($P < 0.05$). An ANOVA was used to determine if the fixed effects were significant; LSD used to compare the means.

3.2. Horse Location via GPS

Over the 28 days treatments were in effect, each GPS unit was expected to record 144 positions daily and 4,032 total. The Trak-4 GPS units contained hardware and logical processing to calculate position based on GPS satellite signals, tracking location by user-selected time interval and movement of unit, potentially producing more or less than the expected number of positions. Four of the six units either reached or exceeded the expected number of positions, delivering an average of 4,128 ± 40.9. The remaining two units generated 97.7 and 98.1%, respectively, of the expected positions. Thus, results were calculated based on the daily expected number of positions.

All six horses were located within 23m of all three elements totaling 22.7 to 29.6% of the overall GPS positions recorded. Element did have an effect on horse presence within 23m, with concentrate feeding area being the most frequented ($P = 0.0002$) followed by water, then shelter (14.7%, 10.4%, and 9.4%, respectively; SEM of 0.37). Element CONF and Period also had an effect ($P < 0.0001$; **Table 2**), in which CONF2-B contained the highest recording of locations of horses within 23m (32.6 ± 1.6% in Period 2) of the concentrate feeding area, followed by CONF 3-A (21.6 ± 1.6% in Period 2; 20.6 ± 1.6% in Period 4).

Table 1: Nutritive values of forage available by Period within pasture plots of repeated CONF, in which Periods 1 and 3 had identical CONF as did Periods 2 and 4. Data are presented as LSM.

	Plot	NDF (%)*	ADF (%)*
Period 1	CONF2-A	77.0[a]	39.4[a]
	CONF1-B	73.4[a]	41.3[a]
	CONF3-B	69.4[a]	41.4[a]
Period 3	CONF2-A	67.6[a]	42.9[b]
	CONF1-B	69.5[a]	45.2[b]
	CONF3-B	53.1[a]	46.5[b]
Period 2	CONF1-A	75.3[a]	47.9[a]
	CONF3-A	89.6[a]	48.4[a]
	CONF2-B	74.1[a]	53.3[a]
Period 4	CONF1-A	67.4[a]	48.6[a]
	CONF3-A	67.6[b]	45.6[b]
	CONF2-B	70.1[a]	54.2[a]

*Standard error of all LSM were 6.9 and 0.63, respectively.

[ab]Values with differing letters within rows of repeated CONF are significantly different ($P < 0.05$). An ANOVA was used to determine if the fixed effects were significant; LSD used to compare the means. Data are presented as LSM with SEM error bars.

Table 2: Percent of time horses spent 23m from each element on a daily basis within each of the six CONF. Data are presented as LSM.

			Day							
		Element	1	2	3	4	5	6	7	Avg.(%)*
Period 1	CONF2-A	Feed	5.9	10.8	6.9	6.6	6.3	8.7	4.9	7.1[a]
		Shelter	4.2	3.5	6.6	0.7	5.6	4.2	10.1	5.0[a]
		Water	13.2	10.4	3.8	5.6	4.5	6.9	6.6	7.3[a]
	CONF1-B	Feed	25.7	16.0	14.6	11.1	15.3	12.8	10.8	15.2[a]
		Shelter	4.5	5.9	7.3	5.2	4.5	5.2	1.0	4.8[b]
		Water	15.3	7.6	6.9	1.0	15.6	16.3	13.9	11.0[a]
	CONF3-B	Feed	3.5	9.7	6.6	2.4	3.1	2.8	1.7	4.3[b]
		Shelter	3.8	7.3	18.8	1.7	19.4	11.5	11.8	10.6[a]
		Water	7.6	3.8	9.0	5.6	11.1	13.5	9.4	8.6[a]
Period 2	CONF1-A	Feed	9.0	18.4	13.5	10.4	14.2	11.8	18.4	21.6[a]
		Shelter	15.3	10.8	13.9	16.7	13.5	15.6	22.9	7.4[b]
		Water	39.2	28.8	25.0	21.2	19.1	22.9	27.1	3.9[b]
	CONF3-A	Feed	28.8	26.0	16.0	20.3	25.3	19.8	14.9	32.6[a]
		Shelter	17.7	3.5	9.0	6.9	3.8	4.9	6.3	6.9[b]
		Water	0.3	1.0	7.3	3.5	3.1	6.3	5.9	4.6[b]
	CONF2-B	Feed	45.8	40.3	27.8	32.6	40.3	19.1	22.2	13.7[b]
		Shelter	8.3	5.9	6.3	6.9	7.6	5.9	7.3	15.5[b]
		Water	3.5	1.0	6.9	5.6	4.9	3.8	6.3	26.2[a]
Period 3	CONF2-A	Feed	9.4	10.1	7.6	2.4	11.8	21.2	2.8	9.3[b]
		Shelter	16.3	21.9	11.5	14.2	16.0	16.3	17.7	16.3[a]
		Water	4.9	2.4	5.2	2.8	1.0	3.1	1.7	3.0[c]
	CONF1-B	Feed	13.5	8.3	13.2	5.6	12.5	8.3	1.4	9.0[b]
		Shelter	13.9	8.0	3.8	3.8	11.1	3.5	7.3	7.3[b]
		Water	27.4	20.8	16.0	22.9	16.7	13.9	21.5	19.9[a]
	CONF3-B	Feed	15.6	6.6	8.0	11.1	13.5	8.0	10.1	10.4[a]
		Shelter	7.6	9.4	2.4	3.8	7.3	5.6	5.2	5.9[b]
		Water	6.9	7.6	7.6	4.5	5.2	5.6	4.9	6.1[ab]
Period 4	CONF1-A	Feed	18.1	21.2	26.4	14.9	17.4	16.7	11.8	18.1[a]
		Shelter	18.4	15.6	14.2	14.6	14.0	12.4	10.4	14.2[b]
		Water	21.2	13.9	11.5	18.8	14.6	12.5	13.2	15.1[ab]
	CONF3-A	Feed	17.0	9.0	21.2	21.5	21.5	22.2	31.6	20.6[a]
		Shelter	16.3	14.6	8.3	9.7	12.8	4.9	3.1	10.0[b]
		Water	9.7	8.7	3.1	7.6	5.9	6.6	3.5	6.4[b]
	CONF2-B	Feed	17.4	13.5	9.4	10.1	15.3	14.9	22.6	14.7[a]
		Shelter	11.8	9.7	4.9	6.9	8.3	13.2	10.4	9.3[b]
		Water	11.1	21.5	14.9	16.7	9.4	9.4	8.0	13.0[ab]

*Standard error of all LSM was 1.6.

[abc]Average values within CONF of respective Period not connected by the same letter are statistically different ($P < 0.05$). An ANOVA was used to determine if the fixed effects were significant; LSD used to compare the means. Data are presented as LSM with SEM error bars.

3.3. Grazing Behavior

Observers monitored the behavior of the six mares for a total of 168 h, with grazing activity averaging 76.9% daily, thus, grazing activity was, therefore, the most observed behavior. Behavior did vary within pasture plots and Period, in which CONF3-A yielded the most grazing (84.7%; $P = 0.01$) followed by CONF2-B (82.1%), both in Period 4 (**Figure 4**). Grazing frequency increased from Period 1 to 3 in CONF1-B ($P < 0.0001$; 71.3% to 77.6%) and CONF3-B ($P < 0.0001$; 71.7% to 78.8%). A similar increase in time spent grazing was observed from Period 2 to 4 in CONF3-A ($P < 0.0001$; 70.9% to 84.7%) and CONF2-B ($P < 0.0001$; 72.7% to 82.1%). No difference in grazing activity was observed between the three observation times (MOR; NOON; EVE).

The second most occurring behavior was standing/resting followed by free movement and eating grain (11.4%, 5.1%, and 3.1%, respectively; SEM of 1.4; **Figure 5**). The remaining non-grazing activities (drinking, social interaction, biting at flies/insects, lying down/rolling, and licking salt block) occurred less than 0.7% of the time observed. There were no differences in these behaviors across the three observation times, as well as no correlation of behaviors within plots of identical configuration.

4. Discussion

4.1. Pasture

A total of five forage species were found in the pastures and identified in the majority of plots within each period. Bermudagrass served as the most dominant forage throughout the course of the study with slight increases in the presence of cool-season forages such as Tall Fescue and White Clover. Minor changes in forage quality were also noted with more differences observed in ADF content from Period 1 to 3. Composition and quality changes could have been due to environmental conditions as the study was conducted between late summer and early fall [3]. The forage changes may impact grazing or non-grazing activities and thus influence the time spent around the maintenance elements [1,3]. However, to the authors' knowledge, the effect forage composition and quality have on equine location and behavior around feed, shelter, and water is minimal and should be further investigated.

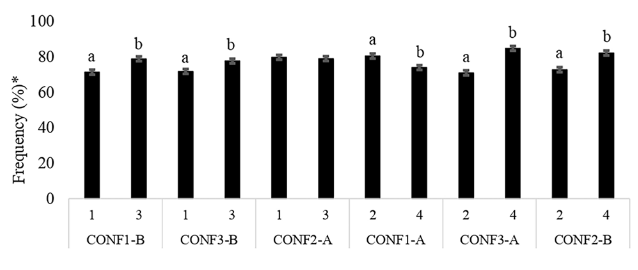

Figure 4: Grazing behavior of horses by Period (1-4) within pasture plots of repeated CONF, in which Periods 1 and 3 had identical CONF as did Periods 2 and 4. Data are presented as LSM with SEM error bars.

*Standard error of all LSM was 1.4.

abAverage values within repeated CONF of respective Periods not connected by the same letter are statistically different ($P < 0.05$). An ANOVA was used to determine if the fixed effects were significant; LSD used to compare the means.

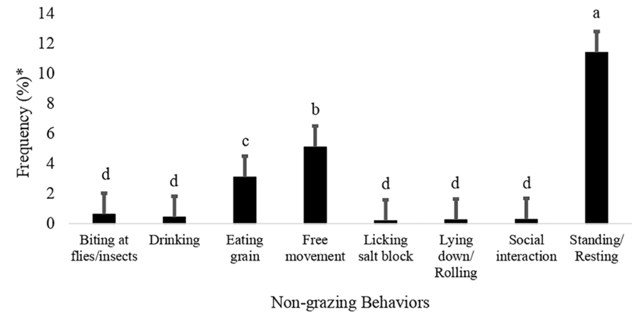

Figure 5: Frequency of horses performing non-grazing behaviors across observation times. Data are presented as LSM with SEM error bars.

*Standard error of all LSM was 1.4.

abcdBehaviors not connected by the same letter are significantly different ($P < 0.05$). An ANOVA was used to determine if the fixed effects were significant; LSD used to compare the means.

4.2. Horse Location via GPS

As hypothesized equine location within a pasture was affected by altering position of the maintenance elements feed, shelter, and water. Location in respect to the elements varied within each Period as well as CONF in which horses spent the most time within proximity of the feed element. Configuration 3-A contained the second and third highest counts of horses within 23m of the concentrate feeding area. Due to the lack of literature regarding the effect required elements have on the grazing distribution of horses, appropriate comparisons to the current trial were made using previous findings in cattle. A study evaluating the grazing distribution of cattle with dehydrated molasses supplement blocks observed a greater forage utilization of cattle across pastures with the dietary supplement than those without [5]. Forage utilization and stubble height measurements showed cattle grazed more heavily within 20 to 200 m from the dietary supplement than in corresponding control areas [5]. McDougald *et al.* [13] investigated the use of a dietary supplement, to manipulate cattle grazing location into less productive pasture areas. They determined, by moving supplemental feeding location away from water sources and into underutilized areas, the impact of cattle on residual dry matter in riparian pasture areas was greatly reduced from 48 to 1% over a three-year period. The current study did not determine use of feeding location in less desirable pasture areas or impact on plant or soil health, yet movement of supplemental feeding into such areas provides opportunity for future research.

Ares [14] found similar results by distributing the grazing efforts of cattle through the placement of a meal-salt ration and compared this in relation to positioning of water. This study found an 84% increase in use of pasture when the meal-salt was located away from the water as opposed to next to it and determined this positioning of the feed supplement resulted in the most efficient grazing of range forage. This preference for spending time near concentrate feeding area was also observed in the current study; however, placement of feed near water was not investigated as each element was a consistent 56 m apart. The lesser influence of water in comparison to feed on P23 in the current study, however, did conflict with Ganskopp [4] who found the movement of water to be the most effective tool for altering cattle distribution where a dietary supplement, salt, had less of an impact. It should be noted that pastures evaluated in this cattle study were much larger than those in the current study, 800 ha versus < 1 ha, respectively. The location of elements in the much larger area may have adverse effects on grazing distribution than when confined to much smaller areas. Additionally, differences between the previous studies and current could be due to the preferences of the dietary supplement types by cattle compared to horses as well as the time of year, and lack of shelter.

The use of the man-made shelters was minimal in the current study, yet horses were not timid of the structures and were occasionally visually observed grazing under and around them. Heleski and Murtazashvili **[15]** discussed that type of artificial shelter in addition to its isolation, ventilation, and orientation could affect the horses' decision to use. Snoeks *et al.* **[7]** found domestic horses used shelter approximately half of the observed time, with increased values seen in study determined cold and hot temperatures. A potential reason for the conflicting use of shelters with the current study could be due to the average temperature not exceeding the horses' thermal neutral zone of 25°C **[16]**. Holcomb *et al.* **[17]** determined that individually housed horses preferred foraging in shaded areas. That study was conducted on dry-lots in which forage was provided under open-sided shade structures, indicating there was likely limited forage, which was not the case in the current study. Despite the lack of shelter use observed in the current study, providing shade is still warranted to ensure best management practices, especially in extreme weather conditions as can be observed in the Southeast, United States.

4.3. Grazing Behavior

It should be noted that horses did tend to visually remain in eyesight of pairs within other pasture plots. However, no matter the configuration that elements were placed within pasture plots, horses spent more time grazing in comparison to other activities. Grazing was expected to be the most frequently occurring behavior, as horses graze between 14 to 17 hours a day **[18–20]**. Snoeks *et al.* **[7]** determined grazing to be the most observed behavior, with 'standing' closely following as in the current study. In natural conditions, Preswalski's horses grazed, rested, and moved more than 90% of the time observed, as also comparable to the current study where horses completed the same behaviors in a pasture environment for just over 90% of their daily allowance **[21]**. Furthermore, as the study progressed, horses were observed to spend an increased amount of time grazing in the majority of configurations. The increase in grazing frequency over time may have been attributed to improved comfortability with the movement of elements as the trial continued and different configurations were presented. Thus, in the current study, grazing behaviors of horses did not change by introducing an altered pasture management technique. While it was hypothesized behavior would be affected by altering element location, the frequency of grazing observed indicated the movement of elements may support horses' natural grazing behaviors within a pasture environment.

4.4. Limitations

While the current study provides horse owners with valuable insight on pasture management practices, the authors recognize the trial contained limitations. For instance, the study was completed in a specific time of year and geographical location, potentially limiting the forage composition and quality of pasture the horses had access to. Repeating the trial within different seasons and locations is encouraged to further validate the effects of altering element location within an equine pasture. In addition, the experimental design focused on the replication of elements configurations which resulted in every pair of horse to graze each configuration only once, in four of the six pasture plots. An improved timeline may allow for configurations and horse access to configurations to be tested in duplicate to strengthen horse response to the movement of feed, shelter, and water.

5. Conclusion

The aims of the current trial were to determine if altering positions of feed, shelter, and water affected horse location, within a pasture, in relation to these elements in addition to grazing behavior of horses. Results indicated that moving feeding location frequently may alter equine grazing location, as horses were found nearest the feed rather than shelter and water. In addition, natural grazing behaviors were not diminished with the manipulation of required element position as horses continued to graze more often than other behaviors. Therefore, the implementation of moving feeding location could be meaningful husbandry technique to distribute animal concentration accordingly and decrease the risk of overgrazing desired areas. Altering feeding location frequently may also provide equine owners with an alternative or serve as a complement to previously existing pasture management techniques. Further research is warranted to determine the effects that required elements have in varying seasons and forage availability.

Authors' Contributions

All authors contributed to the conception and writing of the paper. Perron BS had the primary responsibility for the development of the methodology and manuscript preparation with intellectual input from all other authors at all stages of the research process. The experiment was performed by Perron BS. Data was analyzed by Bridges WC and Perron BS.

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Data Availability Statement

The data supporting the findings of this study are available within the article.

Funding

The research has not received any funding.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

This research was approved by the Institutional Animal Care and Use Committee of Clemson University (IACUC Protocol #: 2020-037).

References

[1] Martinson KL, Siciliano PD, Sheaffer CC, McIntosh BJ, Swinker AM, Williams CA. A review of equine grazing research methodologies. Journal of Equine Veterinary Science 2017;51:92–104. https://doi.org/10.1016/j.jevs.2017.01.002.

[2] Davies NB, Krebs JR. An introduction to behavioural ecology. 3rd ed. Oxford: Blackwell Science; 1993.

[3] Bott RC, Greene EA, Koch K, Martinson KL, Siciliano PD, Williams C, *et al*. Production and environmental implications of equine grazing. Journal of Equine Veterinary Science 2013;33:1031–43. https://doi.org/10.1016/j.jevs.2013.05.004.

[4] Ganskopp D. Manipulating cattle distribution with salt and water in large arid-land pastures: a GPS/GIS assessment. Appl Anim Behav Sci 2001;73:251–62. https://doi.org/10.1016/s0168-1591(01)00148-4.

[5] Bailey DW, Welling GR. Modification of cattle grazing distribution with dehydrated molasses supplement. Journal of Range Management 1999;52:575–82. https://doi.org/10.2307/4003626.

[6] Crowell-Davis SL, Houpt KA, Carnevale J. Feeding and drinking behavior of mares and foals with free access to pasture and water. J Anim Sci 1985;60:883–9. https://doi.org/10.2527/jas1985.604883x.

[7] Snoeks MG, Moons CPH, Ödberg FO, Aviron M, Geers R. Behavior of horses on pasture in relation to weather and shelter—A field study in a temperate climate. Journal of Veterinary Behavior 2015;10:561–8. https://doi.org/10.1016/j.jveb.2015.07.037.

[8] Abaye AO, Allen VG, Fontenot JP. The double Dafor scale: a visual technique to describe botanical composition of pastures. Proceedings of the American Forage and Grassland Council Annual Conference 1997:96–100.

[9] Virostek AM, McIntosh B, Daniel A, Webb M, Plunk JD. The effects of rotational grazing on forage biomass yield and botanical composition of horse pastures. Journal of Equine Veterinary Science 2015;35:386. https://doi.org/10.1016/j.jevs.2015.03.015.

[10] Weinert JR, Williams CA. Recovery of pasture forage production following winter rest in continuous and rotational horse grazing systems. Journal of Equine Veterinary Science 2018;70:32–7. https://doi.org/10.1016/j.jevs.2018.06.017.

[11] Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

[12] Altmann J. Observational study of behavior: sampling methods. Behaviour 1974;49:227–67. https://doi.org/10.1163/156853974x00534.

[13] McDougald NK, Frost WE, Jones DE. Use of supplemental feeding locations to manage cattle use on riparian areas of hardwood rangelands. In: Abell, Dana L, Technical Coordinator 1989 Proceedings of the California Riparian Systems Conference: Protection, Management, and Restoration for the 1990s; 1988 September 22-24; Davis, CA Gen Tech Rep PSW-GTR-110 Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, US Department of Agriculture; p 124-126 1989;110.

[14] Ares FN. Better cattle distribution through the use of meal-salt mix. Journal of Range Management 1953;6:341–6.

[15] Heleski CR, Murtazashvili I. Daytime shelter-seeking behavior in domestic horses. Journal of Veterinary Behavior 2010;5:276–82. https://doi.org/10.1016/j.jveb.2010.01.003.

[16] Morgan K. Thermoneutral zone and critical temperatures of horses. Journal of Thermal Biology 1998;23:59–61. https://doi.org/10.1016/S0306-4565(97)00047-8.

[17] Holcomb KE, Tucker CB, Stull CL. Preference of domestic horses for shade in a hot, sunny environment1. Journal of Animal Science 2014;92:1708–17. https://doi.org/10.2527/jas.2013-7386.

[18] Duncan PB. Horses and grasses: the nutritional ecology of equids and their impact on the Camargue. New York: Springer-Verlag; 1992.

[19] Fleurance G, Farruggia A, Lanore L, Dumont B. How does stocking rate influence horse behaviour, performances and pasture biodiversity in mesophile grasslands? Agriculture, Ecosystems & Environment 2016;231:255–63. https://doi.org/10.1016/j.agee.2016.06.044.

[20] Edouard N, Fleurance G, Dumont B, Baumont R, Duncan P. Does sward height affect feeding patch choice and voluntary intake in horses? Appl Anim Behav Sci 2009;119:219–28. https://doi.org/10.1016/j.applanim.2009.03.017.

[21] Van Dierendonck MC, Bandi N, Batdorj D, Dügerlham S, Munkhtsog B. Behavioural observations of reintroduced Takhi or Przewalski horses (Equus ferus przewalskii) in Mongolia. Applied Animal Behaviour Science 1996;50:95–114. https://doi.org/10.1016/0168-1591(96)01089-1.

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Impact of Forage Presentation on the Equine Brachiocephalicus Mechanical Nociceptive Threshold (MNT) and Forelimb Kinematics

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Abstract

Aims: This investigation aimed to specify whether haynet feeding or floor feeding causes different areas of sensitivity/tensions in the *m. brachiocephalicus*. The secondary aim was to elucidate whether specific areas of tension within the *m. brachiocephalicus* would affect the protraction and retraction of the forelimb. **Methodology:** Ten horses (seven geldings; three mares) were used in the study with an age range of 614. Horses were split into two groups of their already established feeding methods (five haynet feeders; five floor feeders). Each horse was assessed for points of sensitivity in the *m. brachiocephalicus* at its origin, insertion, and muscle belly, by the use of a pressure algometer. The horse was then walked past a camera for kinematic analysis. Motion analysis software was used to measure the protraction and retraction of each forelimb. Shapiro-Wilk tests were used to measure normal distribution. Data that was deemed normally distributed was analyzed using Independent T-Tests. Data that was not deemed normally distributed was analyzed using Mann-Whitney U tests. **Results:** The results of the study suggest that the use of haynet feeding has a negative impact on the muscular tensions of the *m. brachiocephalicus*, most significantly at its insertion. Additionally, haynet groups indicated increased levels of tension in both the muscle belly and origin. It was not significant the effect of the points of tension seen throughout the *m. brachiocephalicus* have over the kinematics of the forelimb. It can be concluded that haynet feeding increases *m. brachiocephalicus* sensitivity/tension, which could impact horse welfare and performance.

Keywords

Feeding practice; horse; muscle tension; pressure algometer

1. Background

A UK survey on the practice of feeding hay concluded that many people preferred to feed hay on the floor rather than a haynet [1]. This was further supported by [2], that reported that 57% of Irish racing stables chose the same method as [1]. Alternatively, in a more general survey given out to the UK population on choice of hay-feeding method, 90.5% of the respondents preferred to feed from a haynet [3]. It is postulated by [4], that despite the more natural feeding posture floor feeding allows, the use of a haynet is preferred as it reduces the risk of wastage and contamination with faeces and urine. However, the use of the haynet has its own added disadvantages as it is suggested that it may adversely affect muscles and nerve function [5].

As of yet, only two studies have been able to definitively define the role that the Brachiocephalic (*BC.m*) has to play in forelimb protraction [6,7]. In a systematic review by McAteer, the literature available on the topic of the *m. brachiocephalicus* showed a severe lack of knowledge surrounding the functionality of the muscle and its role in equine biomechanics [8]. The topic is still largely under debate amongst many industry professionals. Any evidence that is readily accessible to the industry is severely lacking in evidence-based findings as many conclusions are ambiguous with anecdotal and circumstantial findings to back up observations and key points. Only a handful of sources have identified the *m. brachiocephalicus* as a muscle that plays a key role in the biomechanics of the forelimb [7,9] and more specifically the protraction phase [6]. A more recent study further suggests that *m. brachiocephalicus* muscle activities can be associated with stride frequency and speed of racing thoroughbreds when fatigued. Therefore, furthering the understanding of the effects deficits could place on the functionality of the muscle [10].

Localized muscle soreness can lead to a varying amount of subtle performance problems such as reluctance to work on the bit/accepting contact and slight gait abnormalities. Furthermore, they are also known to have a shortening effect over the cranial phase of the stride ipsilateral to the afflicted muscle [11]. These observations, however, tend to be based on experience in the industry and would require quantifiable research in order to back up this statement. Scientific research is still severely lacking on this topic. The clinical significance of localized muscle tensions is rather poorly understood and badly documented which can be related to its subjective nature and lack of standardized procedure. Palpation is a keyway in determining the location and severity of musculoskeletal ailments, however, this technique is highly subjective to the assessor [12]. Pressure algometry (PA) allows for the quantifiable results of musculoskeletal tenderness with many human studies providing its reliability and validity [13–16]. An investigation [17] studied the reliability and validity of PA by using Pearson correlations tests to compare force plate readings with maximum PA readings. The authors concluded that application was deemed relatively consistent, signifying that the device could lead to reliable and repeatable results. However, it is difficult to trust the conclusion of the investigation as a lack of presentation of results, increases the risk of author bias. In contrast to this, Varcoe-Cocks *et al.* [12] aimed at investigating mechanical nociceptive thresholds (MNTs) using PA in order to correlate severity of clinical signs and subjective scoring of palpable muscle pain in horses with presumed sacroiliac dysfunctions. The study concluded that in cases of presumed sacroiliac dysfunctions, horses displayed lower MNTs suggesting increased levels of pain supporting the role a PA has in providing non-subjective methods in producing quantifiable results for musculoskeletal pain.

In order to perform effective and efficient movements, it is imperative that the soft tissue is functioning properly. Impairment to the soft tissue structures within the musculoskeletal system will directly affect the quality and efficiency of any movement. Dysfunctional tissue is non-pathological meaning it is free from disease, inflammation, and non-injured and instead refers to pain or tension within the structures [18].

A preliminary investigation on the effects of head and neck position during feeding on the alignment of the cervical vertebrae in horses has shown that when housing practices such as feeding from haynets and haybars are compared to floor feeding notable differences in muscle tensions along the neck were examined. More specifically, when horses fed from a haynet were compared with horses fed from the floor, more unilateral abnormalities were felt across the neck [19]. Another investigation aimed at looking at BC.*m* tenderness found that asymmetrical muscle tenderness in BC.*m* may have an influence over forelimb kinematic, concluding that further research is recommended with a larger population and a defined trot speed to establish the full extent of its influence.

The main aim of this investigation is to specify whether haynet feeding or floor feeding causes different areas of tension in the *m. brachiocephalicus*. Then, the additional aim of this investigation is to specify whether specific areas of tension within the *m. brachiocephalicus* correlate with differences in the protraction and retraction angles of the forelimb from points of tension found in the muscle.

Our hypothesis was that haynet feeding techniques have an effect on the tension in the *m. brachiocephalicus* when compared with floor feeding, which will then impact protraction/retraction of the forelimb.

2. Materials and Methods

The investigation was an observational study as no intervention was included. Participants were measured with their regular feeding technique which they had been using for at least a year. The participants were split into two groups of their already established feeding methods, five haynet feeders (HF) and five floor feeders (FF).

2.1. Ethics Approval

The data has been acquired according to modern ethical standards and has been approved by the Animal Welfare and Ethics Committee of Writtle University College. The approval number is 98360253/2019. A written informed consent was obtained from the owners of the participants of the study. Prior to the investigation, all horses were assessed for signs of lameness. Horses were introduced to any devices used throughout the trial and the environment in which the assessment was being held, to reduce anxiety and prevent injury.

2.2. Animals

Sample size in an investigation is key to the investigation's success. In order to ensure the correct sample size is chosen, a sample size calculation was carried out. This ensured that not too few participants were selected, reducing scientific validity and questioning the reliability of results. Similarly, it additionally ensured that not too many participants were selected bringing about false positive conclusions and breaching ethics. As the study at hand investigated tension within a muscle of a haynet feeder and a floor feeder, two independent groups were deemed necessary. As such the resource equation was used [20] and a number of five horses per group; floor feeders and haynet feeders were defined. To ensure an accurate population representation ten participants (seven geldings; three mares) were included in this study. Eight horses were still in ridden work whereas two had been retired due to the owners' personal situation. five HF participants were in moderate workloads within a riding school where as only three FF participants were in the same level of work. Horses ages ranged between 6-14 years old, to gain a representative sample group of those having reached skeletal maturity. This age range was chosen specifically as it is noted by [21] that growth plates within the vertebrae take 3.5-6 years to close, therefore, to ensure skeletal maturity, each participant had to be a minimum of six years old. It was noted by [22] that in older horses, age can influence musculoskeletal functionality, and as such the maximum age of participants was 14 years old. The participants were split into two groups of their already established feeding methods, which they had been using for at least a year; five haynet feeders (HF) age 9.8 ± 1.9 y/o, and five floor feeders (FF) age 10.6 ± 1.6 y/o. Haynets utilized were of all the same size holes (2 inches) hung 1.5 metres. All horses included are regularly shoed and checked by a veterinary professional on a semestral basis. Each horse was assessed by a qualified Veterinary Physiotherapy practitioner.

2.3. Pressure Algometer

Pressure algometry (PA) allows for the quantifiable results of musculoskeletal tenderness with many human studies providing its reliability and validity **[13–16]**. Mechanical nociceptive threshold (MNT) of the *m. brachiocephalicus* were carried out using a pressure algometer (FDX 100 Algometer, Wagner Instruments) with a blunt 1cm^2 probe and results were noted in Newton (N). MNTs were measured on three defined points of the *m. brachiocephalicus*. These points included the muscle origin and insertion and the muscle belly as similarly used in previous investigations **[19,23]** **(Figure 1)**. Clavicular insertions were not used in this investigation as there was a preference for assessing the muscle belly of the *m. brachiocephalicus* as a whole and not individual muscles as stated in **[24]**.

All algometric measurements were carried out by one investigator (AM), and the pressure threshold was measured. Values were then noted by an observer without the investigator (AM) seeing the values ensuring blinded therefore bias within pressure applied. Horses were restrained using their own head collar and lead rope appropriately fitted and held by an assistant before readings were taken bilaterally down the neck. The head of each horse was held in a neutral position above vertical by the assistant whilst the investigator (AM) took each measurement. The pressure algometer was placed perpendicular to each measuring point to measure the tension on three allocated points **(Figure 2)**. At first, the pressure algometer was held in light contact with the skin for about 3 s, to reduce any reaction due to startling effects. Afterwards the pressure was gradually increased in 2 s–3 s intervals based on the studies **[25,26]**. As a positive reaction indicative of reaching MNT behaviors (muscle twitch, head moving abruptly, nose flare, eye tensing) was noted, the pressure was stopped at the value noted. Each point was measured three times in order to ensure consistency in measurement across horses, thus allowing for a more reliable method and valid results. The measurements began at the origin, then the muscle belly, and finally the insertion, first the right *m. brachiocephalicus* then the left one.

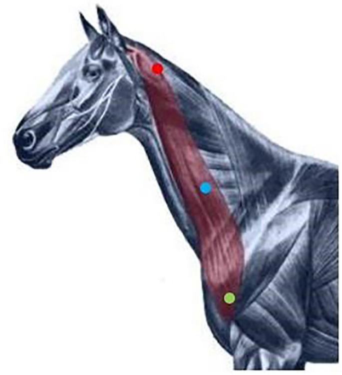

Figure 1: Points of measurement for the pressure algometer on the *m. brachiocephalicus*. Origin: distally to the deltoid tuberosity. Insertion: caudal to the wing of atlas. Muscle Belly: proximal to C5.

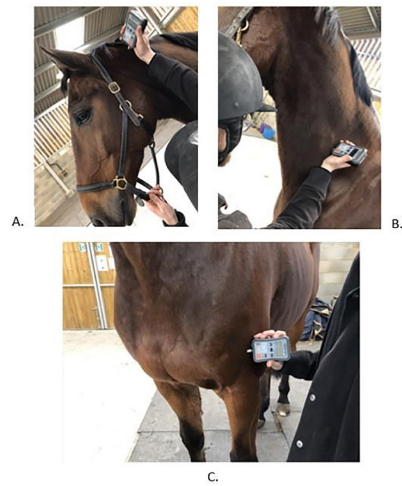

Figure 2: Images were taken throughout the trial, indicating pressure algometer measurement points p. **(A)** insertion of *m. brachiocephalicus*: caudal to the wing of atlas; **(B)** belly of *m. brachiocephalicus*: proximal to C5; **(C)** origin of *m. brachiocephalicus*: distally to the deltoid tuberosity.

2.4. Video Collection

Reflective markers were positioned on the horses' forelimbs to allow precise measurement of protraction and retraction angles. The markers were placed on each horse's scapula spinae tuber and coronary band by the same researcher (AM). Videos for forelimb kinematics analysis were recorded at 240fps (iPhone 8, Apple). The camera was placed on a tripod, 5 m away from the walking area **(Figure 3)**. Horses were videoed at walk six times, three videos recording the left side and three videos recording the right side. All subjects were walked at their comfortable speed and efforts to maintain their pace were made by the handler, so as to ensure full assessment of the influence of the *m. brachiocephalicus* had over the limb. The surface used was a soft rubber matting to ensure no slipping on concrete flooring affecting the results of the angles of protraction and retraction or injuring the individual.

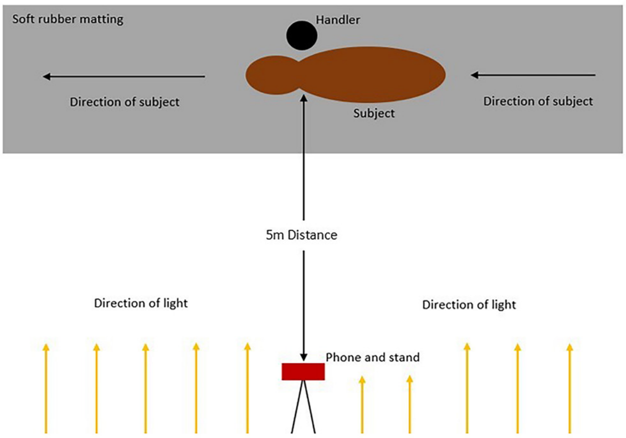

Figure 3: Layout of the videoing for kinematic analysis.

2.5. Kinematics Analysis

A motion analysis software (Quintic Biomechanics v31, Quintic Consultancy, Birmingham, UK) was used to analyze the protraction and retraction of each horse from the videos recorded. Budras *et al.* **[24]** evidenced the role of the *m. brachiocephalicus* plays in protraction of the forelimb, as such, any restriction in the muscle may have an overall effect on the limb's ability to efficiently complete a cycle.

Protraction and retraction angles were measured against the vertical line **(Figure 4)**. Limb protraction and retraction angles were defined by the angle formed by the limb's axis relative to the vertical during the stride. The limb's axis was defined for the entire limb from the segment formed by the two markers placed, spine of scapula and coronary band.

2.6. Statistical Analysis

Intra-operator reliability was assessed using intra-class correlation coefficient (ICC). ICC was determined for the assessor on all anatomic landmarks and all horses The data obtained from each point (three algometer readings) and each protraction and retraction angle (three videos) were averaged. The data was analyzed statistically with SPSS (v.26, IBM *SPSS* Statistics for Windows. Armonk, NY). Continuous variables with normal distribution were presented as mean±standard deviation; non-normal variables were reported as median (interquartile range [IQR]). Shapiro-Wilk test was used to assess data for normality. Data that was deemed to be of normal distribution by the analysis of the Shapiro-Wilk normality test was analyzed using an independent t-test to compare the two independent groups. On other hand, non-parametric data was analyzed using Mann-Whitney U test. The significance level was set at 95% ($p < 0.05$).

Figure 4: Protraction and retraction angles measured against the vertical.

3. Results

3.1. Pressure Algometer Scores

The ICC coefficient for the single assessor, considering all points measured, was 0.066 (p <.001).

Data of five haynet feeding (HF) and five floor feeding (FF) participants were analyzed. The differences in MNT between FF and HF at the origin of the *m. brachiocephalicus* were found to be statistically significant on both sides. Data included is mean ± standard deviation, unless otherwise stated. On the left side, the FF group (19.28 ± 3.48N/cm^2) had a mean difference of 6.50 N/cm^2 more MNT in relation to the HF group (12.78 ± 2.67 N/cm^2), $t(8)$= -3.103, p = 0.015 **(Figure 5a)**. Likewise, the right side has shown a significantly increased MNT on the FF animals (18.00 ± 4.44N/cm^2) in relation to horses having forage presented on a haynet (12.26 ± 1.47 N/cm^2) ($t(8)$ = 3.103, p = 0.015) **(Figure 5b)**.

At the muscle belly, there was a significant increase in MNT for floor feeders (FF) (13.48 ± 0.63N/cm^2) in relation to haynet feeders (HF) (8.70 ± 2.69 N/cm^2) on the left brachiocephalicus ($t(8)$ = -4.156, p = 0.003) with difference of 4.78 N/cm^2 between feeding practices (**Figure 6a**). Likewise, on the right side, there was a significantly higher MNT in the FF group (Median= 14.20 (3.4) N/cm^2) when compared with the HF group (Median=8.9 (2.0) N/cm^2) at the muscle belly level (U = 25.00, p = 0.008) with an overall difference of 3.40N/cm^2 (**Figure 6b**).

Likewise, the insertion of the *m. brachiocephalicus* has shown significant differences between FF and HF. At the right brachiocephalicus, the HF MNT (7.68 ± 1.30N/cm^2) was statistically significantly lower than the FF MNT (13.08 ± 1.12 N/cm^2), with a mean difference of 5.40N/cm^2 between groups ($t(8)$ = -7.008, p = 0.000112) **(Figure 7b)**. On the left side, the trend was similar, with horses being fed forage at the floor showing higher MNT (Median=11.20 (3.6) N/cm^2) than the horses feeding from haynets (Median=7.8 (2.9) N/cm^2) (U = 25, p = 0.008) **(Figure 7a)**.

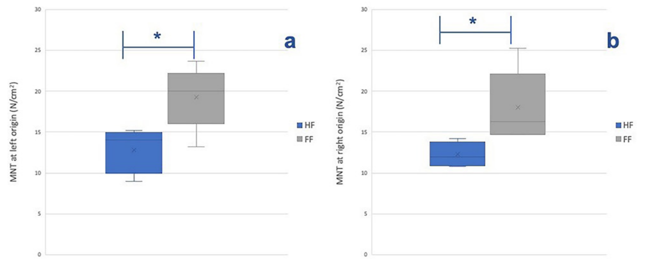

Figure 5: Mechanical nociceptor threshold (MNT) at the origin of the *m. brachiocephalicus* for horses that are floor feeders (FF) (n=5) and haynet feeders (HF) (n=5). The bottom and top of the box are the first and third quartiles, the band inside the box is the second quartile (the median), and the 'x' is the mean. The lines extending vertically from the boxes (whiskers) indicate the minimum and maximum of all of the data. *represents significant differences between HF and FF groups ($p < 0.05$).

3.2. Forelimb Kinematics

Forelimb protraction was not statistically significantly different between FF and HF for the left ($t(8) = -0.048$, $p = 0.963$) and right ($t(8) = 0.866$, $p = 0.412$) forelimbs. Retraction was also not statistically significantly different between groups on the left ($t(8) = 0.156$, $p = 0.880$) and right ($t(8) = 0.213$, $p = 0.836$) forelimbs. **Table 1** shows mean±SD for protraction and retraction angles.

4. Discussion

The purpose of this investigation was to specify whether haynet feeding or floor feeding caused different areas of tension within the *BC.m* and whether these changes had an effect on the disputed role the *BC.m* plays on the kinematic of the forelimbs of the horse. The study was carried out following a systematic review **[8]**, which concluded that the effects of extrinsic factors on the *m. brachiocephalicus* is still largely misunderstood within the industry as evidence available is largely anecdotal and unreliable. The purpose of this investigation was to specify whether haynet feeding or floor feeding caused different areas of sensitivity within the *m. brachiocephalicus* and whether these changes had an effect over the role of the *m. brachiocephalicus* on kinematic of the forelimbs of the horse.

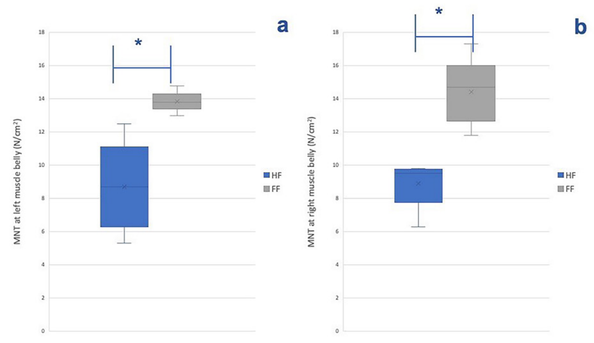

Figure 6: Mechanical nociceptor threshold (MNT) at the belly of the *m. brachiocephalicus* for horses that are floor feeders (FF) (n=5) and haynet feeders (HF) (n=5). The bottom and top of the box are the first and third quartiles, the band inside the box is the second quartile (the median), and the 'x' is the mean. The lines extending vertically from the boxes (whiskers) indicate the minimum and maximum of all of the data. *represents significant differences between HF and FF groups ($p < 0.05$).

Our hypothesis that forage feeding practice would affect *m. brachiocephalicus* sensitivity was confirmed in this study, with horses feeding forage from haynets showing a significant increase in muscle sensitivity. However, unexpectedly we have seen that this increased sensitivity did not affect the forelimb movement in terms of protraction and retraction at walk.

In summation, the results of this study show that whilst tension may be more significant in the *m. brachiocephalicus* for haynet feeders when compared to the floor feeder, this tension does not show a significant effect over the kinematics of its associated limb. Therefore, it is possible to suggest that domestication practice of haynet feeding is more likely to negatively impact musculature such as the *m. brachiocephalicus*. However, it is not currently possible within this investigation to define the *m. brachiocephalicus* as a muscle that may affect forelimb kinematics as previous studies have indicated **[6,10,23]**.

The findings indicate that the use of a haynet feeding method is a potential cause for increased tension of the *m. brachiocephalicus*. Similar findings have only been observed in another study. Speaight *et al.* **[19]**, preliminary study indicated that there was increased *m. brachiocephalicus* tension in horses that fed from both haynet and haybar feeding methods when compared to the floor feeding participants. It is difficult to draw comparisons with the current study as points of tension measured were vague (poll, neck, and shoulder) and lacked presentation of data. Regardless, there is evidence to suggest that feeding methods may affect neck muscle tension in both the current study and Speaight's investigation.

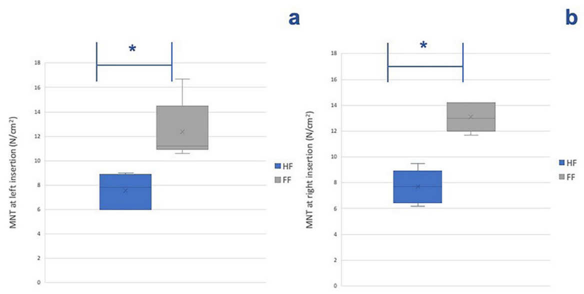

Figure 7: Mechanical nociceptor threshold (MNT) at the insertion of the *m. brachiocephalicus* for horses that are floor feeders (FF) (n=5) and haynet feeders (HF) (n=5). The bottom and top of the box are the first and third quartiles, the band inside the box is the second quartile (the median), and the 'x' is the mean. The lines extending vertically from the boxes (whiskers) indicate the minimum and maximum of all of the data. *represents significant differences between HF and FF groups ($p < 0.05$).

Significant decreases in MNT were noted among haynet feeders in comparison to floor feeders on both sides of the neck and in all three points studied. This demonstrated that in HF the muscle presented with a lower sensitivity threshold, signifying more sensitivity. Across both the groups, it was noted that insertion of the muscle (wing of atlas) accounted for the highest levels of sensitivity. Raw data demonstrated, the pressure algometer scores at the insertion for haynet feeders ranged from 6.0N to 9.5N whereas, for floor feeders ranged from 10.7N to 16.7N indicative of the possible effects feeding methods may have on this specific point. It has been expressed that underlying tissue thickness is an important factor in the production of MNTs, where significant differences in spinal MNT values have been reported for landmarks over muscle and bone **[25]**, which is why within the study it was expressly noted that all points were measured away from bony landmarks and nerve bundles to eliminate such discrepancies. It can be suggested that a primary reason for increased tension at the wing of atlas (insertion) is due to the anatomical structure and function of the atlantooccipital and atlantoaxial joints. Together, they allow the horse to move its head up and down as well as side to side in order to facilitate daily functions such as grazing or feeding from a haynet **[27]**. Furthermore, Hodgson *et al* **[28]**, investigated the forces required to feed from a haynet concluding that haynets hung lower required greater force to feed than haynets hung higher. As such the increased torque required to pull hay from the haynet may explain the increased sensitivity in this area. It is important to note that the results of this investigation mirror that at hand suggesting the point at the wing of atlas has an increased sensitivity in comparison to all other points due to anatomical function.

In general, it was likewise noted that at the muscle belly, haynet feeders showed an overall increased sensitivity. In the context of feeding, it may be suggested that increased sensitivity of muscle belly of the haynet group, when compared to the floor feeding group, may be caused by either a favored side or positioning of the haynet. Floor feeders, on the other hand, do not have this issue when it comes to feeding as the head and neck remain straighter and require less force to obtain hay as evidenced by the two non-working floor feeders.

The findings of the statistical analysis concluded that an increased sensitivity of the *m. brachiocephalicus* does not have an effect over kinematics of the forelimb. Kinematic data within this study noted no significant differences between protraction and retraction angles between groups. However, limited research exists that collectively defines the role that the *m. brachiocephalicus* plays in forelimb kinematics. From an investigation by **[6]** it can be assumed that the *BC.m* is categorized as a forelimb muscle and not a cervical muscle as electromyography readings evidenced that muscle activity of the *m. brachiocephalicus* during the limb protraction phase of locomotion was highest aiding in the protraction of that limb through water than on land. Previous studies **[7,23]** inferred that tensions within the *m. brachiocephalicus* had an effect over the kinematics of the forelimb. However, this was resultant of a hyperflexed head and neck position causing an anteversion of the forelimb or horses being ridden on the contact, making it difficult to compare results to the investigation at hand. This shows that the evidence that is available to the scientific and veterinary industry can only be assumed by gathering conclusions from other studies inclusive of the current investigation.

The main limitations within this investigation were the small participant yield used for each group as well as the range of levels of work each horse was in. Therefore, the study, as a whole, may not have been representative of the equine population when compared to a larger participant yield as well as other extraneous variables. The conclusions drawn from this study may be difficult to generalize to the wider equine population. A limitation in terms of the kinematic data may have been introduced by the lack of control over the walking speeds of each subject within the trial. The aim of the investigation was to analyze the horse walking at its standard speed in order to evaluate the effect the *BC.m* had over natural gait without the interference of speed control from the handler or rider. Where this was optimal to evaluate the full effect that the *BC.m* had over forelimb kinematics, a lack of standardization of the horses' natural speed resulted in a variety of speed produced by each horse meaning that a true correlation between subjects is difficult to determine at this time. Nevertheless, a standardized speed would not have been appropriate as the selection of participants in the investigation ranged from 12hh ponies to 17hh horses, therefore, a speed suitable for one may not be suitable for another. The lack of speed calculations completed within the kinematic analysis of each subject meant that mean speed of each subject could not be measured. As a result of lack of speed control, there may be an unforeseen effect over the stride length, therefore, the lack of speed calculations increases the risk of anomalous results that may affect conclusions derived from the investigation at hand.

Table 1: Mean±SD of right and left forelimbs protraction and retraction angles (°) for horses that are floor feeders (FF) (n=5) and haynet feeders (HF) (n=5).

		Floor feeders (n=5)	Haynet feeders (n=5)
Protraction (°)	Left forelimb	20.19±3.14	20.11±1.58
	Right forelimb	19.32±2.07	20.59±2.55
Retraction (°)	Left forelimb	15.24±2.56	15.02±1.78
	Right forelimb	14.70±3.05	15.05±2.11

The evidence of the effects of domestication practices, such as feeding methods, on the equine species can be summarized by culminating research that is collectively available, however, what is available to the industry is often conflicting and lacking in accuracy. As such, the investigation at hand begins to evidence the effects such practices have on the muscles of the equine neck, setting out a foundation for further investigations to take place. From data accrued throughout this investigation, it was possible to support that haynet feeders are more prone to increased sensitivity throughout the *m. brachiocephalicus* as a collective muscle. The insertion of the *m. brachiocephalicus* is the most susceptible to these changes in tension. This may be due to the role played by the atlantoaxial and atlantooccipital joints which aid feeding behavior. The conclusions derived from the muscle origin and belly, however, cannot be clinically reasoned due to a number of extraneous variables not being eliminated due to a lack of additional parameters not included. The effect of the tension in *m. brachiocephalicus* had over kinematics of the forelimb could not be accurately determined. It is therefore not currently possible to deduce the effect feeding methods have over the kinematics of the forelimb. This study does however add to the understanding of the effects human domestication has had on the body of the equine. It therefore can set out an understanding of how to better manage the horse within both companion and competitive environments.

Authors' Contributions

AM- data collection, data analysis, conceptualization, paper writing. RG- supervision, conceptualization, paper review. RFG- conceptualization, data analysis, paper review.

Data Availability

The data that support the findings of this study are available from the corresponding author, RFG, upon reasonable request.

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This research has not received any funding.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

The material in this manuscript has been acquired according to guidelines set by The Animal (Scientific Procedures) Act 1986 and the Declaration of Helsinki and has been approved by the Animal Welfare and Ethics Committee of Writtle University College. The approval number is 98360253/2019. A written informed consent was obtained from the owners of the participants of the study.

References

[1] Brown J, Smith P. Horse and Stable Management. 1994.

[2] Townson J, Dodd V, Brophy P. A survey and assessment of racehorse stables in Ireland. Irish Vet 1995;48:364–72.

[3] Ministry of Agriculture, Fisheries and Food: Statistics (Census and Prices) Division: Agricultural and Horticultural Census Datasets 1997.

[4] Harris PA. Review of equine feeding and stable management practices in the UK concentrating on the last decade of the 20th century. Equine Vet J Suppl 1999:46–54. https://doi.org/10.1111/j.2042-3306.1999.tb05156.x.

[5] Hintz HF. Hay Racks vs. Feeding Hay on the Stall Floor. Equine Practice (USA) 1997.

[6] Tokuriki M, Ohtsuki R, Kai M, Hiraga A, Oki H, Miyahara Y, *et al*. EMG activity of the muscles of the neck and forelimbs during different forms of locomotion. Equine Vet J Suppl 1999:231–4. https://doi.org/10.1111/j.2042-3306.1999.tb05224.x.

[7] Kienapfel K. The effect of three different head-neck positions on the average EMG activity of three important neck muscles in the horse. J Anim Physiol Anim Nutr (Berl) 2015;99:132–8. https://doi.org/10.1111/jpn.12210.

[8] McAteer A. A systematic review investigating the brachiocephalicus muscle mass in relation to stride length and/or protraction of forelimb in adult horses. Writtle University College, 2019.

[9] Payne R, Veenman P, Wilson A. The role of the extrinsic thoracic limb muscles in equine locomotion. J Anat 2005;206:193–204. https://doi.org/10.1111/j.1469-7580.2005.00353.x.

[10] Takahashi Y, Mukai K, Ohmura H, Takahashi T. Do muscle activities of M. splenius and M. brachiocephalicus decrease because of exercise-induced fatigue in Thoroughbred horses? Journal of Equine Veterinary Science 2020;86:102901. https://doi.org/10.1016/j.jevs.2019.102901.

[11] Dyson SJ. Lesions of the equine neck resulting in lameness or poor performance. Vet Clin North Am Equine Pract 2011;27:417–37. https://doi.org/10.1016/j.cveq.2011.08.005.

[12] Varcoe-Cocks K, Sagar K, Jeffcott L, McGowan C. Pressure algometry to quantify muscle pain in racehorses with suspected sacroiliac dysfunction. Equine Veterinary Journal 2006;38. https://doi.org/10.2746/042516406x154804.

[13] Fischer AA. Pressure Threshold measurement for diagnosis of myofascial pain and evaluation of treatment results. The Clinical Journal of Pain 1986;2:207.

[14] Magora A, Vatine JJ, Magora F. Quantification of musculoskeletal pain by pressure algometry. Pain Clinic 1992;5:101–4.

[15] Bendtsen L, Jensen R, Jensen N, Olesen J. Pressure-controlled palpation: A new technique which increases the reliability of manual palpation. Cephalalgia : An International Journal of Headache 1995;15. https://doi.org/10.1046/j.1468-2982.1995.015003205.x.

[16] Brown FF, Robinson ME, Riley JL, Gremillion HA, McSolay J, Meyers G. Better palpation of pain: reliability and validity of

a new pressure pain protocol in TMD. Cranio 2000;18:58–65. https://doi.org/10.1080/08869634.2000.11746114.

[17] Kinser AM, Sands WA, Stone MH. Reliability and validity of a pressure algometer. J Strength Cond Res 2009;23:312–4. https://doi.org/10.1519/jsc.0b013e31818f051c.

[18] Wathen D. Book Review: The Basics of Strength Training, 2nd edition. Strength and Conditioning Journal 2002;24:76.

[19] Speaight E, Routledge N, Charlton S, Cunliffe C. A preliminary study on the effects of head and neck position during feeding on the alignment of the cervical vertebrae in horses. Journal of Veterinary Behavior 2016;15:93. https://doi.org/10.1016/j.jveb.2016.08.061.

[20] Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. The Malaysian Journal of Medical Sciences : MJMS 2017;24:101. https://doi.org/10.21315/mjms2017.24.5.11.

[21] Bennet D. The evolution of the horse. In: Evans JW, editor. Horse Breeding and Management, Amsterdam, The Netherlands: Elsevier; 1992, p. 1–37.

[22] Fortin M, Videman T, Gibbons LE, Battié MC. Paraspinal muscle morphology and composition: a 15-yr longitudinal magnetic resonance imaging study. Med Sci Sports Exerc 2014;46:893–901. https://doi.org/10.1249/MSS.0000000000000179.

[23] Purchas A. Do Asymmetries in Pressure Do asymmetries in pressure Algometer readings taken from the left and right equine brachiocephalicus muscle correspond with asymmetries in forelimb kinematics and rider hand grip strength? Furthermore, what are average hand grip strength readings for equestrian athletes? BPP University, 2015. https://doi.org/10.13140/RG.2.1.4739.4325.

[24] Budras K, Bergh R, Rock S, Sack W, Horowitz A. Anatomy of The Horse. 6th ed. 2009.

[25] Haussler KK. Pressure algometry for the detection of mechanical nociceptive thresholds in horses. Animals (Basel) 2020;10:2195. https://doi.org/10.3390/ani10122195.

[26] Pongratz U, Licka T. Algometry to measure pain threshold in the horse's back – an in vivo and in vitro study. BMC Veterinary Research 2017;13. https://doi.org/10.1186/s12917-017-1002-y.

[27] Pilliner S, Elmhurst S, Davies Z. The Horse in Motion: The Anatomy and Physiology of Equine Locomotion. Wiley-Blackwell; 2002.

[28] Hodgson S, Bennett-Skinner P, Lancaster B, Upton S, Harris P, Ellis AD. Posture and pull pressure by horses when eating hay or haylage from a hay net hung at various positions. Animals (Basel) 2022;12:2999. https://doi.org/10.3390/ani12212999.

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Original Article Open Access

Muscle Stress and Thermal Discomfort of Equines in a Brazilian Rodeo-Style Sport (Vaquejada)

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Abstract

This study aimed to evaluate the effect of the exercise performed under the semi-arid weather on horses submitted to a Rodeo-style Sport (Vaquejada) through hemogasometric, clinical, and thermographic variables. To conduct this study, a Vaquejada simulation test (VST) was conducted in agreement with the rules of the Brazilian Association of Vaquejada. Physiological, thermographic, hemogasometry, and biochemistry data were collected before and after a single race. After it, significant changes were observed for thermographic, physiological, hemogasometric, and biochemical variables. The general surface temperature (ST) and those by body region differed ($P < 0.01$) between pre- and post-exercise conditions. The RR, HR, CK, Lactate, K^+, and AG increased ($P < 0.01$) after the exercise. A negative correlation between the maximum lactate concentration and the reduction ($P < 0.01$) of pH, HCO_3^-, TCO_2, and EB was also observed after the exercise. No significant changes were observed in PO_2, PCO_2, SO_2, and other serum electrolytes after the exercise. The thermographic profile evidences the environment of thermal, critical, and dangerous discomfort to which the animals are exposed. Thus, we believe horses used in this activity should not be submitted to consecutive and immediate exercises so that pathological complications from repetitive exertion are avoided.

Keywords

Blood gas analysis; thermal comfort; physical performance

1. Introduction

The Vaquejada, a manifestation of the culture in the Brazilian Northeast, emerged in the mid-twentieth century from a simulation of cattle management performed by a cowboy (local rural worker) and his horse. This competition consistently reduces gas variables, such as EB, HCO_3^-, and TCO_2 after high-intensity exercises [1], which is equivalent to high-goal polo [2] and high-speed treadmill activities [3]. In addition, physical-motor injuries, such as tendinitis and tenosynovitis associated with biochemical and hematological imbalances due to intense exercise, have already been described [4–7].

In addition to the intense physical activity, the high environmental temperatures of the event sites can negatively influence the welfare and performance of animals [8]. Therefore, meteorological variables and bioclimatological indexes, such as the temperature-humidity index (THI), comfort index (CI), and degree of comfort of the animal, combined with infrared thermography (IT); a noninvasive and precise method that detects minimal variations in surface temperature; have been used in the monitoring of animal welfare **[9–11]**. In this context, the analysis of the thermograms integrated with the changes in the physiological responses of the animals allows for a better understanding of thermoregulatory mechanisms because of the increase in surface temperature and the action of the environment on the thermal condition of animals **[12]**.

When considering the significant impact that climatic variables may have on sport horses and their physiological responses, we measured the impact of the environment and physical exercise on the physiological responses of vaquejada equines through gasometric, thermographic, and clinical biochemical analyzes before and after the intense physical activity of the event.

2. Material and Methods

2.1. Study Site

The experimental phase was a cross-sectional study, conducted in São Mamede, a municipality in the semi-arid region of northeastern Brazil, with a climate characterized as hot and dry with an average annual rainfall of 780.8 mm and average temperature ranging from 21°C (minimum) to 33°C (maximum), according to data from the National Institute of Meteorology (INMET). The study site was a vaquejada park (Lat: -6.925144°; Long: -37.098774°) with a track measuring approximately 160 m in length, with variations in width, covered in fine sand with a thickness of about 50 cm.

2.2. Animals, Management, and the Vaquejada Simulation Test

Twelve Quarter Horses were divided into six pairs, composed of the pull and helper horses, comprising five males and seven females, with an average age of 3.6±1.2 years and a weight of 305.6±49.8 kg, active in the vaquejada events, with training three times a week in fine sand for 60 minutes through walking, trotting, galloping, and racing activities. All animals belonged to private breeders in São Mamede. Their diet consisted of chopped elephant grass (*Pennisetum purpureum* Schum.) or Tifton (*Cynodon dactylon*) divided into two (5 AM and 5 PM) daily portions and commercial concentrate[1] (1.0 kg/100 kg body weight, with 12% crude protein) provided fractionally. An inorganic mineral salt mixture was not offered and water was always available. The criterion for the selection of healthy horses was based on clinical and laboratory aspects by consulting the histories of the animals on the veterinarian's domain of the properties. The clinical aspects of the physical examination involved measurement of rectal temperature, heart and respiratory rates, skin turgor, capillary refill time, evaluation of lymph nodes, inspection of mucous membranes, cardiac and pulmonary auscultation, abdominal palpation, and inspection of the external genitalia and laboratory (blood count), whose variables analyzed were red blood cell count (He), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (CHCM), total and differential leukocyte count and platelet count. In this study, animals with a recent history of pathologies, therapeutic treatment, and with reports of recent participation (two weeks) in sports competitions were removed from the experimental group. Furthermore, all selected animals were evaluated for hematological parameters (complete blood count) one week before the simulation experiment and immediately before the race. As there were no significant differences between the pre- and post-race moments, they were not described. The animals were brought to the site of the vaquejada simulation test (VST) by their halters, from nearby properties (400 meters). The time interval between going to the test site and data collection was two hours. The official vaquejada park is the same place used for training the selected animals. The VST was conducted between 9 AM and 1 PM.

The VST consisted of the execution of a single race without successive repetitions as occurs in the official events (a cycle of three races), as regulated by the Brazilian Association of Vaquejada **[13]**. The pull horse/cowboy and helper horse/cowboy ran on a 160-m long soft sand track after a bovine specimen (bull). The pull horse/cowboy pulls the bull down after 100 m of running and the other helper horse/cowboy helps the first one to keep the bull running in a straight line. The pull horse gallops at approximately 8.0 m/s, which is completed with a tug until the bull lies on the track, while the helper horse gallops at the same speed to the place where it is laid **[14,15]**. Each bull was used only once, and the horses were ridden by their usual riders.

2.3. Thermographic Data

The surface temperature (ST) of each animal was measured with an infrared thermographic camera (Fluke Ti 25®) while the animals were immobile, with no restriction, and with little manipulation to avoid possible stress. Images were taken from the right lateral plane, the first in resting conditions in the shade (pre-exercise) and the second after the vaquejada exercise, under direct sunlight (post-exercise). Later, the thermograms were analyzed by the Smartview software version 3.1, for obtaining the general average temperature and that for each body region, that is, of the thigh, rump, buttock tip, neck, and head, to calculate the representative surface temperature of the animals, considering an emissivity of 0.98 (**Figure 1**).

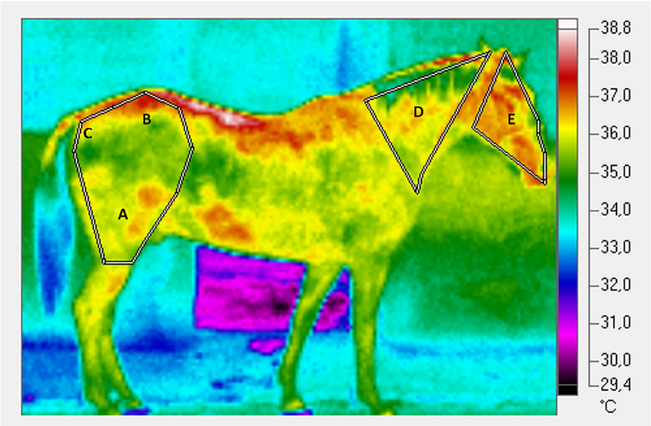

Figure 1: Equine infrared thermography. Images were obtained from the right lateral plane of the following body regions: thigh **(A)**, rump **(B)**, buttock tip **(C)**, neck **(D)** head **(E)**, analyzed using Smartview version 4.1 (Fluke® Ti25 thermographic camera).

The environmental data included air temperature (AT), relative humidity (RH), and maximum (Tmax) and minimum (Tmin) temperatures, obtained from the meteorological station of the National Institute of Meteorology (INMET), in Patos, the closest city (24 km) with a climate similar to that of the study site. From these climatic variables, two climatic condition indices were calculated. The Temperature-Humidity Index (THI) was calculated from the equation developed by **[16]** for dairy cows: THI = AT + 0.36Td + 41.5, where: AT = air temperature (°C) and Td = dew point temperature (°C). THI values up to 70 indicate a nonstress environment, between 71 and 78 indicate a critical one, between 79 and 83 a dangerous one, and above 83, an emergency condition **[16,17]**. To characterize whether the thermal environment during the collection period was stressful or not for equines, we used the Comfort Index (CI), employed by **[9]**, with the following formula: CI = Air Temperature (°F) + Relative Humidity (%).

2.4. Sample Handling and Analysis

Physiological data and blood samples were collected immediately before and after the exercise. The heart and respiratory rates were obtained with the aid of a stethoscope. Blood samples were collected anaerobically by puncturing the external jugular vein after physical containment of the animal and local disinfection. A 5.0 mL collection was made in a tube with sodium fluoride (BD Vacutainer®)[3] to perform the glucose and lactate dosage in plasma and another 5.0 mL collection was made in a tube with lithium heparin to perform hemogasometry and serum and electrolytic biochemistry. Blood gas analysis was performed immediately after collection by direct aspiration in a piece of portable equipment. The remaining blood was stored and transported refrigerated to the Laboratory of Veterinary Clinical Pathology of the University Veterinary Hospital (LPCV/HUV) of the Federal University of Campina Grande (UFCG) where they were processed. The plasma was obtained by centrifugation at 600 g for 10 minutes and analyzed.

Gasometric analysis was performed on-site in heparinized whole venous blood, using a portable hemogasometer[4] (AGS-22, Drake®). The hydrogen ion concentration (pH), partial pressure of carbon dioxide (PCO_2), and partial pressure of oxygen (PO_2) were determined by specific electrodes. The concentrations of bicarbonate (HCO_3-), excess base (EB), total carbon dioxide (TCO_2), and oxygen saturation (%) were calculated indirectly through the equipment software. The biochemical analysis was performed using a Cobas C111 automated biochemical apparatus[5] (Roche®) using spectrophotometry in enzymatic or colorimetric kinetic assays when using the commercial biochemical kits from the manufacturer (Roche Diagnostics, Mannheim, Germany) to measure Aspartate Aminotransferase (AST), Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Glucose, Total Calcium (tCa), and Lactate. The electrolytic analysis was performed in an automated analyzer for electrolytes[6] (Max Ion - Med max®) by the direction selectivity method for Sodium (Na), Potassium (K), Chlorine (Cl), and Ionized Calcium (iCa). The anion gap was calculated using the values of sodium, chlorine, and bicarbonate.

2.5. Statistical Analysis

The statistics evaluated the effect of the main factors, sport modality (pull and helper), experimental period (pre- and post-exercise), and the possible interaction between them, also considering the possibility of nonparametric effects for the nonsignificant variables in the ANOVA F-test. The thermographic, physiological, biochemical, and hemogasometric parameters were interpreted by analyzing parametric variance using the F-test and comparing the means by the Tukey test at 5%, and by the paired t-test at 5% for data that did not present parametric organization. Analyses were performed using the Graph Pad Prism software for Windows[7](GraphPad Software version 5.01).

3. Results

At the time of the experiment, the average ambient temperature (AT) was 32.5°C, the maximum temperature (Tmax) was 37.0°C, the minimum temperature (Tmin) was 29.0°C and the relative humidity was 45%. The comfort index (CI) was 135.5 and the temperature-humidity index (THI) was 74, which reached 79 when obtained from the maximum temperature. The physiological, hemogasometric, and biochemical variables investigated were significantly affected only by the exercise factor, with a tendency to rise. The RR and HR increased ($P < 0.01$) after exercise **(Table 1)**. The surface temperature (ST), generally and by regions, differed ($P < 0.01$) between pre- and post-exercise conditions **(Table 1)**, except for the T in the head region (H) which did not differ significantly between the moments.

The significant elevation rates were 111.0% and 1,062% for CK and Lactate, respectively **(Table 2)**.

Table 1: Respiratory rate (RR), heart rate (HR), general surface temperature (ST), and ST by body regions: thigh (RT), buttock tip (BT), rump (R), and head (H) of the equine pre- and post-exercise.

Physiological responses	Pre-exercise	Post-exercise	P	Reference
RR (bpm)**	31.0^b	39.7^a	0.0001	8-16#
HR (bpm)**	49.0^b	106.2^a	0.0073	30-40#
Surface temperatures (°C)				
sT**	37.7^b	40.3^a	0.00008	na
sT (RT)**	37.5^b	40^a	0.00004	na
sT (BT)**	37.9^b	40.5^a	0.00012	na
sT (R)**	38.3^b	43.7^a	0.00003	na
sT (H)ns	37.6	38.3	0.08888	na

Means followed by the same letters do not differ by the Tukey test with 5% probability; (**) significance at $P \leq 0.01$; (ns) not significant; bpm - beats per minute or breaths per minute; (na) not available; (#)**[18]**.

Table 2: Serum enzymes and metabolites of the equine pre- and post-exercise.

	Pre-exercise	Post-exercise	*P*	Reference[#]
$CK_{(U/L)}$ **	283.2±160.9^b	599.0±340.5^a	0.0056	108.0-380.0
$AST_{(U/L)}$ ns	271.1±112.0	286.3±118.3	0.7544	220.0-600.0
$LDH_{(U/L)}$ ns	378.5±95.2	446.4±112.3	0.1307	160.0-410.0
$Lactate_{(mmol/L)}$ **	0.83±0.45^b	9.69±5.32^a	0.0001	1.11-1.80
$Glucose_{(mmol/L)}$ ns	5.78±0.84	6.08±0.88	0.4087	4.20-6.40

Means followed by equal letters do not differ from the Tukey test with 5% probability; (**) significance with $P \leq 0.01$; (ns) not significant; (#) [18].

In the hemogasometric study, pH, HCO_3-, TCO_2, K^+, and AG were significantly affected **(Table 3)**. A slight acidification of the blood after physical activity was observed, followed by a 39% reduction in bicarbonate concentration. The venous oxygen saturation was reduced by 39% and AG presented an increase of 85%. A negative correlation ($P < 0.001$) between the maximum lactate concentration and the pH value, HCO_3-, EB, and AG after exercise was observed. A significant elevation ($P < 0.05$) for the electrolyte K^+ was verified, however, physiologically, it remained within the reference range as well as the other electrolytes after exercise.

4. Discussion

The CI obtained in the experimental period classifies the environment as of thermal discomfort according to [9], since the limit value that would demonstrate nonactivation of the animal thermoregulatory system is 130. The THI values between 74-79 demonstrate an environment that can vary from critical to dangerous for the animals [17]. The physiological parameters (RR and HR) at rest exceeded the reference range of 8 to 16 breaths min^{-1} and 30 to 40 beats min^{-1}, respectively, according to [18]. These data are related to the THI value obtained, because when animals are inserted in an unfavorable thermal environment, there is the activation of the thermoregulatory center, with increased peripheral sweating and vasodilation, followed by increased HR and subsequent elevation of RR [19]. The ST, in general, and in different body regions studied, was higher due both to the influence of the thermal environment and the thermal energy generated by the muscle contraction required in high-speed running. The heat production, even being an expected physiological event, in most cases is greater than the dissipation capacity of the body, causing it to experience increased temperature [20]. The heat produced by exercise in a horse can increase its body temperature between 3.0 to 5.0 °C. If effective heat dissipation does not occur, this temperature may rise to a risk of thermal shock or integrated physiological disturbances [21]. Unfortunately, thermographic data collections in a second post-race moment were not performed. Thus, the effective heat dissipation and the return to the physiological state of thermal comfort have not been evaluated.

Associated with the environmental thermal stress detected, intense exercise promoted a proportional increase in the respiratory rate in response to high blood flow velocity in the pulmonary artery to the increase in output and heart rate as observed by [22]. Thus, there was a reduced time of erythrocytes in alveolar capillaries, which decreased the absorption time of O_2. Consequently, the association of reduced PO_2 values with intense exercise caused a change in the production of aerobic muscle energy of myocytes by an anaerobic metabolic pathway with massive lactate production [23]. Glucose, an important source of energy for muscle activity, however, did not undergo significant serum changes since, in intense exercise, much of the energy is generated from muscle glycogen hydrolysis through anaerobic glycolysis.

Despite the nonsignificant reduction of PO_2 and the saturation of O_2 in venous blood after only one event, the lactate level approaches the values of equines in longer activities, such as cross-country races [24] and high-speed treadmill [3]. Combined with changes in O_2 pressure, hypercapnia can occur with values above 50 mmHg [25]. However, this was not observed in our study since the activity was extremely short, with less than a minute between the start and rest in the post-exercise period.

The transient metabolic acidosis profile is well-known in sports medicine for equines [26], including with vaquejada animals at milder temperatures [1]. Transient acidosis in sports horses, which have hypoxemia because of high-intensity exercise, is correlated with post-exercise lactate levels. Lactate levels and HCO_3- concentration are inversely proportional because bicarbonate is consumed in the processing of Lactic Acid buffering [27]. According to [28], the increase in AG levels and reduction of EB and TCO_2 is justified by the reduction of bicarbonate levels consumed in the buffering of the excess lactic acid produced. After just one race, we observed a significant reduction in HCO_3- associated with reduced pH levels, so that, given a greater effort, as in consecutive races, a possible picture of marked metabolic acidosis may arise. Although consecutive runs are infrequent, it is common during unofficial vaquejadas, despite restrictions issued by the Brazilian Association of Vaquejada.

Table 3: Blood gas and serum electrolytes of the equine pre- and post-exercise.

	Pre-exercise	Post-exercise	*P*	Reference#
pH**	7.44±0.08a	7.21±0.07b	0.0001	7.32-7.46
$pCO_{2\,(mmHg)}$ ns	39.33±3.58	41.17±3.75	0.2341	38.0-46.0
$pO_{2\,(mmHg)}$ ns	58.42±16.83	57.33±16.51	0.8751	35.0-45.0
Sat O_2 ns	86.83±18.11	74.75±15.59	0.0937	80.0-90.0
HCO_3^- (mmol/L) **	27.75±4.48a	16.75±2.71b	0.0001	23.0-32.0
BE (mmol/L) **	4.58±0.70a	-11.5 ±-1.76b	0.0001	0.0-6.0
$TCO_{2\,(mmol/L)}$ **	28.92±4.55a	17.5±2.75b	0.0001	22.0-31.0
$K^+_{(mmol/L)}$ *	4.02±0.18b	4.18±0.19a	0.0497	3.0-5.0
$Na^+_{(mmol/L)}$ ns	129.22±1.84	129.74±1.55	0.5113	132.0-146.0
$Cl^-_{(mmol/L)}$ ns	108.04±4.63	107.99±4.32	0.9797	98.0-110.0
$iCa^{+2}_{(mmol/L)}$ ns	1.40±0.07	1.37±0.06	0.3397	1.40-1.70
$tCa^{+2}_{(mmol/L)}$ns	2.80±0.13	2.74±0.18	0.3602	2.80-3.44
AG (mEq/L) **	-6.36±-0.88b	5.58±0.77a	0.0001	10.0-25.0

Means followed by equal letters do not differ from the Tukey test with 5% probability; (*) significance with $P \leq 0.05$; (**) significance with $P \leq 0.01$; (ns) not significant; (#)[18].

According to [29], depending on the time of duration and the type of physical exertion, an acute inflammatory process of physiological character is produced, altering the cellular permeability of myocytes. This change in permeability of the muscle enzymes CK, AST, and LDH, transfers them from the intracellular space to the extracellular one, causing an increase in serum level. The CK values in the pre-test were similar to those from [14]. Sodré *et al.* [15] found, for vaquejada horses, even with a single run, that the results in the post-test were higher than those observed in a post-test with successive runs. This significant increase in CK may be related to an intense and continuous muscular injury [30], or the lack of physical conditioning of some animals that made up the experimental groups and that, consequently, increased the CK values in the post-race.

The elevation of K^+ levels in maximal exertion activities may occur because of the transition of cations from the intracellular medium to the extracellular one, by the action of α-adrenergic receptors and some catecholamines present in red blood cells, liver, and muscle tissues [31]. Regarding the harmful effects on animals exposed to vaquejadas, it is pertinent to report that there will be a significant reduction 30 minutes after the completion of the activity, which will occur due to the same catecholamines that will act on β-adrenergic receptors, thereby correcting the hyperkalemia [32].

5. Conclusions

For the vaquejadas performed in the Brazilian Northeast, thermal discomfort characterized by a critical to dangerous environment for animals was observed in the thermographic analysis. The association of this thermal stress with high-intensity physical exertion caused transient metabolic acidosis with a marked base deficit and acute energetic and muscle tissue stress. Because of this, we believe that consecutive races in vaquejadas should be discouraged because they may involve muscle damage and acid-base imbalance, which can represent the genesis of contusions, strains, and myopathies by exertion in sports horses.

Authors' Contributions

Thiago A. Gurjão, Gabriella S.B. Souto, Cledson C. Oliveira and Maycon R. Da Silva contributed to study design and execution, data analysis and interpretation, and preparation of the manuscript. Bonifácio B. de Sousa contributed to study execution. Antônio F.M. Vaz contributed to study design, data analysis and interpretation and preparation of the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

The data supporting the findings of this study are available within the article.

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Manufacturers

- [1]Rações Supra Alisul Alimentos S. A, São Leopoldo, RS, Brazil.
- [2]Fluke Corporation, Everett, Washington, USA.
- [3]BD Vacutainer, Franklin Lakes, NJ, USA.
- [4]Drake, São José do Rio Preto, SP, Brazil.
- [5]Fritz Hoffmann-La Roche, Basel, Switzerland.
- [6]Medmax, Barueri, SP, Brazil.
- [7]GraphPad Software, San Diego, CA, USA.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

The research project was approved by the Research Ethics Committee (REC) of the Federal University of Campina Grande with protocol number 306/2015. All procedures for handling animals related to this study were conducted in accordance with national or institutional guidelines for the care and use of animals.

References

[1] Arruda SSB, Huaixan LN, Barreto-Vianna ARC, Godoy RF, Lima EMM. Clinical and blood gasometric parameters during Vaquejada competition. Pesq Vet Bras 2015;35:889–92. https://doi.org/10.1590/S0100-736X2015001100003.

[2] Ferraz GC, Soares O a. B, Foz NSB, Pereira MC, Queiroz-Neto A. The workload and plasma ion concentration in a training match session of high-goal (elite) polo ponies. Equine Veterinary Journal 2010;42:191–5. https://doi.org/10.1111/j.2042-3306.2010.00278.x.

[3] Tavares Miranda AC, Padilha FGF, Ramos MT, Dimache LAG, Nascimento Godoi F, Galina Filho A, *et al.* Hemogasometry of eventing horses before and after intense exercise on a high speed treadmill. Livestock Science 2016;186:85–7. https://doi.org/10.1016/j.livsci.2015.07.020.

[4] Lopes KRF, Batista JS, Dias RVC, Soto-Blanco B. Influence of vaquejada competitions on stress parameters in equines. Ci Anim bras 2009:538–43.

[5] Melo UP, Santiago RMFW, Júnior RAB, Ferreira C, Bezerra MB, Palhares MS. [Biometry and changes in foot balance in equines used in vaquejada]. Acta Veterinaria Brasilica 2011;5:368–75. https://doi.org/10.21708/avb.2011.5.4.2261.

[6] Rodrigues BCC, Borges MDL, Lacerda M a. S, Schellin PC, Leite JEB. [Fractures in crossbred horses used in vaquejadas: predominant location and type]. Revista de Educação Continuada Em Medicina Veterinária e Zootecnia Do CRMV-SP 2019;17:78–78.

[7] Melo UP, Ferreira C. Back pain in vaquejada horses: clinical, ultrasound findings and therapeutic outcome in 25 cases. R Bras Ci Vet 2020;27:193–9.

[8] Tanda G. Skin temperature measurements by infrared thermography during running exercise. Experimental Thermal and Fluid Science 2016;71:103–13. https://doi.org/10.1016/j.expthermflusci.2015.10.006.

[9] Moura DJ de, Maia AP de A, Vercellino R do A, Medeiros BBL, Sarubbi J, Griska PR. [Use of infrared thermography in the analysis of thermoregulation in training horses]. Eng Agríc 2011;31:23–32. https://doi.org/10.1590/S0100-69162011000100003.

[10] Sykes DJ, Couvillion JS, Cromiak A, Bowers S, Schenck E, Crenshaw M, *et al.* The use of digital infrared thermal imaging to detect estrus in gilts. Theriogenology 2012;78:147–52. https://doi.org/10.1016/j.theriogenology.2012.01.030.

[11] Menegassi SRO, Barcellos JOJ, Dias EA, Koetz C, Pereira GR, Peripolli V, *et al.* Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. Int J Biometeorol 2015;59:357–64. https://doi.org/10.1007/s00484-014-0847-z.

[12] Silva LKX, Martorano LG, Silva WC da, Reis AB, Silva FP da, Fernandes GB, *et al.* [Physiological responses associated with thermal variations diagnosed by infrared thermogram in equines submitted to intense physical effort]. Revista Agroecossistemas 2018;10:265–79. https://doi.org/10.18542/ragros.v10i1.5063.

[13] Brazilian Association of Vaquejada. General regulations of vaquejada (ABVAQ). 2020. https://www.abvaq.com.br/regulamento (accessed April 16, 2020).

[14] Hunka M, Lima L, Souza L, Silva C, Silva E, Manso H, *et al.* Heart rate and velocity in Vaquejada horses during field tests. Comparative Exercise Physiology 2017;13:25–30. https://doi.org/10.3920/CEP160027.

[15] Sodré TDRP, Sousa LN, Silva CA, Santos JM, Sampaio MQ, Coni ROS, *et al.* Is there an ideal rest interval between races during vaquejada in which it would be possible to associate best performance and welfare? Journal of Equine Veterinary Science 2020;91:103141. https://doi.org/10.1016/j.jevs.2020.103141.

[16] Buffington DE, Collazo-Arocho A, Canton GH, Pitt D. Black globe-humidity index (BGHI) as comfort equation for dairy cows. American Society of Agricultural and Biological Engineers 1981:711–4.

[17] Baêta FC, Souza CF. [Ambience in rural buildings thermal comfort]. Viçosa, Brazil: UFV; 1997.

[18] Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary Medicine. W. B. Saunders; 2017. https://doi.org/10.1016/B978-0-7020-5246-0.00033-4.

[19] Marai IFM, Haeeb AAM. Buffalo's biological functions as affected by heat stress — A review. Livestock Science 2010;127:89–109. https://doi.org/10.1016/j.livsci.2009.08.001.

[20] Carvalho T de, Mara LS de. [Hydration and Nutrition in Sport]. Rev Bras Med Esporte 2010;16:144–8. https://doi.org/10.1590/S1517-86922010000200014.

[21] Seabra JC, Dittrich JR. Thermoregulatory system of sport horses. Equine Science Academic Journal 2017;1.

[22] Wilkins PA, Gleed RD, Krivitski NM, Dobson A. Extravascular lung water in the exercising horse. J Appl Physiol (1985) 2001;91:2442–50. https://doi.org/10.1152/jappl.2001.91.6.2442.

[23] Watanabe MJ, Thomassian A, Teixeira Neto FJ, Alves ALG, Hussni CA, Nicoletti JLM. [Changes in arterial pH, P O2 and P CO2 and blood lactate concentration in Arabian horses during high-speed treadmill exercise]. Arq Bras Med

Vet Zootec 2006;58:320–6. https://doi.org/10.1590/S0102-09352006000300006.

[24] Muñoz A, Riber C, Santisteban R, Rubio M, Agüera E, Castejón F. Cardiovascular and metabolic adaptations in horses competing in cross-country events. The Journal of Veterinary Medical Science 1999;61. https://doi.org/10.1292/jvms.61.13.

[25] Poole DC, Erickson HH. Heart and vessels: function during exercise and response to training. Journal of Equine Veterinary Science 2004;24:288–94. https://doi.org/10.1016/j.jevs.2004.06.013.

[26] Evans DL. The cardiovascular system: anatomy, physiology, and adaptations to exercise and training. In: Rose RJ, Hodgson DR, editors. The Athletic horse, Philadelphia: Saunders; 1994.

[27] Carlson GP. Interrelationships between fluid, electrolyte and acid-base balance during maximal exercise. Equine Veterinary Journal 1995;27:261–5. https://doi.org/10.1111/j.2042-3306.1995.tb04933.x.

[28] Dumont CBS, Leite CR, Moraes JM, Moreira M, Moscardini ARC, Godoy RF, *et al*. [Osmolarity, anion gap, hydrogenion potential and measurable plasma ions of thoroughbred Arabian horses finalists in 90 km endurance races]. Pesq Vet Bras 2012;32:542–6. https://doi.org/10.1590/S0100-736X2012000600012.

[29] Sales JVF, Dumont CBS, Leite CR, Moraes JM, Godoy RF, Lima EMM. [Expression of Mg+2, CK, AST and LDH in enduro horses finalists]. Pesq Vet Bras 2013;33:105–10. https://doi.org/10.1590/S0100-736X2013000100019.

[30] Smith BP. Large Animal Internal Medicine. 5th Edition. Elsevier; 2015.

[31] Aguilera-Tejero E, Estepa JC, López I, Bas S, Mayer-Valor R, Rodríguez M. Quantitative analysis of acid-base balance in show jumpers before and after exercise. Res Vet Sci 2000;68:103–8. https://doi.org/10.1053/rvsc.1999.0341.

[32] Harris P, Snow DH. Plasma potassium and lactate concentrations in thoroughbred horses during exercise of varying intensity. Equine Vet J 1992;24:220–5. https://doi.org/10.1111/j.2042-3306.1992.tb02819.x.

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Competition Anxiety in Equestrians Across Different Disciplines and Performance Levels

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Abstract

Competition anxiety among equestrians has been examined very little so far. Therefore, this study investigated the extent and distribution of competition anxiety in female and male equestrians. Furthermore, it was examined whether there are specific differences among equestrians in different disciplines (dressage, show jumping, western, recreational, and eventing) and performance levels. The sample ($N = 406$) consisted of female ($n = 385$) and male ($n = 21$) German competition riders ($mean_{age} = 34.84$). Competition anxiety (somatic anxiety, concern, concentration (decrease)) was measured with the Competition Anxiety Inventory Trait (German: Wettkampf-Angst-Inventar/WAI-T). The data analysis was based on *t*-tests, analyses of variance (ANOVAs) including post-hoc tests and correlation analyses. The results reveal that equestrians in higher-level performance classes (2, 3, and 4) had lower perceptions of somatic anxiety ($mean_{class\ 2} = 8.70$; $mean_{class\ 3} = 9.13$; $mean_{class\ 4} = 9.79$) than respondents in lower-level classes ($mean_{class\ 6} = 11.76$, $p < .001$). There were differences among the disciplines in concern ($p < .01$) and concentration (decrease); ($p < .001$). Dressage riders showed higher concern scores (mean = 10.67) than western riders (mean = 7.90). Furthermore, dressage riders scored higher on concentration (decrease) (mean = 7.57) compared to show jumping (mean = 6.69) and eventing (mean = 5.76). Equestrians competing in show jumping (mean = 6.69) had significantly lower concentration (decrease) scores than in recreational riding (mean = 8.22). Female riders (mean = 1.71) rated worrying thoughts as more performance-inhibiting compared to male respondents (mean = 2.19, $p < .01$). Dressage riders (mean = 1.74) reported feeling more physical arousal before competitions than show jumpers (mean = 1.71). These results underline the importance of considering competition anxiety to adapt training for riders, minimize risks of injuries in equestrian sports, and improve performance. Results may also be of interest for selecting advice and coaching processes.

Keywords

Stress; equestrian sports; concentration (decrease); concern; somatic anxiety; coping

1. Introduction

Anxiety is accompanied by negative feelings of tension and is geared toward a threat in the future [1]. Since it occurs immediately and automatically as emotions, in contrast to cognitions [2], it can protect people from danger or actions with negative consequences. Conversely, anxiety, like stress, has evolved as a response pattern to better cope with challenging situations [3] and thus get the best out of people. Anxiety plays an important role in almost all competitive sports. Competition anxiety can be protective as well as motivating [3]. Competitions are associated with stress and anxiety in athletes, which may affect their performance [4]. Thus, competition anxiety has already been examined in various sports, such as bowling [5], athletics [6], basketball and volleyball [7], ice skating [8], golf [9], swimming [10], football and rugby [11] as well as tennis [12].

Competition anxiety [13] can be divided into state anxiety and trait anxiety. State anxiety consists of an emotional response (anxiety) caused by a particular competitive situation and by worries about not meeting others' expectations. Trait anxiety is considered a personality trait and influences how a competitive situation is perceived [14]. Furthermore, competition anxiety can be understood as a multidimensional construct [15]. Somatic anxiety refers to the physiological response to anxiety. In addition, anxiety manifests itself cognitively or through concentration problems [14]. Specifically for cognitive aspects, the individual experience of competition anxiety is oftentimes assessed using questionnaires. The validated *Wettkampf-Angst-Inventar* (WAI-T; Competition Anxiety Inventory Trait) [16], which is available in German, considers the subdivisions into state and trait anxiety as well as a multidimensional perspective of competition anxiety. Therefore, it was used in the present study.

Despite equestrian sport being of the most popular sports in Germany [17], there are only a few studies (with comparatively small numbers of participants). Horses are flight and prey animals. Thus, athletes expose themselves to additional dangers during competition and depend on their equine partner to perform optimally. Due to the severity of injuries and high mortality, horseback riding is considered a risky sport with numerous accidents reported [18–23]. Therefore, it is not surprising that equestrian sports are found to be anxiety-inducing [24]. It was reported that the perceived severity of horseback riding accidents and injuries correlates with cognitive and somatic competitive anxiety [25].

Unlike in many other sports, which are primarily about speed, strength, or precision and may use inanimate equipment, which is in this sense unresponsive, equestrians interact with their horses via non-verbal channels and view their horses with their individual characteristics and sensations as partners. Equestrians and horses need to work together in harmony to optimize not only their performance but also their welfare, health, and safety [26]. In equestrian sports, the interaction between rider and horse plays an important role, as animals can sense and react to human emotions [25,27]. Critically, it has been pointed out that riders' interpretations of horses' affective state are oftentimes misguided [28] and, hence, increases the risk of miscommunication and, consequently, of injuries. In addition, horses have been suggested to be able to sense their rider's fear and the rider's arousal can be transferred to horses as shown by heart rate measurements [29,30], potentially causing dangerous situations. Therefore, in this study, the extent of competition anxiety in equestrians was examined.

Increasing a rider's somatic arousal can also negatively impact horse-rider interaction and performance. Relaxed, confident riders should be able to execute correct movements to which the horse reacts appropriately. For equestrians who show symptoms of somatic arousal, it could be that previously trained signals are unclear, e.g., due to muscle tension or increased heart rate. This should lead to a decrease in performance. In advanced stages of equestrian sports, demands on the equestrian-horse dyad become more sophisticated and require greater precision motor skills and better differentiation between the various aids [31]. For those riders who had a better assessment of their riding abilities and more training sessions, less nervousness was transferred to the respective horse [30]. Moreover, competition riders showed significantly higher values of self-efficacy and the assessment of their riding skills than recreational riders and, thus, had more confidence in their abilities. Those who considered themselves to be good riders also had higher self-efficacy values compared to riders who considered themselves less proficient. In addition, self-efficacy correlated with riding experience, the number of horses and, in the case of competition riders, the number of competitions [32]. That is, the performance depends to some extent on the interaction between rider and horse involving communication in both directions and psychological states such as self-efficacy or anxiety.

Investigating the interaction between horses and dressage riders using inertial sensors showed that the riding level influenced the rider's posture. Differences could be detected with contemporary available sensor technology and methods. Professional dressage riders and dressage beginners were examined during the study. Professional equestrians held their pelvis closer to the middle position and further forward whereas beginners tilted their pelvis further to the right and further back. The coupling intensity of the horse and rider showed differences between gaits. Although this study did not explore competition anxiety, differences between professionals and beginners were found [33]. Another study emphasized the importance of developing resilience, i.e., mental resistance, for competitive riders and suggested that developing performance strategies can help develop resilience. Overall, more significant correlations between performance strategies were demonstrated among professionals than among novices [34]. Regarding competition anxiety, Wolframm *et al.* [35] argued that pre-competition mood, i.e., competition anxiety, improves performance in advanced riders compared to novice riders because it presumably leads to higher processing efficiency and task-specific concentration in advanced riders. However, it must be emphasized that the associated study involved only 26 dressage riders and no riders from other disciplines. Another study investigating mood and psychological skills in N = 54 elite and sub-elite equestrian riders (disciplines: show jumping and dressage) found that elite riders scored higher in anxiety management than non-elite riders [36].

Equestrians with low levels of confidence have been shown to be more likely to feel unable to control their horse [37]. Self-confidence may therefore be an indicator of how well equestrians rate their control, which in turn may be associated with equestrian anxiety. These two constructs of self-confidence and particularly competition anxiety were investigated in another study. Therefore, pre-competition anxiety and self-confidence in 57 eventing equestrians (including dressage, show jumping, and cross-country) were analyzed. Respectively different disciplines identified the show-jumping phase as having the largest impact on somatic anxiety and cognitive anxiety. The participants' self-confidence was highest for the cross-country phase (which is often associated with the most dangerous phase) [38]. In the cross-country phase, equestrians need to trust in themselves and their abilities to complete the course safely and successfully (in addition to their riding skills).

Regarding the psychological profile, Wolframm and Micklewright [39] investigated the effects of a six-week mental skills training on precompetitive anxiety and performance in non-elite equestrian dressage riders. Ten Dutch non-elite dressage riders who competed regularly in Dutch amateur levels (B = beginner, L = easy, M = medium, and Z = advanced) participated in that study [39]. Findings suggest that mental skills training has a positive effect on competitive dressage performance in non-elite equestrians. These findings illustrate the importance of studying competition anxiety in equestrians and deriving practical implications from the findings. Results of another study by Wolframm and Micklewright [31] showed lower somatic arousal and higher self-confidence in elite compared with non-elite riders ($N = 40$; $n_{female} = 28$, $n_{dressage} = 12$, $n_{show\ jumping} = 17$, and $n_{eventing} = 11$). Negative correlations between cognitive arousal and self-confidence were found among elite equestrians, non-elite equestrians, show jumpers, and female equestrians. According to another study, there were differences in self-efficacy in different riding disciplines. Eventers rated their confidence the highest, and recreational riders the lowest. Due to riding ability, show jumpers rated their riding competence the highest, and recreational riders rated their riding ability the lowest [32]. While most of the studies mentioned above had rather small sample sizes, interesting results were found. Nevertheless, the findings indicate further research possibilities. Since various psychological constructs were found to differ between equestrian performance classes and disciplines in these studies, these aspects are also part of the present study.

There are some studies in the field of competitive anxiety dealing with differences between females and males, but the findings are not consistent. Russell *et al.* [7] found a higher degree of cognitive and somatic anxiety in females before competitions. Male competitors exhibited higher levels of self-confidence and lower levels of cognitive anxiety than females [40]. Meta-analytic research revealed that the mean effect size for self-confidence on performance in males was greater than that in females, suggesting males generally exhibit greater self-confidence than females [41]. Fernandes *et al.* [42] found higher expression only for cognitive anxiety, while other results demonstrated higher expression of somatic anxiety and concentration problems in females [43]. In addition, Correia and Rosado [43] found higher general competitive anxiety for females. This finding, again, contrasts with the findings of Aşçi *et al.* [44], who showed no differences in either cognitive or somatic anxiety between males and females. Iungano et al. [34] studied 101 show jumpers and found that male and female riders used different performance strategies. Male riders seemed to use the "activation" strategy more often, which showed a positive correlation with resilience. In contrast, female riders more often use the "negative thinking" strategy, which correlates negatively with resilience. Furthermore, sex differences have received little attention in equestrian sports research, which has mostly focused on a small number of male riders (e.g., [31–33,38]). Therefore, the present study also aimed to add to the literature by investigating possible sex differences by investigating a larger sample of athletes.

Focusing on competition anxiety in riders is required to be better able to minimize risks and prevent accidents but also to mentally strengthen individuals. It is also argued that anxiety can affect equestrian athletes who resume riding before they have psychologically recovered from a previous riding accident, affecting their own performance and that of their horse [45].

The present study investigated whether there are differences in competition anxiety among equestrians of different performance classes, and disciplines, and also whether there are sex differences. Differences would have implications for the psychological profile of athletes and may, hence, lead to consequences for practice, training procedures, and coaching. For example, athletes with higher trait anxiety may benefit from adequate coping strategies whereas athletes with lower trait anxiety may devote more cognitive resources to technical skills (motor control & interaction with the horse).

In the present study it was hypothesized that there are differences between male and female riders (hypothesis H1), equestrian disciplines (H2), and performance classes (H3) regarding competition anxiety. Furthermore, a negative correlation was expected between the assessment of one's own riding abilities and competition anxiety (H4). In addition, it should be investigated whether equestrians' values of competition anxiety were similar to those of the norm sample, which included other athletes, or whether there were differences. These hypotheses are derived to contribute to answering the following research questions: Are there differences between female equestrians' and male equestrians' competition anxiety? Do equestrians in various performance classes have different competition anxiety scores? Is competition anxiety found to be different in equestrians of various disciplines (dressage, show jumping, western, recreational[1], eventing)? Do equestrians who rate their own riding abilities lower than those who do not show higher competition anxiety scores? Do equestrians differ in their competitive anxiety scores from other athletes that belong to a norm sample?

2. Methods

2.1. Sample

A total of $N = 406$ competition riders participated in this study. Participants were 385 females (95%) and 21 males with a mean age of 34.84 ($SD = 11.18$) years. They were either horse owners ($n = 371$) and had $M = 2.27$ horses of their own ($SD = 2.42$) or did not own a horse but rode a foster horse or a school horse ($n = 35$). Participants were recruited via various platforms (social media, notices in riding stables, and universities). An online survey design was used (via "Unipark") over a period of eight weeks. Participants were able to withdraw from the study at any time. In accordance with the ethics code of the American Psychological Association and the ethical standards of the sixth revision of the guidelines of the Declaration of Helsinki [46], participants of the research were volunteers and gave their written informed consent. To protect human welfare, all applicable international, national, and/or institutional guidelines for human participants were followed. Institutional ethics was granted by the local

[1]Recreational equestrians are respondents stating that they take part in competitions from time to time, but do not assign themselves to a specific discipline.

university (Hochschule Fresenius, University of Applied Sciences). Data were treated anonymously.

2.2. Design

A between-subjects design was used in which levels of competition anxiety were compared according to sex (male vs. female), discipline (dressage vs. show jumping vs. eventing vs. western vs. recreational riding), and performance class (2-7).

A standardized, German-language questionnaire with various subscales (see below) was used to measure competition anxiety, namely the Competition Anxiety Inventory Trait (WAI-T) **[16]**, which is based on the Sport Anxiety Scale (SAS) by **[47]**. The WAI-T comprises 12 items, with four each belonging to the subscales of somatic anxiety, concern, and concentration (decrease) (four-point scale from "not at all" to "very much"). It is a psychometrically tested questionnaire with a structure corresponding to the SAS questionnaire. Three components of competition anxiety are assessed (somatic anxiety, concern, and concentration (decrease)). Somatic anxiety refers to the component of anxiety that is physically felt and associated with various signs of anxiety (palpitations, sweaty hands, queasy feeling in the stomach). This anxiety component includes athletes' propensity to have negative expectations, specific concerns, or self-doubt before a competition. The concentration (decrease) component is connected to being distracted by external influences during the ongoing competition (e.g., by spectator reactions). In addition to concern, this also includes the cognitive component of competition anxiety. Internal consistencies of the scales are generally $\alpha = .81$ for the somatic anxiety component, $\alpha = .83$ for concern scale, and $\alpha = .77$ for difficulty concentrating scale. Overall, these can be rated as satisfactory to good. In the present study, internal consistencies for both the somatic anxiety and concern components are $\alpha = .84$ and for concentration (decrease) scale $\alpha = .70$.

Additionally, there are two items in the questionnaire not related to the three sub-constructs. Employing these questions, the extent to which physical arousal on the one hand, and worrying thoughts on the other hand are perceived as performance-inhibiting vs. performance-enhancing is assessed. In addition to demographic variables, respondents were also asked whether they participate in horse shows (filter question) and if so, how many usually (prior to the COVID-19 pandemic), how many years of riding experience they have, which discipline they primarily belong to (e.g., dressage, show jumping, western, eventing) and which performance class they are in (7 = lowest, 1 = highest). Furthermore, participants were asked to state whether they had already experienced a critical situation or a loss of control with the horse and to what extent they considered themselves good riders.

2.3. Data Analysis

Items belonging to each of the three subscales were averaged for further calculations. One-way ANOVA tests were used to examine differences in equestrian disciplines and performance classes. Post-hoc paired-sample *t*-tests were performed using a Bonferroni corrected alpha level of 0.05 to indicate statistical significance. A *t*-test was used to analyze sex differences. Pearson's correlations tests were conducted between each of the WAI-T subscales according to equestrians' riding experience (in years), number of own horses, number of horse shows (per year), age, and riding expertise (self-assessment). Scores within this sample were compared to those of the norm sample by **[16]**. An α level of 0.05 was used to indicate statistical significance. Effect sizes were interpreted using Cohen's guidelines **[48]**. Eta squared values were consequently interpreted as follows: .01 = small effect, .06 = moderate effect, and .14 = large effect. Statistical analyses were conducted using SPSS 28.

3. Results

For this study, 406 competition riders were recruited (95% female, M_{age} 34.84, $SD = 11.18$). Percentages of equestrian disciplines and performance classes can be found in Figures **1** and **2**.

Participants had an average of $M = 25.15$ years ($SD = 10.70$) of riding experience. They took part in $M = 9.72$ ($SD = 7.73$) shows per year. Most respondents stated an experience of a critical (dangerous) situation ($n = 370$, 91.1%) and a loss of control with the horse ($n = 353$, 86.9%). For the three sub-constructs, whose values were derived from the sum scores of the respective items, a value of $M = 10.13$ ($SD = 3.12$) was found for somatic anxiety, $M = 10.21$ ($SD = 3.15$) for experience of concerns and $M = 7.27$ ($SD = 2.67$) for concentration (decrease). The normative sample refers to 414 athletes. These include 169 female (40.8% of the sample) and 245 male (59.2% of the sample) athletes from different sports and different performance levels **[16]**. Descriptive statistics can be found in Tables **1**, **2**, and **3**.

Female and male riders' values were above those of the norm sample of **[16]**. There were no significant differences between male and female riders for the three sub-constructs. However, analyzing the items on competition anxiety (the extent to which physical arousal and worrying thoughts are perceived as performance-inhibiting vs. -enhancing) revealed a significant difference in worrying thoughts before a competition between male and female riders with a moderate effect size, $t(404) = -2.91$, $p < .01$, Cohen's $d = -.62$. Female participants ($M = 1.71$, $SD = .74$, $n = 385$) were more likely to rate worrying thoughts as inhibiting performance compared to male participants ($M = 2.19$, $SD = .81$, $n = 21$). Compared to female and male respondents of the norm sample, participants' scores with regard to the three subcomponents are shown in **Table 1**.

Between the most frequently mentioned disciplines (dressage, show jumping, western, recreational, eventing), a univariate analysis of variance revealed significant differences for the concern component with a small effect, $F(5, 400) = 3.60$, $p < .01$, $\eta^2 = .04$, and for the concentration (decrease) subconstruct with moderate effect size, $F(5, 400) = 4.79$, $p < .001$, $\eta^2 = .06$. Post-hoc tests showed that differences in concern were between the dressage ($M = 10.67$, $SD = 3.18$, $n = 209$) and western ($M = 7.90$, $SD = 2.60$, $n = 7$) disciplines. Dressage riders showed higher concern scores than western riders.

Table 1: Descriptive results of investigated sample and normative sample.

	Study				Normative sample			
	Female equestrians		Male equestrians		Women		Men	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Somatic anxiety	10.19	3.14	9.05	2.64	9.16	2.75	7.91	2.33
Concerns	10.24	3.12	9.62	2.77	9.06	2.70	7.64	2.34
Concentration (decrease)	7.28	2.70	7.10	2.23	6.39	2.32	6.09	2.10

Note: *M* = Mean; *SD* = Standard Deviation

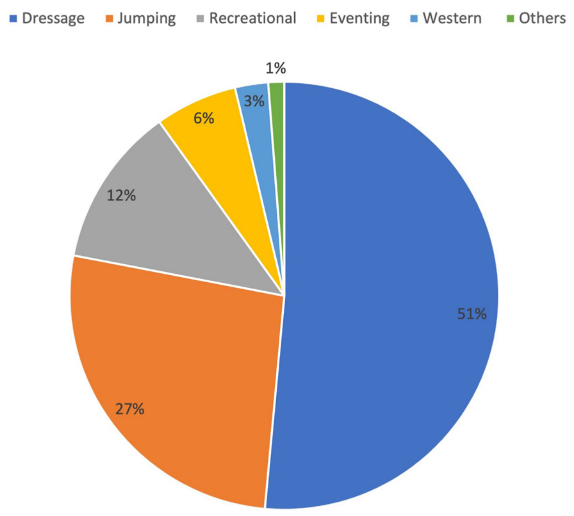

Figure 1: Divisions of the equestrian disciplines.

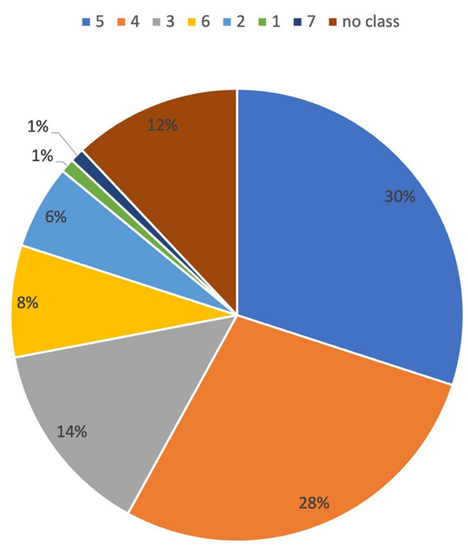

Figure 2: Divisions of the equestrian performance classes (7 = lowest, 1 = highest).

For concentration (decrease), differences were found between dressage (*M* = 7.57, *SD* = 2.68, *n* = 209) vs. show jumping (*M* = 6.69, *SD* = 2.34, *n* = 108) vs. eventing (*M* = 5.76, *SD* = 2.42, *n* = 25) and between show jumping vs. recreational riding (*M* = 8.22, *SD* = 2.95, *n* = 49). Consequently, dressage riders reported having greater difficulties with concentration than show jumpers and eventers, who reported this component as the least pronounced. In addition, recreational riders reported having far more difficulties with concentration than show jumpers.

Both of the other items related to competitive anxiety were also examined in individual disciplines. A significant result between the groups was found for physically felt arousal before a competition with a small effect size, $F(5, 400) = 2.53$, $p < .05$, $\eta^2 = .03$. Post-hoc tests yielded a difference between show jumpers (*M* = 1.71, *SD* = .75, *n* = 108) and dressage riders (*M* = 1.74, *SD* = .75, *n* = 209). Dressage riders reported feeling more physically perceived arousal before competitions than show jumpers.

Due to unequal sex distribution, a further calculation was conducted exclusively based on the female riders' data (*n* = 385). Similar results were found here. For the concern component, there was a significant result $F(5, 379) = 3.68$, $p < .01$, $\eta^2 = .05$, as well as for concentration (decrease), $F(5, 379) = 4.89$, $p < .001$, $\eta^2 = .06$. Post-hoc test results showed similar values as in the first calculation including male respondents (higher concern scores in dressage riders vs. western riders; higher concentration (decrease) scores in dressage riders vs. show jumpers vs. eventers; higher concentration (decrease) scores in recreational riders vs. show jumpers). In contrast, for the concern component, the difference between respondents of the disciplines dressage and western was not significant. For physically perceptible arousal before competitions, there was a non-significant result without the male riders' data, $F(5, 379) = 2.19$, $p = .054$ (higher scores in dressage riders vs. show jumpers).

In relation to different performance classes, a further ANOVA showed a significant difference in somatic anxiety with moderate effect size, $F(7, 398) = 3.86$, $p < .001$, $\eta^2 = .06$. The post-hoc test yielded differences between respondents in performance class 6 (*M* = 11.76, *SD* = 2.63, *n* = 34) compared to riders in performance classes 4 (*M* = 9.79, *SD* = 2.93, *n* = 114), 3 (*M* = 9.13, *SD* = 2.95, *n* = 56) and 2 (*M* = 8.70, *SD* = 3.31, *n* = 23). Equestrians having a lower-performance class showed higher somatic anxiety than those in higher performance classes.

There were no significant results regarding any other sub-constructs. Due to unequal sex distribution, only the female equestrian data was used to test the third hypothesis. Similar results were found here. For somatic anxiety, a significant result was obtained $F(7, 377) = 3.41$, $p < .001$, $\eta^2 = .06$. Post-hoc tests showed similar values as in the first calculation including male respondents (higher scores in equestrians with better performance classes).

Table 2: Descriptive results of disciplines.

	Constructs					
	Somatic anxiety		Concerns		Concentration (decrease)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Dressage	10.20	3.16	10.67	3.18	7.57	2.68
Show jumping	9.74	3.18	9.73	2.96	6.69	2.34
Western	10.30	3.40	7.90	2.60	6.70	2.54
Recreational	11.06	2.74	10.40	2.91	8.22	2.95
Eventing	9.56	2.83	9.44	3.65	5.76	2.42

Note: M = Mean; SD = Standard Deviation

Weak, negative correlations were also found between the assessment of one's own riding expertise and the three subconstructs of somatic anxiety (r = -.13, p < .01), concern (r = -.22, p < .01), and concentration (decrease) (r = -.13, p < .05). Equestrians having lower self-evaluations had higher anxiety scores. In addition, correlations were found between the scales examined, as can be seen in **Table 4**.

4. Discussion

The purpose of the present study was to investigate differences in competition anxiety among riders of various performance levels, disciplines, and sexes. New insights into the distribution of competition anxiety among riders in Germany were gained. These findings have theoretical and practical implications. Subcomponents of somatic anxiety, concern, and concentration (decrease) were found to be more pronounced in equestrians than in the normative sample **[16]**. Participants analyzed in the normative sample were non-equestrian athletes. In support of our hypotheses, equestrians might show higher competition anxiety scores than non-equestrian athletes due to higher potential risks associated with riding horses **[18–20,22,23]**.

In accordance with our hypothesis, female and male equestrians differed in worrying thoughts before competitions. This was one of the two additional questions in the questionnaire (WAI-T) that are not part of the three subcomponents (somatic anxiety, concern, and concentration (decrease)). Female respondents were more likely to rate worrying thoughts as inhibiting performance compared to male respondents. This result is in line with findings on sex differences in competition anxiety, including differences between female and male athletes **[7,42,43]**. Greater self-confidence scores in men than in women might be another explanation. However, the subcomponents of competition anxiety did not yield an (expected) difference between female and male equestrians. Aşçi *et al.* **[44]** reported no sex differences in either cognitive or somatic anxiety. As in other studies (e.g., **[31–33,38]**) the small number of male equestrians participating in the present study must be taken into account. Although this distribution is ecologically valid (the distribution of the sexes is in accordance with the rider population in German-speaking countries), the reason why no sex differences were found needs to be established by future research.

Importantly, there were significant differences among disciplines for components of concern and concentration (decrease), but not for somatic anxiety. Concern and concentration are mental aspects and they correlated with each other. Somatic anxiety involves physical aspects that are often related to mental dimensions, but obviously not always. Thus, a higher level of concern was associated with a higher decrease in concentration. Here, dressage riders reported being more anxious before horse shows than western riders. Concentration (decrease) was also significantly higher for dressage riders – in comparison to show-jumping riders and eventers, who had the lowest scores of all disciplines. In addition to dressage and show-jumping competitions, eventing competitions also include cross-country competitions, which represent an additional challenge and may require more courage because of the partially high obstacles and the higher speed. An assumption might be that fearful riders do not belong to these disciplines, but rather more courageous riders with less fear or those who have more self-confidence. Consistently, event riders showed better concentration performance as indicated by low values in concentration (decrease). Further differences related to riders in show jumping and recreational riding, with show jumpers having significantly lower scores.

Another difference was found in the reported physically noticeable arousal between dressage riders and show jumpers; arousal was higher for dressage riders. Schütz **[32]** also found the highest self-evaluation of self-efficacy among eventers compared to equestrians in other disciplines. In the same study, show-jumping riders rated their riding abilities highest. Dressage riders rated themselves as less self-efficacious and less good at riding. These findings could be used to explain the results of the present study. In this context, another study can also be referred to, which only focused on eventers **[38]**. The show-jumping phase had the largest impact on somatic anxiety and cognitive anxiety. The cross-country phase had the highest self-confidence mean score. Competition anxiety is thus also among eventers (as in the present study), but there is a lack of comparative possibilities with riders of other disciplines to be able to classify the equestrians interviewed by McGinn *et al.* with regard to their anxiety. The previous study can also be considered, at least to a limited extent, as an explanation of the present results, even though only show jumpers were examined here and therefore no differences between the disciplines could be focused on. Wolframm and Micklewright **[39]** investigated the effects of mental training on dressage riders. They did not give any reasons why they did not include equestrians from other disciplines in the study. This research could also serve as an explanation for the findings in the present study.

Table 3: Descriptive results of performance classes (7 = lowest, 1 = highest).

	Constructs					
	Somatic anxiety		Concerns		Concentration (decrease)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Performance class 1	8.75	2.99	10.75	1.26	9.25	3.40
Performance class 2	8.70	3.31	9.09	3.81	6.43	2.17
Performance class 3	9.13	2.95	9.54	2.64	7.11	2.51
Performance class 4	9.79	2.93	10.16	3.20	7.10	2.61
Performance class 5	10.54	3.16	10.45	3.29	7.23	2.68
Performance class 6	11.76	2.63	11.21	2.95	8.15	3.00
Performance class 7	11.00	2.94	11.00	2.16	6.50	1.91

Note: M = Mean; SD = Standard Deviation

Regarding the performance classes, a significant difference in somatic anxiety was recorded. Equestrians with the lower-performance class 6 had higher somatic anxiety than respondents with the higher performance classes 4, 3, and 2 (with no further differentiation among classes 4, 3, & 2). This could be attributed, for example, to less experience and routine in competitive sports for riders in lower-performance classes. Less experience can result in greater arousal. Based on more riding experience, badges in competitive sports as well as successes, higher-performance classes could be reached. This perspective is in accordance with findings by **[35]**, who suggest advanced riders have a better mood and thus less competition anxiety compared to novice riders. Experience and success may in turn be associated with higher self-efficacy and lower anxiety. There might also be an interaction between somatic fear, fine muscle control of a rider, and communication between horse and rider. Horses already sense small changes in the rider's posture **[33]**, for example, when the equestrian changes the muscle tension in a minimal way.

Similarly, riders who assessed their riding abilities better and trained more transferred less nervousness to their horse **[30]**. They were therefore also less nervous overall **[30]**. Münz *et al.* **[33]** found the equestrian level influenced the rider's posture. Professional dressage riders had a different (more efficient) posture compared to novice riders. This may have a positive or negative effect on the interaction with the horse and may be associated with less fear in professional equestrians with better posture. Beauchamp and Whinton **[37]** found equestrians with low levels of confidence to be more likely to feel unable to control their horse. This may be associated with higher anxiety. Meyers *et al.* **[36]** found elite riders score higher in anxiety management than non-elite riders, which is consistent with the present results. In addition to equestrians, their horses should also be taken into account. Most horses showing at higher levels probably also have more show experience than those horses competing at lower levels. Therefore, horses competing at lower levels may be more nervous, which could negatively impact their (probably) less experienced riders (increased rider anxiety).

Table 4: Correlations of the subcomponents of competition anxiety (1) with other variables (2-8).

	1	2	3	4	5	6	7
1 Somatic anxiety	-						
2 Concern	.60**	-					
3 Concentration (decrease)	.40**	.49**	-				
4 Riding experience (years)	-.13**	-.21**	-.04	-			
5 Number of own horses	-.18**	-.17**	-.07	.16**	-		
6 Number of horse shows	-.23**	-.07	-.13**	-.05	.28**	-	
7 Age	-.08	-.15**	.02	.84**	.13*	-.01	-
8 Riding expertise	-.13**	-.22**	-.13*	.16**	.05	.10*	-.01

Note: ** p <.01, * p <.05

4.1. Limitations

Although the sex distribution in the present sample corresponded to the sex distribution in equestrian sports, the comparatively low number of male respondents should be noted. In 2020, the proportion of women in competitive sports was around 87%. Similarly, most members (79%) of the German Equestrian Federation (FN, 2020) were female (n = 539,607). The distribution of horses by sex in 2019 also shows this trend (87% female; FN, 2020). It should be considered this study's results are primarily based on data from female equestrians and therefore cannot be simply extrapolated to male equestrians. Similarly, among males aged 14 and over, 0.32 million owned at least one horse; among females, the figure was twice as high at 0.64 million **[49]**. Furthermore, we would like to note that comparatively few female and male western riders participated in the study.

4.2. Directions for Future Research and Implications for Practice

In follow-up studies, aspects mentioned in the limitation section should be further considered, for example, a greater number of participants with respective subgroups regarding disciplines, performance classes, and sex distribution. In addition, the human-horse relationship and other facets of riding and riding skills should be included. It could also be investigated to what extent the respondents have already actively worked on their competition anxiety and whether this was effective. Furthermore, besides the characteristics of equestrians in competitions, attributes of horses could also be investigated, such as the horses' sex **[50]**.

In this context, longitudinal studies would be informative in addition to experimental designs. An experimental group could receive a treatment such as special anti-anxiety training and could be compared with a control group without treatment over a longer period of time with several measurement points.

People who did not participate in horse competitions were not included in this study. For them, a construct to be investigated would therefore not be competition anxiety, but riding anxiety as such. Nevertheless, it would be interesting to investigate to what extent they have a fear of riding per se - also in direct comparison with those who participate in competitions. Furthermore, a comparison between different sports in terms of fear of competition and related injuries would provide further insight. It could also be investigated to what extent fear plays a central role in human-horse interaction and the actual successes at horse shows. Dressage riders in particular were more anxious before competitions and had more concentration problems. For this reason, it is particularly advisable for female dressage riders to work on their anxiety and concentration problems. One possibility is mental training, which can be integrated into regular training and is associated with performance increases for dressage riders **[39]**. Wolframm and Micklewright **[39]** argued that improving mental skills like goal-setting or self-talk may reduce distracting thoughts and could be an explanation of better performance. Future research should also investigate the effect of levels of cognitive and somatic arousal on competitive performance, including the potentially moderating variable of self-confidence, as stated by Brand et al. **[31]**.

Differences found between disciplines could serve as a basis for specific training to specifically promote riders depending on the discipline. The results of the present study could also be passed on to riding coaches so that they can adapt their riding lessons accordingly. Focusing on the mental states of riders could also have a positive impact on competition success and training to control one's emotions and develop coping strategies. Further on, a discipline-specific, psychological profile (incl. competition anxiety) could also be included in the selection and promotion of squad members.

In practice, equestrians could be targeted by psychological profiling to identify personal risks, derive recommendations for appropriate disciplines, and design suitable opportunities for support. If equestrians, coaches, or support staff are better educated about the underlying causes of their fear and ways to improve coping processes, they can benefit not only regarding the personal experience of fear but also in terms of the interaction between humans and horses. Thus, the human-horse bond could be strengthened, which could prevent accidents. Besides risk minimization, the connection to this sport could also be increased. McGinn *et al.* **[38]** also refer to the idea of coaches or sports psychologists considering how to help equestrians manage their level of arousal according to the competition phase. For example, coping strategies can be developed and promoted to improve self-awareness. Consequently, arousal can have an optimal influence on the equestrian's performance. As a result, communication between horses and equestrians can be improved.

5. Conclusion

The present findings call for further research on (competitive) anxiety in equestrian athletes, which is increasingly being investigated but less so as in other sports. Specific differences among sexes and disciplines are reported and should be taken into account as critical aspects of an optimal psychological profile. These findings are relevant for athletes and professionals who aim for optimal (mental) training, competition preparation, and risk reduction in equestrian sports.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

In accordance with the ethics code of the American Psychological Association and the ethical standards of the sixth revision of the guidelines of the Declaration of Helsinki **[46]**, participants of the research were volunteers and gave their written informed consent. In order to protect human welfare all applicable international, national, and/or institutional guidelines for human participants were followed, and institutional ethics was granted by the local university. Data were treated anonymously.

References

[1] Becker E. Angst. München: Ernst Reinhardt; 2011.

[2] Betsch T, Funke J, Plessner H. [On the role of emotions in decisions]. In: Betsch T, Funke J, Plessner H, editors. [Thinking – judging, deciding, problem solving], Heidelberg: Springer; 2011, p. 123–34.

[3] Nesse RM. Evolutionary explanations of emotions. Human Nature (Hawthorne, NY) 1990;1. https://doi.org/10.1007/BF02733986.

[4] Ford JL, Ildefonso K, Jones ML, Aryinen-Barrow M. Sport-related anxiety: current insights. OAJSM 2017;8:205–12. https://doi.org/10.2147/OAJSM.S125845.

[5] Woodman T, Albinson JG, Hardy L. An investigation of the zones of optimal functioning hypothesis within a multidimensional framework. Journal of Sport and Exercise Psychology 1997;19:131–41. https://doi.org/10.1123/jsep.19.2.131.

[6] Gould D, Tuffey S, Hardy L, Lochbaum M. Multidimensional state anxiety and middle distance running performance: An exploratory examination of Hanin's (1980) zones of optimal functioning hypothesis. Journal of Applied Sport Psychology 1993;5:85–94. https://doi.org/10.1080/10413209308411307.

[7] Russell WD, Robb M, Cox RH. Sex, sport, situation, and competitive state anxiety. Percept Mot Skills 1998;86:816–8. https://doi.org/10.2466/pms.1998.86.3.816.

[8] Martin KA, Hall CR. Situational and intrapersonal moderators of sport competition state anxiety. Journal of Sport Behavior 1997;20:435–47.

[9] Moore LJ, Wilson MR, Vine SJ, Coussens AH, Freeman P. Champ or chump?: Challenge and threat states during pressurized competition. Journal of Sport and Exercise Psychology 2013;35:551–62. https://doi.org/10.1123/jsep.35.6.551.

[10] Burton D. Do anxious swimmers swim slower? Reexamining the elusive anxiety-performance relationship. Journal of Sport & Exercise Psychology 1988;10:45–61. https://doi.org/10.1123/jsep.10.1.45.

[11] Lavallée L, Flint F. The Relationship of Stress, Competitive Anxiety, Mood State, and Social Support to Athletic Injury. Journal of Athletic Training 1996;31:296.

[12] Hoja S, Jansen P. Mindfulness-based intervention for tennis players: a quasi-experimental pilot study. BMJ Open Sport & Exercise Medicine 2019;5:e000584. https://doi.org/10.1136/bmjsem-2019-000584.

[13] Ehrlenspiel F, Graf K, Kühn C, Brand R. [Stability and variability from competition anxiety]. Zeitschrift Für Sportpsychologie 2011;18:31–43. https://doi.org/10.1026/1612-5010/a000034.

[14] Patel DR, Omar H, Terry M. Sport-related performance anxiety in young female athletes. Journal of Pediatric and Adolescent Gynecology 2010;23:325–35. https://doi.org/10.1016/j.jpag.2010.04.004.

[15] Cheng W-NK, Hardy L, Markland D. Toward a three-dimensional conceptualization of performance anxiety: Rationale and initial measurement development. Psychology of Sport and Exercise 2009;10:271–8. https://doi.org/10.1016/j.psychsport.2008.08.001.

[16] Brand R, Ehrlenspiel F, Graf K. [Competitive Anxiety Inventory. Manual for comprehensive initial diagnostics of competition anxiety, competition anxiety and anxiety management mode in sport]. Köln: Sportverlag Strauß; 2009.

[17] [Most popular types of sport in Germany from 2019 to 2021, based on the population's interest in sport]. IfD Allensbach 2022. https://de.statista.com/statistik/daten/studie/171072/umfrage/sportarten-fuer-die-besonderes-interesse-besteht/ (accessed November 16, 2022).

[18] Bentley T, Page S, Meyer D, Chalmers D, Laird I. How safe is adventure tourism in New Zealand? An exploratory analysis. Applied Ergonomics 2001;32:327–38. https://doi.org/10.1016/S0003-6870(01)00011-4.

[19] Fleischer L, Faschingbauer M, Seide K, Kienast B. [Injury patterns in riding accidents]. Dtsch Z Sportmed 2016;2016:270–5. https://doi.org/10.5960/dzsm.2016.249.

[20] Heitkamp H-C, Horstmann T, Hillgeris D. Riding and handling injuries in experienced riders. Unfallchirurg 1998;101:122–8. https://doi.org/10.1007/s001130050244.

[21] Schröter C, Schulte-Sutum A, Zeckey C, Winkelmann M, Krettek C, Mommsen P. [Equestrian accidents]. Unfallchirurg 2017;120:129–38. https://doi.org/10.1007/s00113-015-0074-z.

[22] Sorli JM. Equestrian injuries: a five year review of hospital admissions in British Columbia, Canada. Inj Prev 2000;6:59–61. https://doi.org/10.1136/ip.6.1.59.

[23] Wallin L, Strandberg E, Philipsson J, Dalin G. Estimates of longevity and causes of culling and death in Swedish warmblood and coldblood horses. Livestock Production Science 2000;63:275–89. https://doi.org/10.1016/S0301-6226(99)00126-8.

[24] Tenenbaum G, Lloyd M, Pretty G, Hanin YL. Congruence of actual and retrospective reports of precompetition emotions in equestrians. Journal of Sport and Exercise Psychology 2002;24:271–88. https://doi.org/10.1123/jsep.24.3.271.

[25] Gomolla A. [Psychotherapy: Horses have a mirror function]. Deutsches Ärzteblatt 2014;12:356–7.

[26] McGreevy PD. The advent of equitation science. The Veterinary Journal 2007;174:492–500. https://doi.org/10.1016/j.tvjl.2006.09.008.

[27] Smith AV, Proops L, Grounds K, Wathan J, McComb K. Functionally relevant responses to human facial expressions of emotion in the domestic horse (Equus caballus). Biology Letters 2016;12:20150907. https://doi.org/10.1098/rsbl.2015.0907.

[28] Bornmann T, Randle H, Williams J. Investigating equestrians' perceptions of horse happiness: an exploratory study. Journal of Equine Veterinary Science 2021;104:103697. https://doi.org/10.1016/j.jevs.2021.103697.

[29] Keeling LJ, Jonare L, Lanneborn L. Investigating horse–human interactions: The effect of a nervous human. The Veterinary Journal 2009;181:70–1. https://doi.org/10.1016/j.tvjl.2009.03.013.

[30] von Borstel UU, Duncan IJH, Shoveller AK, Millman ST, Keeling LJ. Transfer of nervousness from competition rider to the horse. the Conference Proceedings 3rd International Equitation Science Conference, East Lansing: Michigan State University, MI USA; 2007.

[31] Wolframm I, Micklewright D. Pre-competitive levels of arousal and self-confidence among elite and non-elite equestrian

riders. Comparative Exercise Physiology 2008;5:153–9. https://doi.org/10.1017/S1478061509356133.

[32] Schütz K. [Believe in yourself and your horse! Expectations of self-efficacy and assessment of riding competence among riders]. Zeitschrift Für Psychologie Im Reitsport 2021:1–10.

[33] Münz A, Eckardt F, Witte K. Horse–rider interaction in dressage riding. Human Movement Science 2014;33:227–37. https://doi.org/10.1016/j.humov.2013.09.003.

[34] Iungano HM, Lancaster BE, Wolframm I. Relationship between performance strategies, resilience qualities, riding experience and competitive performance of show jumping riders. Comparative Exercise Physiology 2019;15:69–76. https://doi.org/10.3920/CEP180019.

[35] Wolframm IA, Shearman J, Micklewright D. A preliminary investigation into pre-competitive mood states of advanced and novice equestrian dressage riders. Journal of Applied Sport Psychology 2010;22:333–42. https://doi.org/10.1080/10413200.2010.485544.

[36] Meyers M, Bourgeois A, Leunes A, Murray N. Mood and psychological skills of elite and sub-elite equestrian athletes. Journal of Sport Behavior 1999;22:399–409.

[37] Beauchamp M, Whinton L. Self-efficacy and other-efficacy in dyadic performance: riding as one in equestrian eventing. Annual Conference of the British-Psychological-Society 2005;27:245–52. https://doi.org/10.1123/jsep.27.2.245.

[38] McGinn S, Alcock D, Cameron L j. A retrospective investigation of the impact of 'eventing phase (dressage, show-jumping and cross-country)' on pre-competition anxiety and self-confidence. Comparative Exercise Physiology 2019;15:269–81. https://doi.org/10.3920/CEP190007.

[39] Wolframm IA, Micklewright D. The effect of a mental training program on state anxiety and competitive dressage performance. Journal of Veterinary Behavior 2011;6:267–75. https://doi.org/10.1016/j.jveb.2011.03.003.

[40] Jones G, Swain A, Cale A. Gender differences in precompetition temporal patterning and antecedents of anxiety and self-confidence. Journal of Sport & Exercise Psychology 1991;13:1–15.

[41] Woodman T, Hardy L. The relative impact of cognitive anxiety and self-confidence upon sport performance: a meta-analysis. Journal of Sports Sciences 2003;21. https://doi.org/10.1080/0264041031000101809.

[42] Fernandes MG, Nunes SAN, Raposo JV, Fernandes HM. Factors influencing competitive anxiety in Brazilian athletes. Brazilian Journal of Kinanthropometry and Human Performance 2013;15:705–14. https://doi.org/10.1590/1980-0037.2013v15n6p705.

[43] Correia ME, Rosado A. Anxiety in athletes: Gender and type of sport differences. International Journal of Psychological Research 2019;12:9–17. https://doi.org/10.21500/20112084.3552.

[44] Aşçi FH, Koca C, Demirhan G, Dinç SC. Precompetitive anxiety and affective state of climbers in indoor climbing competition. Percept Mot Skills 2006;102:395–404. https://doi.org/10.2466/pms.102.2.395-404.

[45] Hogg RC, Hodgins GA. The relationship between trait anxiety, trait competitive anxiety, riding experience, accident/injury status, and state competitive anxiety for equestrian athletes. Journal of Veterinary Behavior 2010;5:211–2. https://doi.org/10.1016/j.jveb.2009.11.011.

[46] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for Medical Research involving human subjects. JAMA 2013;310:2191–4. https://doi.org/10.1001/jama.2013.281053.

[47] Smith RE, Smoll FL, Schutz RW. Measurement and correlates of sport-specific cognitive and somatic trait anxiety: The sport anxiety scale. Anxiety Research 1990;2:263–80. https://doi.org/10.1080/08917779008248733.

[48] Cohen J. Statistical power analysis for the behavioral sciences. Hillsdale: NJ: Erlbaum; 1988.

[49] Number of people who personally own a horse by gender in 2019. Statista 2019. https://de.statista.com/statistik/daten/studie/959897/umfrage/umfrage-in-deutschland-zum-persoenlichen-besitz-eines-pferdes-nach-geschlecht (accessed November 16, 2023).

[50] Whitaker TC, Olusola O, Redwin L. The influence of horse gender on eventing competition performance. Comparative Exercise Physiology 2008;5:67–72. https://doi.org/10.1017/S1478061508017039.

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Review Article Open Access

Donkeys in Brazil: Bibliometric Mapping and Breed Information

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Abstract

In this paper, we looked at the distribution of donkeys (*Equus asinus*) in Brazil as well as the research being carried out with them. We used bibliographic mapping techniques to identify major themes, timelines, and research groups working with these animals. Donkeys are essential for producing mules (*Equus mulus*), and, especially in the Northeast region, they are vital in cattle production in Brazil. Nevertheless, there is little research being carried out, which may affect their survival in the long term, thereby impacting agricultural systems. Most research is on health and reproduction issues, and little is seen in production aspects related to genetics and nutrition. The body indices for Brazilian donkeys show that they vary from medium (Paulista) to small (Northeastern and Pêga) animals with an aptitude for strength (Body Index >0.90). The Northeastern and Pêga tend to have poorer thoracic development, but all show good traction capabilities (Dactyl Thoracic Index, Conformation Index). Northeastern donkeys are smaller animals and have lower load-carrying capabilities. This paper shows various areas where research in donkeys could be expanded.

Keywords

Citation analysis, *Equus mulus*, local breed, Northeastern, Paulista, Pêga

1. Introduction

In the letter from Pero Vaz de Caminha (the clerk of the fleet commanded by Pedro Álvares Cabral, who arrived in Brazil in April 1500) to the King of Portugal, it is evident that at that time farm animals did not exist in Brazil.

They neither plough nor sow. There are no bulls, cows, goats, sheep, chickens, or any other animal here, which is customary for men to live. Nor do they eat anything but this yam, which has been here for a long time, and this seed and fruit, which the earth and the trees shed. And with this they go so and so upright and so naive, that we are not so much, with how much wheat and vegetables we eat.

The colonization of America meant that the Portuguese and Spaniards brought animals from the Iberian Peninsula and North Africa to the new continent [1].

Donkeys belong to the kingdom Animalia, phylum Chordata, class Mammalia, order Perissodactyls, family Equidae, genus Equus. In 2003, the International Commission on Zoological Nomenclature decided that if domestic and wild species are considered subspecies of each other, the scientific name of the wild species takes precedence. This meant that the traditional name of the donkey, *Equus asinus asinus,* was changed to *Equus africanus asinus* [2]. The donkeys are divided into two trunks: the European trunk, *Equus asinus europeans*, probably originating in the Mediterranean region, and the African trunk, *Equus asinus africanus*, originating in North Africa, the Nile basin or Abyssinia (present-day Ethiopia) [3,4].

Donkeys are thought to have been domesticated in northeastern Africa [5,6]. The oldest Egyptian monuments showed illustrations of donkeys from Abraham's journey to Egypt, as the donkey is mentioned in the book of Genesis (Genesis 12:16; Genesis 22:3) [7], confirming that the domestication of the donkey predated that of the horse. Domestication was thought to have occurred around 7,000 years ago [8]. The occurrence of domestic donkeys is thought to date from 4500 BC [9], with these animals appearing on an Egyptian palette dated to c. 3100 BC. Pathological damage from working donkeys was seen in animals buried with an Egyptian king outside Abydos c. 3000 BC. Donkeys were also found in the Near East around this time. Nomadic populations brought donkeys across the Sahara to Egypt and these populations still use donkeys today [10]. Regarding domestication, the prevailing idea is that the donkey, although used later than the horse in Europe, was used more remotely in Africa and Asia [8]. Greenfield *et al.* [11] showed the use of mouth bits as early as 2800-2600 BC in the Middle East.

The donkey (*Equus asinus*), also called asinine, ass, or donkey is a medium-sized perissodactyl mammal with a long snout and ears, used since prehistoric times as a pack animal. Its origin is linked to Abyssinia, where it was known as an onager or wild ass [12]. Evidence also shows that the African wild ass (*Equus africanus*) is the donkey's ancestor [5] and, in Western Asia, wild onagers were later crossed with donkeys [13]. These animals arrived in Europe brought by Greek wine merchants [14] and, according to this author, in Greece and Rome, all forms of equine medicine were called mulomedicina.

Christopher Columbus took donkeys on his first journeys to the American continent [15]. The discovery of this region and the later establishment of trade between Europe and the Americas meant that donkeys spread across the Americas. Both Portuguese and Spanish colonizers introduced the species during this period. The donkeys brought from the Madeira and Canary Islands by Martin Afonso de Souza were the first to be introduced in Brazil around 1534 [16,17], to the town of São Vicente (São Paulo State, Brazil). Later, Tomé de Souza brought donkeys from Cape Verde to Bahia State (in the year 1549) in the caravel [1] "Golf". The Spaniards introduced the species through two principal routes: i) the Bay of Panama, with animals introduced in the Antilles, which led to the spread of donkeys throughout Northern South America (Colombia, Venezuela, Ecuador, Peru, and northern Brazil), and (ii) the Rio de la Plata region (Argentina), introducing these animals to the southern cone of the continent [18,19]. At the same time, livestock were also introduced in areas of Portuguese domination. Brazil's southeastern and northeastern regions received these animals during the 15th and 16th centuries [20]. At the end of the last century [4], Italian and Spanish donkeys were imported and promoted by immigrants and the Ministry of Agriculture.

According to [21], the human movement in Brazil in the 19th century depended on the use of mules. After introducing these animals into the Brazilian economic structure during the colonial period, mules became the means of transport *par excellence* in the non-coastal regions of imperial Brazil [22]. The coffee and sugarcane crops depended on the services of the troops, both for the flow of production, as well as for the regional supply of products from other locations. The mule troops were reared mostly in the southern region of Brazil, so these animals traveled a long and very difficult route until they reached the places of demand for their services, which were mainly in the provinces of São Paulo and Minas Gerais. A route called "Caminho das Tropas" was created, linking the regions south and centersouth of the Brazilian territory [21], with the aim of facilitating the movement of the animals.

Overall, 14.5% of the equine population in Brazil are mules, and 8.9% are donkeys [23]. The distribution varies by region and state **(Figure 1)**, varying from 60% of the equines in the northeast and 3% in the south, with the highest percentages in the states of Ceara, Paraiba, and Piaui. Almost 100% of farms in the NE have these types of equines, compared with 6% in the South. There are approximately 380,000 donkeys in Brazil compared to 620,000 mules. While the donkey population is concentrated in the Northeast region, significant mule populations can be found in Minas Gerais (75,000), Mato Grosso (49,000), and Pará (83,000) States, among others. These states are significant for cattle production [24].

In Brazil, the main interest in donkeys is summarized in the donation of semen to produce mules, although donkey herds are of high genetic value [25]. In the Northeast, there are more donkeys than mules (27% more), while in the centerwest there are 90% more mules than donkeys. For centuries, a cross between donkey (2n = 62) and horse (2n=64) has been carried out. The mule (male donkey x female horse) and the reciprocal cross, the hinny (male horse x female donkey) are the most common equine hybrids [26], and both progenies are sterile (2n = 63). These hybrids have been seen to be robust, able to adapt to adverse environments, and docile, and are widely used on farms in Brazil [27]. As such, donkeys can be found on 237,575 farms and mules on 281,491 farms.

The decline in the number of donkeys in Brazil and other equids began with the introduction of engines used in cars. Because of that, according to [28], they began to be abandoned and reproduced indiscriminately, and today they are found in large numbers adrift in the Brazilian semiarid region, causing car accidents and overloading state authorities that are responsible for their capture and care [29].

Starting in 2016, Brazil has exported the hide of donkeys [30] to produce a medicine known as ejiao or *Colla corii asini* – CCA, a gelatine extracted from the skin of donkeys, an ingredient in tonics and face cream used in traditional Chinese medicine, popular in China [31]. There is no scientific proof that it works. Still, in the Asian country, ejiao is consumed in a variety of ways, such as in teas and cakes, and is used with the promise of treating various health problems, including anaemia, circulatory and reproductive problems, especially in women, in addition to insomnia such as irregular menstruation, anaemia, insomnia, and even sexual impotence. The free-ranging donkeys mentioned above were initially siphoned off to meet this trade [32].

[1]small, fast Spanish or Portuguese sailing ship of the 15th–17th centuries.

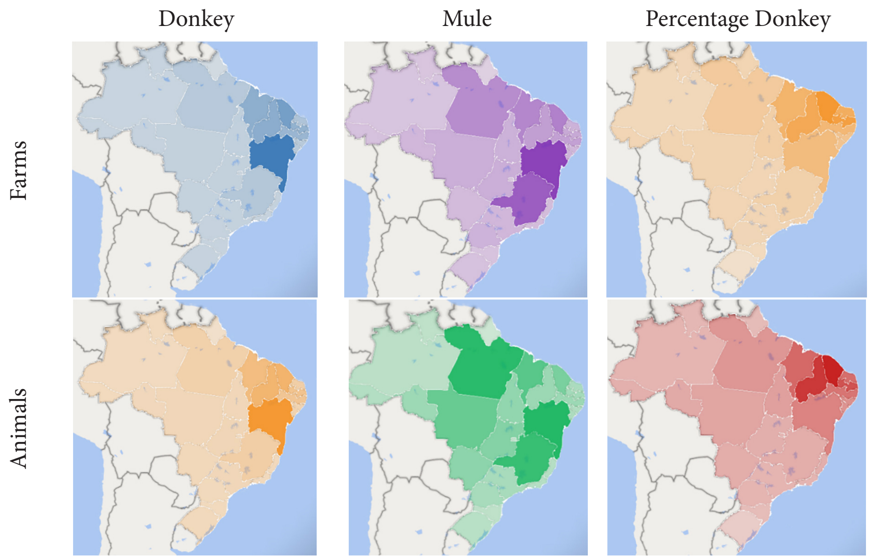


Figure 1. Heat maps for the distribution of donkeys and mules in Brazil.

To manufacture the product, the animals are collected from the caatinga and rural areas of the Northeast in large volumes, without a production chain that renews the herd, being slaughtered faster than the reproduction capacity. Donkey slaughter for the sale of skin on the foreign market, both legal and illegal, is threatening the existence of these animals. Queiroz *et al*. **[33]**, considering only the records of the Ministry of Agriculture, Livestock and Supply (MAPA), showed that slaughter increased by more than 8,000% between 2015 and 2019 when 91,645 animals were killed. Between 2010 and 2014, there were just over 1,000 slaughters across the country. The Federal Court decided to suspend the slaughter of donkeys in Brazil for export to China (3/2/22)[2].

The Brazilian donkey breeds of Brazil, originating from animals brought by colonizers, underwent natural selection, developing adaptation characteristics in different environments **[34]**. There are three main breeds of donkeys in Brazil: Northeast (Nordestino Ecotype), Brazilian or Paulista, and Pêga.

Brazilian donkey breeds have haplotypes of origin in common with Asian and European breeds, originating from the somaliensis trunk **[35]**, although this study only looked at Northeastern donkeys. Jordana *et al*. **[19,36]** also looked at this ecotype and found high admixture. A subdivision was observed in the somaliensis trunk, which suggests the existence of two distinct haplogroups from this origin or the possible presence of more domestication centers. Almeida **[28]** found, comparing the Paulista, Pêga, and Nordestino donkeys, that the Paulista has lower nucleotide diversity in the mtDNA control region than the other breeds. Alves *et al*. **[37]**, looking at the mitochondrial control region (D-loop) in the three breeds, found five mitochondrial haplotypes with 19 polymorphic sites, two of them exclusively found in the Nordestino donkey. This latter group was found to be distinct from the other two groups. This study also found maternal influence of both Nubian and Somali clades in the formation of Brazilian donkeys, with the Pêga and Paulista being closer to the *Equus africanus somaliensis* and the Northeastern closer to *Equus africanus africanus*. According to **[37]**, the greatest genetic distances between groups were observed for the Paulista and Nordestino donkeys, and the smallest distances between Pêga and Paulista. These authors also noted a high level of structuring and differentiation among Brazilian donkey breeds. It is important to note that these genetic studies may be influenced by sampling bias (for example, **[35]** only sampled in Ceará state).

[2]https://www.yourhorse.co.uk/news/donkey-slaughter-banned-brazil/#:~:text=Brazil%20has%20reinstated%20a%20ban,in%20Brasilia%20on%203%20February

1.1. Northeastern Donkey

These animals were important in the regional development of the northeastern region of Brazil and are considered a cultural symbol **[29]**. It is believed that the Northeastern (Nordestino) donkeys descend from North African animals, via the Portuguese islands such as Madeira, Santiago de Cabo Verde, and São Tomé. It is currently not a breed *per se*, but a group of animals that vary phenotypically (ecotype) in terms of height, color, head size, etc. Alves *et al*. **[37]** found high haplotype and nucleotide variability in this ecotype, indicating a lower level of artificial selection.

This ecotype is found from the south of Bahia to Maranhão States. The Northeastern donkey is of great service in that region, where it is used for agriculture and transport with low-income populations. It emerged from the need for a strong, resistant working animal, that was adapted to the harsh Caatinga semiarid biome. It is used for riding, traction, and plowing, although it is occasionally consumed as food in the Northeast **[4]**. They were very abundant, but with the mechanization of the countryside, the use of trucks to transport cargo, and the use of motorcycles as a means of transport, their use became increasingly restricted. Many animals were released and are now roaming free, often causing traffic accidents on northeastern roads and other problems **[29]**.

In 1954, thousands of Northeastern donkeys were used to manufacture rabies vaccines. The donkey also suffered a 75% reduction in its herd between 1967 and 1981 **[28]**. Since then, the population has been decreasing yearly, due to the establishment of slaughterhouses in the region, and the indiscriminate slaughter to export meat for pet food. Much of the Brazilian donkey meat is exported to Russia and Vietnam **[38]**, with Belgium also being a historical importer. These authors also show that Italy and Portugal import raw skins and hides, while Mexico is the main importing country for live animals, but significant volumes (9,000 animals) were only seen in 2019. Exports are also highly variable, due to unconsolidated markets.

1.2. Pêga

The donkeys that gave rise to the Pêga may have been introduced in the time of D. João VI, coming from Egypt directly or via Abyssinia, where Portugal maintained regular trade. If there were crosses with Italians, these were recent. The second theory says that the Pêga descends from the cross between the Italian and Andalusian European breeds and the African donkey. The Egyptian breed is the one that is nearest to the Pêga **[37]**, and there are two points of note:

1. The occurrence of the white coat is frequent in the Egyptian donkey, and no other variety shows this, neither the *Equus asinus africanus* nor the European *Equus asinus*.
2. The presence of a star and white limb ends found on the Egyptian donkey. A mixed origin was admitted, as an exclusive introduction of the African trunk was deemed unacceptable in the breeder's association. There was the introduction of Italian, Andalusian, and Egyptian animals. The characteristics of *Equus asinus africanus* prevail.

The breed originated at the beginning of the last century, around 1810, on the Cardume farm belonging to the priest from Minas Gerais Manoel Maria Torquato de Almeida, in the municipality of Entre Rios de Minas **[39]**. They started crossbreeding using Italian and Egyptian strains and a subsequent selection of the best animals. The breed gained strength on a farm in Lagoa Dourada, a municipality in Minas Gerais near São João del Rei, by Colonel Eduardo José de Resende, owner of the Fazenda do Engenho Grande dos Cataguazes. In 1847, they bought two males and seven females from Father Manoel and continued the improvement in successive crossings, with special care for standardization. Colonel Eduardo donated donkeys to his children, thus preserving the breed's history and formation.

The name Pêga was given by the custom in Lagoa Dourada of branding these animals by the fire with a symbol that looked like the handcuff (two iron rings) used to hold slaves by the ankle, called Pêga. The donkeys that gave rise to the breed were branded by fire by their owner, representing that device. Thus, all the animals of this original group had the Pêga brand and, thus, were recognized as a breed with the same name.

The demand for mule production came with the increase in mining in the 18th and 19th centuries in Minas Gerais, and interest in the Pêga donkey increased. The breed has a march gait and passes this trait on to asinine and mule descendants, who are good for riding and pulling carts, in addition to physical strength and endurance. The animals are rustic. Nowadays, the Pêga donkey is no longer concentrated in Minas Gerais, being found on farms throughout Brazil. Todd *et al*. **[8]** showed that Pêga is genetically linked to Iberian donkeys. According to **[37]**, the variability of the Pêga breed is intermediate between the Paulista and Nordestino.

1.3. Paulista or Brasileiro

As the name says, this breed originates from the State of São Paulo (SP), southeastern Brazil. There is a similarity with the Pêga regarding its suitability for work, used for riding or traction. It may have originated from crosses between Egyptian donkeys and/or from crosses between European Italian (predominantly from Sicily), Andalusian, and African donkey breeds. In Brazil, they were crossed with donkeys from Portugal and called Paulista donkeys **[16]**. According to Glass **[40,41]**, during the Constitutionalist Revolution, a battalion (Rio Pardo Cavalry Regiment) of soldiers mounted on donkeys was formed in Barretos -SP. Alves *et al*. **[42]** found this breed to be genetically close to Italian breeds, while Alves *et al*. **[37]** found low intrapopulation variability thereby indicating the need for studies on maintaining its genetic diversity. This was also seen in **[43]**, using pedigree data, where highly inbred animals and excessive contributions of a few ancestors were found in the breed.

According to **[44]**, the Paulista breed is predominant in the state and is used to produce medium-sized mules. Although the Association establishes a lower height than the exotic donkeys (Poitou, Spanish, and even Italian), it can produce excellent mules when the mares are of good stature. As it is a wider animal (chest and croup) than the Pêga breed, the animal has been used to transport coffee.

The Brazilian Donkeys Association was founded in 1939 **[16,17]**, but there is no record of this association[3]. The Brazilian donkey was included in the Genetic Resources Conservation Program **[1]**, although there are few published studies on the genetics of this breed. According to that institution's website, there was a Nucleus for the Conservation of Brazilian Donkeys of the Animal Science Institute in Sao Paulo State, which is also deactivated.

1.4. Size and Body Indices

The body indices for Brazilian donkeys **(Table 1)** show that they vary from medium (Paulista) to small (Northeastern and Pêga) animals with an aptitude for strength (Body Index >0.90). It should be noted that these indices are calculated using those available for horses, as none are available specifically for donkeys.

The Northeastern and Pêga tend to have poorer thoracic development, but all show good traction capabilities (Dactyl Thoracic Index, Conformation Index). Northeastern donkeys are smaller animals and have lower load-carrying capabilities than the other two breeds.

[3]https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/registro-genealogico/arquivos/AssociaesatualmenteregistradaspeloMAPA1Plan1.pdf

Table 1: Size and Body Indices for Brazilian Donkeys.

	Abbrev	Pêga [45,46]	Northeastern [47,48]	Paulista [49]	Aptitudes
Chest Circumference (m)	CC	1.48	1.26	1.47	
Body Length (m)	BL	1.41	1.14	1.32	
Croup Height (m)	CH	1.36	1.10	1.26	
Withers Height (m)	WH	1.31	1.09	1.20	
Chest Width (m)	CW	0.32	0.25		
Cannon Bone Circumference (m)	CB	0.17	0.14	0.17	
Hip Width (m)	HW	0.46	0.35		
Mid-back Height	MBH	1.34	1.10	1.23	
Space Under Horse	SUH	0.77		0.63	
Weight (kg)	W[1]	290	190	397	Weights > 550 kg are large or hypermetric; between 350 and 550 kg, medium or eumetric; and < 350 kg small or ellipometric.
Calculations are based on [50,51]					
Body Index	BL/CC	0.95	0.91	0.90	Elongated animal < 0.85 (speed); medium animal between 0.86 and 0.88; and short animal > 0.90 (strength).
Thoracic Development (TD)	CC/WH	1.13	1.14	1.23	Values above 1.2 indicate animals with good TD.
Pectoral Index	MBH/SUH	1.74	1.41	1.95	If the mid-back height (MBH) < the space under the horse (SUH), the animal is considered "far from the ground", favoring speed due to relatively long legs.
Conformation Index 1	CC^2/WH	1.67	1.43	1.80	
Dactyl Thorax Index	CB/CC	0.115	0.114	0.123	Not less than 0.105 in light animals, up to 0.108 in intermediary, up to 0.110 in light traction animals, and up to 0.115 in heavy traction equines. This index also indicates thoracic development.
Conformation Index (Baron & Crevat)	CC^2/CH	1.61	1.45	1.72	The saddle horse must show a CI of around 2.1125. Values above this indicate animals for traction.
Load Index 1	$(CC^2*56)/CH$	90	80	96	The weight in kg that an animal can support without exaggerated force at trot or gallop.
Load Index 2	$(CC^2*95)/CH$	153	135	164	The weight in kg that an animal can support without exaggerated force at a walk.
Riding Comfort degree	BH – (WH+CH)/2	1.37	1.06	1.26	The inclination at the point on the back where the saddle sits.
Compact Index 1	(W/CH)/100	2.13	1.80	3.15	Equines for heavy traction > 3.15; Close to 2.75 indicate light traction and 2.60 for saddle animals.
Compact Index 2	[W/(CH-1)]/100	8.06	31.77	15.27	> 9.5 for heavy traction relative to size, 8.0 to 9.5 for light traction, and 6.0 to 7.75 for saddle animals.

[1]CC3*80 when not measured directly.

1.5. Publications About Donkeys in Brazil

A survey of publications on donkeys in Brazil was carried out in SciVal® based on Scopus®. Publications were analyzed in VosViewer® to identify the main authors and themes. A more detailed explanation of this analysis can be found in [52]. The bibliographic mapping (**Supplementary File 1**; **Figure 2**) shows 170 documents, with groups based in the northeast and southeast, with newer groups emerging. Although it is a Brazilian theme, there is interaction with groups abroad, mainly with studies on diseases, reproduction, and genetics.

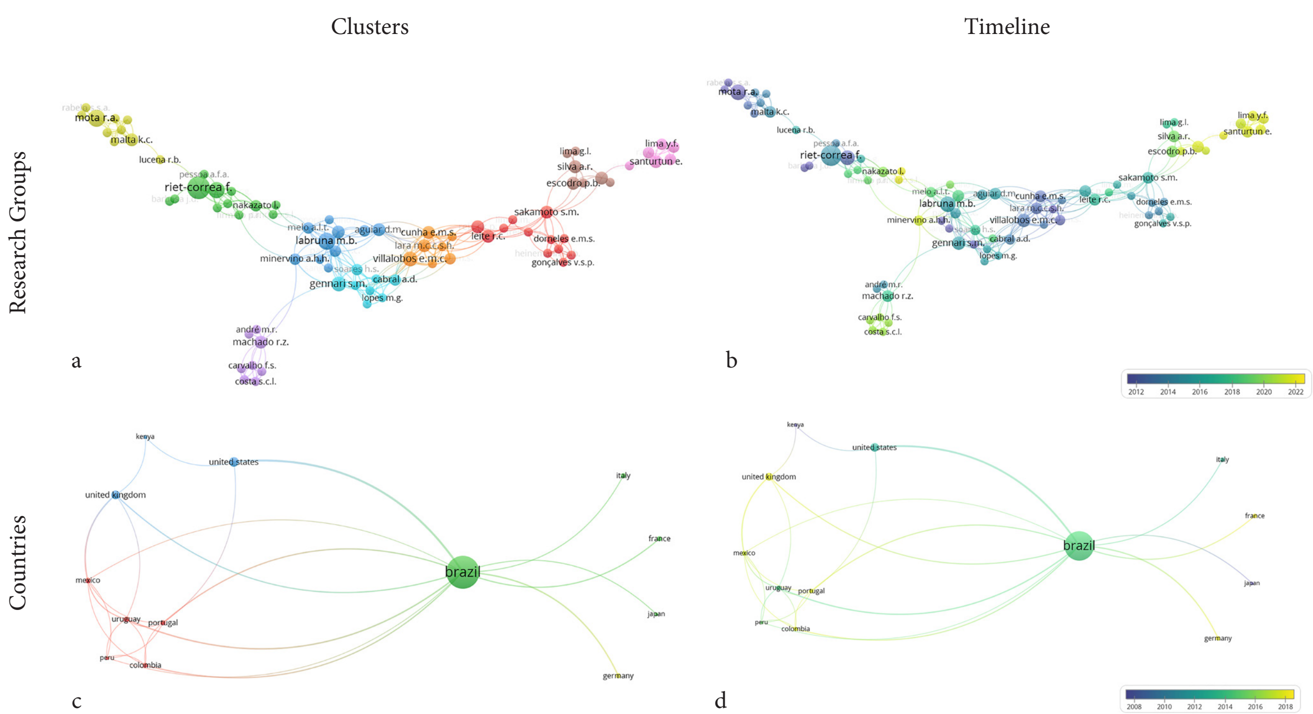
These links are relatively new **(Figure 2d)**. Author groups are clustered by location rather than by topic, including the northeast region (yellow and green) and São Paulo (blue). This may be due to the breed or specific regional issues and production system differences, as stated above. Animal health is the major area of research, followed by reproductive issues. Animal welfare is a recent problem, especially related to ejiao and sustainability.

It is noted that there are few documents on Brazilian (Paulista) donkeys **(Table 2)** and these are concentrated in Brazilian journals **(Figure 3)**. Major financing agencies of these studies are the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 30 papers), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – 19 papers) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG – 9 papers). Only 108 papers have funding information included.

Although the number of documents per year varies widely **(Figure 3d)** there has been a steady increase since 2006 ($y = 0.87x - 1737.3$ and $R^2 = 0.48$), with only 13 papers found before this date. The focus on infection and disease in the titles and keywords in papers is evident (**Figures 3e** and **3f**). This is also evident in the number of citations about donkeys in Brazil **(Table 2)** and cited articles about donkeys in Brazil **(Table 3)**.

Table 2: Authors with the highest number of citations about donkeys in Brazil.

Author	Institution	cluster	Documents	Citations
Labruna M.B.	USP	2	5	229
Gennari S.M.	USP	2	6	184
Horta M.C.	UFVSM	2	2	155
Riet-Correa F.	UFCG	1	12	91
Machado R.Z.	UNESP	7	4	63
André M.R.	UNESP	7	2	60
Freschi C.R.	UNESP	7	2	60
Martins T.F.	USP	2	3	58
Mota R.A.	UFRPE	4	4	56
Cabral A.D.	USP	2	3	46
Soares H.S.	USP	2	3	44
Dantas A.F.M.	UFCG	1	6	42
Lopes M.G.	USP	2	2	42
Pena H.F.J.	USP	2	2	42
Vitaliano S.N.	USP	2	2	42
Villalobos E.M.C.	IB/SP	8	4	41
Melo A.L.T.	UFMT	2	2	32
Pacheco R.C.	UFMT	2	2	32
Lima J.M.	UFERSA	3	2	30
Cavalcante P.H.	INTA	2	2	28



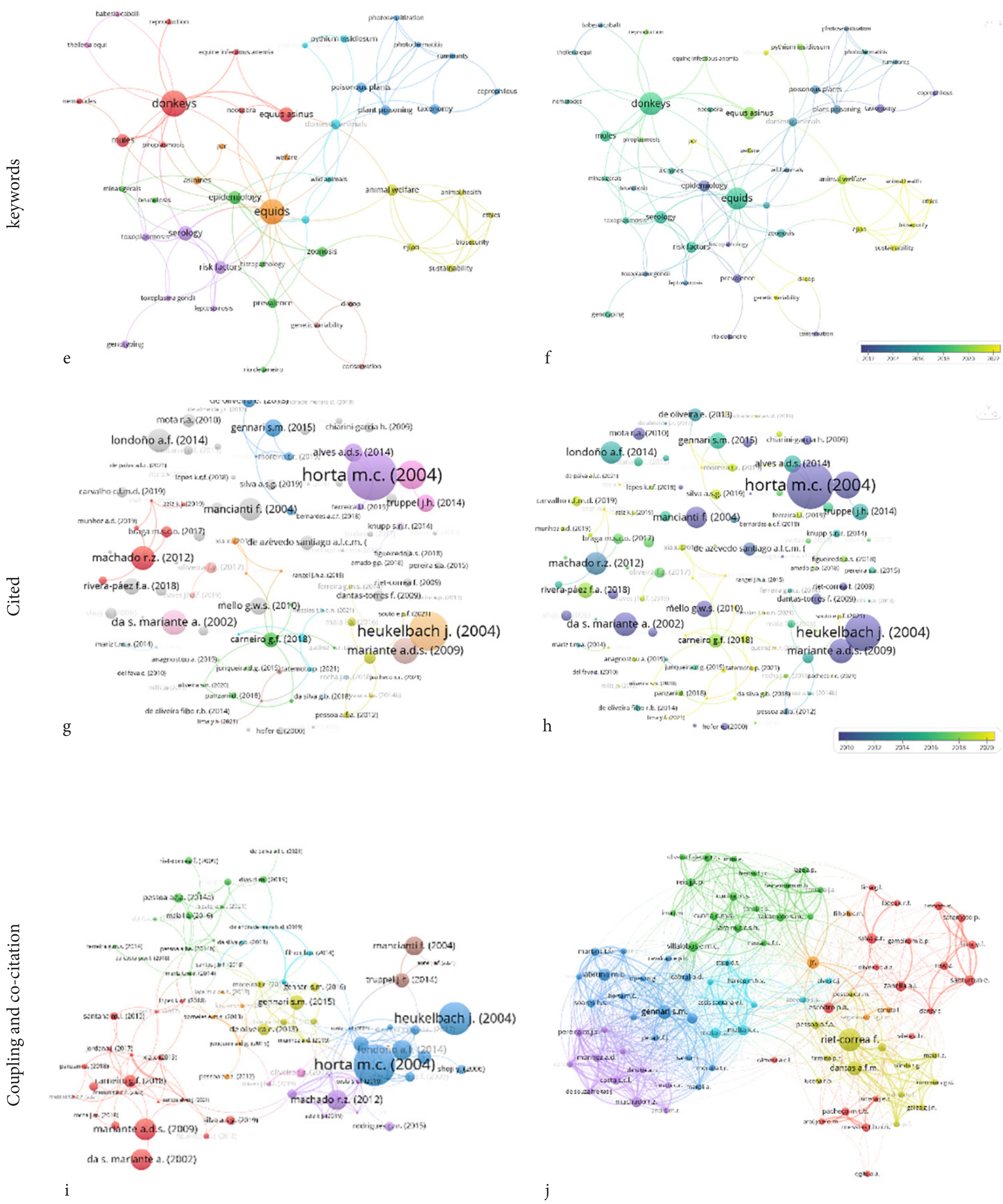


Figure 2: Bibliographic mapping on donkeys in Brazil showing **a)** major research groups, **b)** their evolution, **c)** main countries publishing on Brazilian donkeys, **d)** their evolution, **e)** principal keywords, **f)** their evolution, **g)** major cited papers, **h)** evolution of paper citation, **i)** major papers in bibliographic coupling, and **j)** major papers in co-citation analyses.

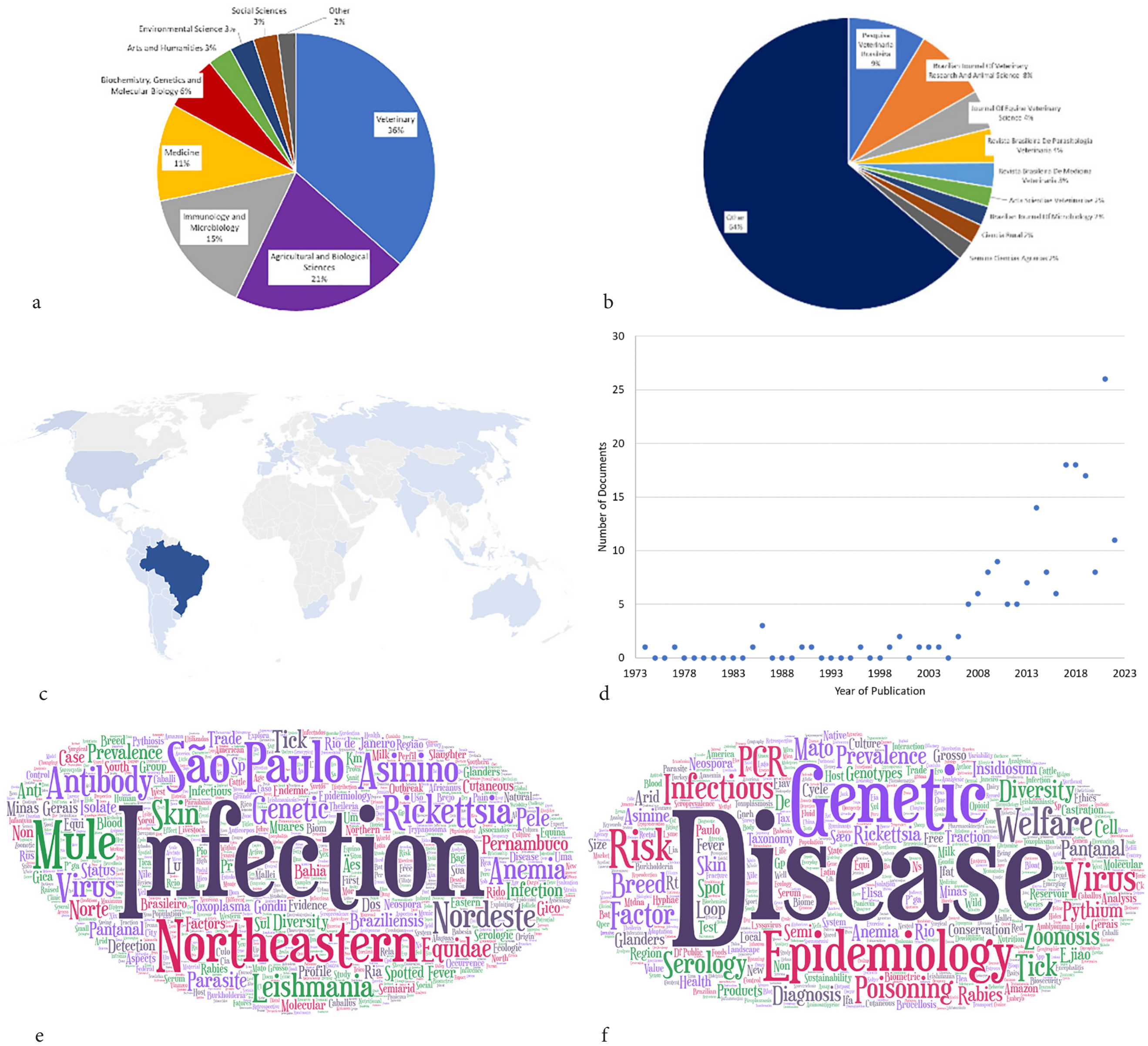


Figure 3: Information on Brazilian publications on donkeys **a)** area of knowledge, **b)** publication source, **c)** collaborating country, **d)** number of papers per year, **e)** cloud of words in the title, and **f)** cloud of words in keywords.

The top cited articles **(Table 3)** show citations for information on the general conservation of genetic resources **[1,53]**, but none on specific issues linked to donkey conservation. Most are linked to various types of viral and other infections and internal and external parasites **[54,55]**, many at a state or municipality level **[56,57]**. These studies show donkeys tend to be rustic, docile animals, used to foraging local native plants, resistant to diseases and parasites, and can undergo physical exertion. As such, the authors show that functional physical conformation for this purpose is preferred in this animal. Several other areas of studies with these animals are not investigated, such as nutrition, production, etc., or do not appear in the Scopus database. This "institutional neglect", as coined by **[58]**, may soon affect the farming structure in Brazil, given the importance of these animals for cattle farming and the production of mules. Unfortunately, recent studies on equine diversity in Brazil using Single Nucleotide Polymorphisms **[59]** did not include donkeys. Genetic studies to date, as stated above, are based on mitochondrial DNA (mtDNA) and microsatellites.

Table 3: Top 20 cited articles about donkeys in Brazil.

Author (year)	URL	Cluster	Citations
Horta M.C. (2004) [60]	https://doi.org/10.4269/ajtmh.2004.71.93	3	127
Heukelbach J. (2004) [61]	https://doi.org/10.1111/j.0269-283x.2004.00532.x	3	91
Mariante A.S. (2009) [1]	https://doi.org/10.1016/j.livsci.2008.07.007	1	50
Mariante A. (2002) [53]	https://doi.org/10.1016/s0093-691x(01)00668-9	1	48
Machado R.Z. (2012) [62]	https://doi.org/10.1016/j.vetpar.2011.11.069	5	45
Londoño A.F. (2014) [63]	https://doi.org/10.1016/j.ttbdis.2014.04.018	3	44
Mancianti F. (2004) [64]		8	41
Alves A.D.S. (2014) [65]	https://doi.org/10.1603/me14042	3	30
Gennari S.M. (2015) [66]	https://doi.org/10.1016/j.vetpar.2015.01.023	4	28
Truppel J.H. (2014) [67]	https://doi.org/10.1371/journal.pone.0093731	8	28
Rivera-Páez F.A. (2018) [68]	https://doi.org/10.1016/j.ttbdis.2017.10.008	3	26
Carneiro G.F. (2018) [4]	https://doi.org/10.1016/j.jevs.2018.03.007	1	23
de Oliveira E. (2013) [66]	https://doi.org/10.1645/ge-3210.1	4	20
Pessoa A.F.A. (2014a) [69]	https://doi.org/10.1590/s0100-736x2014000800006	2	16
Dantas-Torres F. (2009) [56]	https://doi.org/10.4322/rbpv.01803004	3	16
Braga M.S.C.O. (2017) [54]	https://doi.org/10.1590/s1984-29612017046	5	15
Oliveira F.G. (2017) [70]	https://doi.org/10.1016/j.prevetmed.2017.02.015	9	15

Major research groups **(Table 4)** are seen in USP (Universidade de São Paulo), UNESP (Universidade Estadual Paulista), and UFCG (Universidade Federal de Campina Grande).

A limitation of this type of analysis is the failure to highlight more recent publications or research groups that may become important in the future. Recent publications may not appear prominently in our analysis as they have had insufficient time to accumulate the necessary volume to become highlighted in a study of this type. The search may also be limited by a lack of specific terms in the document title, abstract, and keywords which are vital for this type of study. The study is also limited to the Scopus database, so papers in other databases may not be considered.

2. Concluding Remarks

Donkeys are important in the establishment and maintenance of humans in the countryside, traction and transport, and for producing mules which are widely used in cattle production systems. Nevertheless, research in Brazil with these animals is still incipient and centered on health issues with little information on production systems (such as nutrition, genetics, etc.). This failure to recognize their importance for livestock production systems and lack of information overall may affect the maintenance of these animals in production systems in the long term, thereby impacting the efficiency of agricultural systems.

Other uses for donkeys can be explored. Animals with a physical conformation considered pleasant to the eye, with colors and markings in different patterns, could be used as pets or in farm hotels, exhibition shows, visitor attractions, etc. Other uses, such as onotherapy, have been suggested [71], as well as use in guarding sheep and goats [72].

Donkeys can fulfill various functions on and off the farm, such as plowing, traction, locomotion over great distances, and general work activities. There are some studies on the use of these animals in dairy systems [73]. Cheese produced from donkey milk is highly valued [74], reaching up to 1000 euros per kilogram[4] for European pule cheese, but this is still at the research level in Brazil [4]. It is expected that, over time, animals with greater dairy aptitude can be selected. In Brazil, there is almost no culture of consumption of donkey or horse meat, but there are countries where its consumption is common [38], and may provide export opportunities.

As such, increased research on donkey production systems in Brazil is necessary. Issues include alternative uses of the animals and their products such as meat and milk, means of adding value to the production chain, and exploration for local and export markets. For this, research must go beyond current themes such as animal health, and look towards production systems as a whole, including welfare, nutrition, supply chains, and reproduction, as well as genetics and conservation of this valuable Brazilian farm animal genetic resource.

[4]https://www.huffpost.com/entry/most-expensive-cheese-pule_n_2122323

Table 4: Institutions with five or more publications on donkeys in Brazil (Scopus®).

Affiliation	Documents
Universidade de São Paulo	27
Universidade Federal de Campina Grande	16
Universidade Estadual Paulista Júlio de Mesquita Filho	16
Universidade Federal de Minas Gerais	14
Universidade Federal Rural de Pernambuco	12
Universidade Federal de Mato Grosso	12
Universidade Federal da Bahia	10
Universidade Federal da Paraíba	9
Instituto Biologico - Sao Paulo	7
Universidade Federal Rural do Rio de Janeiro	7
Universidade Federal Rural do Semi-Árido	7
Universidade Federal de Santa Maria	7
Universidade Federal de Uberlândia	6
Fundacao Oswaldo Cruz	6
Universidade Federal do Parana	5
Universidade de Brasília	5
Universidade Federal do Piauí	5
Universidade Federal de Pernambuco	5
The Donkey Sanctuary	5

Supplementary Materials

Supplementary Material includes information on the published papers from Scopus used for mapping bibliography on donkeys in Brazil.

Authors' Contributions

Felipe Pimentel responsible for data curation, and visualization; Samuel Paiva was responsible for data curation; Daniel Pimentel responsible for data curation, Writing original Draft; Laila Dias was responsible for validation and methodology; Concepta McManus responsible for supervision, methodology, validation and formal analyses; all authors were responsible for Writing Review & Editing.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethics Approval

The information came from bibliographic sources. No permission was required.

References

[1] Mariante AS, Albuquerque, M.S.M, Egito AA, McManus C, Lopes MA, Paiva SR. Present status of the conservation of livestock genetic resources in Brazil. Livestock Science 2009;120:204–12. https://doi.org/10.1016/j.livsci.2008.07.007.

[2] Grubb P. Order perissodactyla. In: Wilson DE, Reeder DM, editors. Mammal Species of the World: A Taxonomic and Geographic Reference. 3rd Edition, Johns Hopkins University Press; 2005, p. 629–30.

[3] Rosenbom S, Costa V, Al-Araimi N, Kefena E, Abdel-Moneim AS, Abdalla MA, *et al*. Genetic diversity of donkey populations from the putative centers of domestication. Animal Genetics 2015;46:30–6. https://doi.org/10.1111/age.12256.

[4] Carneiro GF, Cavalcante Lucena JE, de Oliveira Barros L. The current situation and trend of the donkey industry in South America. Journal of Equine Veterinary Science 2018;65:106–10. https://doi.org/10.1016/j.jevs.2018.03.007.

[5] Beja-Pereira A, England PR, Ferrand N, Jordan S, Bakhiet AO, Abdalla MA, *et al*. African origins of the domestic donkey. Science 2004;304:1781–1781. https://doi.org/10.1126/science.1096008.

[6] Kimura B, Marshall FB, Chen S, Rosenbom S, Moehlman PD, Tuross N, *et al*. Ancient DNA from Nubian and Somali wild ass provides insights into donkey ancestry and domestication. Proceedings of the Royal Society B: Biological Sciences 2010;278:50–7. https://doi.org/10.1098/rspb.2010.0708.

[7] Clough DL. The Bible and animal theology. In: Marlow H, Harris M, editors. The Oxford Handbook of the Bible and Ecology, Oxford University Press; 2022, p. 400–12. https://doi.org/10.1093/oxfordhb/9780190606732.013.7.

[8] Todd ET, Tonasso-Calvière L, Chauvey L, Schiavinato S, Fages A, Seguin-Orlando A, *et al*. The genomic history and global expansion of domestic donkeys. Science 2022;377:1172–80. https://doi.org/10.1126/science.abo3503.

[9] Rossel S, Marshall F, Peters J, Pilgram T, Adams MD, O'Connor D. Domestication of the donkey: timing, processes, and indicators. Proceedings of the National Academy of Sciences 2008;105:3715–20. https://doi.org/10.1073/pnas.0709692105.

[10] Arnold ER, Hartman G, Greenfield HJ, Shai I, Babcock LE, Maeir AM. Isotopic evidence for early trade in animals between Old Kingdom Egypt and Canaan. PLOS ONE 2016;11:e0157650. https://doi.org/10.1371/journal.pone.0157650.

[11] Greenfield HJ, Shai I, Greenfield TL, Arnold ER, Brown A, Eliyahu A, *et al*. Earliest evidence for equid bit wear in the ancient Near East: The "ass" from Early Bronze Age Tell eṣ-Ṣâfi/Gath, Israel. PLOS ONE 2018;13:e0196335. https://doi.org/10.1371/journal.pone.0196335.

[12] Epstein H, Mason IL. The origin of the domestic animals of Africa. New York: Africana Publishing Corporation; 1971.

[13] Champlot S, Gautier M, Arbuckle B, Balasecu A, Davis S, Eisenmann V. Phylogeography of the small equids, 2010.

[14] Mitchell P. The donkey in human history: an archaeological perspective. Oxford University Press; 2018.

[15] Vega-Pla JL, Calderón J, Rodríguez-Gallardo PP, Alcaide B, Sereno FTPS, Costa MR, *et al*. The Retuertas horse: the "missing

link" in the Iberoamerican horse breeds origin? Publication-European Association for Animal Production 2005:167–76.

[16] Mariante AD, Cavalcante N. [Discovery animals: domestic breeds in the history of Brazil]. 2nd Edition. Embrapa Recursos Genéticos e Biotecnologia; 2006.

[17] Torres AD, Jardim WR. [Horse and other equine breeding]. São Paulo: Nobel; 1987.

[18] Delgado JV, León J, Gomez M, Nogales S, Camacho M. [Iberian sheep breeds and their participation in the colonization of Latin America]. Biodiversidad Ovina Iberoamericana -Caracterización y Uso Sustentable- 2010:17–30.

[19] Jordana J, Ferrando A, Miró J, Goyache F, Loarca A, Martínez López OR, *et al*. Genetic relationships among American donkey populations: insights into the process of colonization. J Anim Breed Genet 2016;133:155–64. https://doi.org/10.1111/jbg.12180.

[20] Primo AT. [Conquest and colonization: The fantastic story of the Iberian conquistadors and their animals in the Age of Discovery]. Movimento; 2004.

[21] Suprinyak CE. [Troops on the march: The market for pack animals in the Center-South of imperial Brazil]. Annablume Editora; 2008.

[22] Salles P de A, Sousa L de O, Gomes LPB, Barbosa VV, Medeiros GR de, Sousa CM de, *et al*. Analysis of the population of equidae in semiarid region of Paraíba. Journal of Biotechnology and Biodiversity 2013;4:269–75. https://doi.org/10.20873/jbb.uft.cemaf.v4n3.salles.

[23] IBGE. Agricultural Census 2017. https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/21814-2017-censo-agropecuario.html?=&t=resultados (accessed March 21, 2023).

[24] McManus C, Barcellos JOJ, Formenton BK, Hermuche PM, Jr OA de C, Guimarães R, *et al*. Dynamics of cattle production in Brazil. PLOS ONE 2016;11:e0147138. https://doi.org/10.1371/journal.pone.0147138.

[25] Canisso IF, Carvalho GR de, Silva EC da, Rodrigues AL, Ker PG, Guimarães JD. [Some biometric aspects of the external genital tract of Pêga breed semen donor donkeys]. Cienc Rural 2009;39:2556–62. https://doi.org/10.1590/S0103-84782009000900027.

[26] Allen WR, Short RV. Interspecific and extraspecific pregnancies in equids: anything goes. J Hered 1997;88:384–92. https://doi.org/10.1093/oxfordjournals.jhered.a023123.

[27] Franco MM, Santos JBF, Mendonça AS, Silva TCF, Antunes RC, Melo EO. Short Communication Quick method for identifying horse (Equus caballus) and donkey (Equus asinus) hybrids. Genet Mol Res 2016;15. https://doi.org/10.4238/gmr.15038895.

[28] Almeida LD de. [The genetic diversity of donkey breeds reared in Brazil, based on analysis of microsatellite loci and mitochondrial DNA. Dissertation (Master) Brasília: Faculty of Agronomy and Veterinary Medicine, University of Brasília]. Genetic diverdity of the donkey breeds created in Brazil, based of analysis of microsatellite loci and MTDNA 2009.

[29] Perozzi Gameiro MB, Clancy C, Zanella AJ. Between freedom and abandonment: social representations of free-roaming donkeys in the Brazilian Northeast. Anthrozoös 2022;35:335–54. https://doi.org/10.1080/08927936.2021.1999607.

[30] Tatemoto P, Lima YF, Santurtun E, Reeves EK, Raw Z. Donkey skin trade: is it sustainable to slaughter donkeys for their skin? Brazilian Journal of Veterinary Research and Animal Science 2021;58:e174252–e174252. https://doi.org/10.11606/issn.1678-4456.bjvras.2021.174252.

[31] Li X, Shi F, Gong L, Hang B, Li D, Chi L. Species-specific identification of collagen components in Colla corii asini using a nano-liquid chromatography tandem mass spectrometry proteomics approach. IJN 2017;12:4443–54. https://doi.org/10.2147/IJN.S136819.

[32] Rodrigues J, Raw Z, Santurtun E, Cooke F, Clancy C. Donkeys in transition: changing use in a changing world. Brazilian Journal of Veterinary Research and Animal Science 2021;58:e174325. https://doi.org/10.11606/issn.1678-4456.bjvras.2021.174325.

[33] Queiroz MR, Gameiro MBP, Zanella AJ. The population of donkeys and mules in Brazil according to agricultural censuses from 1960 to 2017. Brazilian Journal of Veterinary Research and Animal Science 2021;58:e174365–e174365. https://doi.org/10.11606/issn.1678-4456.bjvras.2021.174365.

[34] Egito AD, Albuquerque M, Mariante AD. [Current status of animal genetic characterization at Embrapa Genetic Resources and Biotechnology] 1999.

[35] Xia X, Yu J, Zhao X, Yao Y, Zeng L, Ahmed Z, *et al*. Genetic diversity and maternal origin of Northeast African and South American donkey populations. Animal Genetics 2019;50:266–70. https://doi.org/10.1111/age.12774.

[36] Jordana J, Goyache F, Ferrando A, Fernández I, Miró J, Loarca A, *et al*. Contributions to diversity rather than basic measures of genetic diversity characterise the spreading of donkey throughout the American continent. Livestock Science 2017;197:1–7. https://doi.org/10.1016/j.livsci.2016.12.014.

[37] Santos Alves J, da Silva Anjos M, Silva Bastos M, Sarmento Martins de Oliveira L, Pereira Pinto Oliveira I, Batista Pinto LF, *et al*. Variability analyses of the maternal lineage of horses and donkeys. Gene 2021;769:145231. https://doi.org/10.1016/j.gene.2020.145231.

[38] Gameiro MBP, Rezende VT, Zanella AJ. Brazilian donkey slaughter and exports from 2002 to 2019. Brazilian Journal of Veterinary Research and Animal Science 2021;58:e174697–e174697. https://doi.org/10.11606/issn.1678-4456.bjvras.2021.174697.

[39] Porter V, Alderson L, Hall S, Sponenberg D. Mason's World Encyclopedia of livestock breeds and breeding: 2 volume pack. 2016.

[40] Glass V. [Offspring of the Brazilian Donkey are good for traction and riding]. Folha de São Paulo, AgroFolha n.d. https://www1.folha.uol.com.br/fsp/agrofolh/fa12019902.htm (accessed March 22, 2023).

[41] Glass V. [Crisis means donkeys and asses are back in mode in the countryside]. Folha de São Paulo, AgroFolha n.d. https://www1.folha.uol.com.br/fsp/agrofolh/fa12019901.htm (accessed March 22, 2023).

[42] Alves JS, de Araujo Oliveira CA, Escodro PB, Pinto LFB, Costa RB, de Camargo GMF. Genetic origin of donkeys in Brazil. Trop Anim Health Prod 2022;54:291. https://doi.org/10.1007/s11250-022-03280-x.

[43] Santana ML, Bignardi AB. Status of the genetic diversity and population structure of the Pêga donkey. Trop Anim Health Prod 2015;47:1573–80. https://doi.org/10.1007/s11250-015-0900-x.

[44] Lima MA, Alves B, de Carvalho EC. [Methane emission inventory by livestock (enteric fermentation and animal waste management systems) in the State of São Paulo, 1990 to

2008]. CETESB – Companhia Ambiental Do Estado de São Paulo 2011. https://www.alice.cnptia.embrapa.br/bitstream/doc/921473/1/2011MZ01.pdf (accessed October 22, 2022).

[45] Moreira IL, Campebell RC, Teixeira-Neto AR, Câmara ACL. Orthopedic injury in 156 equids in Federal District, Midwestern Brazil. Brazilian Journal of Veterinary Medicine 2019;41:e095619–e095619. https://doi.org/10.29374/2527-2179.bjvm095619.

[46] Di Filippo PA, Azevedo VM, Quirino CR, Godinho ABFR. Evaluation of angular, linear, and body index measures of Pêga donkeys (Equus asinus) from the Southeast region of Brazil 2022. https://doi.org/10.2139/ssrn.4258746.

[47] Pimentel MML, Pinheiro M, Maia Filho H, Sakamoto SM, Nobre FV, Dias RV da C. [Biometric parameters of donkeys (equus asinus) used in running events in the state of Rio Grande do Norte]. Acta Veterinaria Brasilica 2014;8:136–43.

[48] Escodro P, Mariz T, Dittrich J, Neto M, Lima C, Ribeiro J. [Biometric standard, coupling measurements and load index of urban traction Equide in the municipality of Arapiraca, Alagoas]. Archives of Veterinary Science 2014;19. https://doi.org/10.5380/avs.v19i2.34085.

[49] Schmidek A, Gonçalves ECP, Bárbaro-Torneli IM, Grizotto RK, Miguel FB, Faria MH de. [Phenotypic variability of the body conformation of equines of the Brazilian equestrian breeds, Bretão Postier and Jumento Brasileiro]. Brazilian Journal of Animal and Environmental Research 2022;5:1985–92. https://doi.org/10.34188/bjaerv5n2-042.

[50] McManus C, Falcão RA, Spritze A, Costa D, Louvandini H, Dias LT, *et al*. [Morphological characterization of Campeiro horses]. R Bras Zootec 2005;34:1553–62. https://doi.org/10.1590/S1516-35982005000500015.

[51] McManus CM, Santos SA, Silva JA da, Louvandini H, Abreu UGP de, Sereno JRB, *et al*. [Body indices for the Pantanal horse]. Brazilian Journal of Veterinary Research and Animal Science 2008;45:362–70. https://doi.org/10.11606/issn.1678-4456.bjvras.2008.26677.

[52] McManus C, Pimentel F, Pimentel D, Sejian V, Blackburn H. Bibliographic mapping of heat tolerance in farm animals. Livestock Science 2023;269:105163. https://doi.org/10.1016/j.livsci.2023.105163.

[53] Mariante A da S, Egito AA. Animal genetic resources in Brazil: result of five centuries of natural selection. Theriogenology 2002;57:223–35. https://doi.org/10.1016/s0093-691x(01)00668-9.

[54] Braga M do SC de O, Costa FN, Gomes DRM, Xavier DR, André MR, Gonçalves LR, *et al*. Genetic diversity of piroplasmids species in equids from island of São Luís, northeastern Brazil. Rev Bras Parasitol Vet 2017;26:331–9. https://doi.org/10.1590/S1984-29612017046.

[55] Costa SCL, de Souza Freitas J, Carvalho FS, Pereira MJS, Cordeiro MD, da Fonseca AH, *et al*. Frequency and factors associated of potential zoonotic pathogens (Borrelia spp., Rickettsia spp., Leishmania spp., and Anaplasma phagocytophilum) in equids in the state of Bahia, Brazil. Parasites & Vectors 2021;14:275. https://doi.org/10.1186/s13071-021-04777-4.

[56] Dantas-Torres F. Ticks on domestic animals in Pernambuco, Northeastern Brazil. Rev Bras Parasitol Vet 2009;18:22–8. https://doi.org/10.4322/rbpv.01803004.

[57] Maria Seles Dorneles E, Gomes Fernandes L, Almeida Santana J, Joelson Correa Freitas F, Maria de Lima J, de Oliveira Barros I, *et al*. Anti-Brucella abortus antibodies in free-ranging equids from Mossoró, Rio Grande do Norte, Brazil. Semina Ciências Agrárias 2013;34:1281–6.

[58] Fernando P, Starkey P. Donkeys and development: socio-economic aspects of donkey use in Africa. Donkeys, People and Development. A resource book in the Animal Traction Network for Eastern and Southern Africa (ATNESA). ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA); 2004.

[59] Nogueira MB, de Faria DA, Ianella P, Paiva SR, McManus C. Genetic diversity and population structure of locally adapted Brazilian horse breeds assessed using genome-wide single nucleotide polymorphisms. Livestock Science 2022;264:105071. https://doi.org/10.1016/j.livsci.2022.105071.

[60] Horta MC, Labruna MB, Sangioni LA, Vianna MCB, Gennari SM, Galvão MAM, *et al*. Prevalence of antibodies to spotted fever group rickettsiae in humans and domestic animals in a Brazilian spotted fever-endemic area in the state of São Paulo, Brazil: serologic evidence for infection by Rickettsia rickettsii and another spotted fever group Rickettsia. Am J Trop Med Hyg 2004;71:93–7.

[61] Heukelbach J, Costa AML, Wilcke T, Mencke N, Feldmeier H. The animal reservoir of Tunga penetrans in severely affected communities of north-east Brazil. Medical and Veterinary Entomology 2004;18:329–35. https://doi.org/10.1111/j.0269-283X.2004.00532.x.

[62] Machado RZ, Toledo CZP, Teixeira MCA, André MR, Freschi CR, Sampaio PH. Molecular and serological detection of Theileria equi and Babesia caballi in donkeys (Equus asinus) in Brazil. Veterinary Parasitology 2012;186:461–5. https://doi.org/10.1016/j.vetpar.2011.11.069.

[63] Londoño AF, Díaz FJ, Valbuena G, Gazi M, Labruna MB, Hidalgo M, *et al*. Infection of Amblyomma ovale by Rickettsia sp. strain Atlantic rainforest, Colombia. Ticks and Tick-Borne Diseases 2014;5:672–5. https://doi.org/10.1016/j.ttbdis.2014.04.018.

[64] Mancianti F. [Feline leishmaniasis: what's the epidemiological role of the cat?]. Parassitologia 2004;46:203–6.

[65] Alves ADS, Melo ALT, Amorim MV, Borges AMCM, Gaíva E Silva L, Martins TF, *et al*. Seroprevalence of Rickettsia spp. in equids and molecular detection of 'Candidatus Rickettsia amblyommii' in Amblyomma cajennense sensu lato ticks from the Pantanal region of Mato Grosso, Brazil. J Med Entomol 2014;51:1242–7. https://doi.org/10.1603/ME14042.

[66] Gennari SM, Esmerini P de O, Lopes MG, Soares HS, Vitaliano SN, Cabral AD, *et al*. Occurrence of antibodies against Toxoplasma gondii and its isolation and genotyping in donkeys, mules, and horses in Brazil. Veterinary Parasitology 2015;209:129–32. https://doi.org/10.1016/j.vetpar.2015.01.023.

[67] Truppel JH, Otomura F, Teodoro U, Massafera R, Costa-Ribeiro MCV da, Catarino CM, *et al*. Can equids be a reservoir of Leishmania braziliensis in endemic areas? PLOS ONE 2014;9:e93731. https://doi.org/10.1371/journal.pone.0093731.

[68] Rivera-Páez FA, Labruna MB, Martins TF, Perez JE, Castaño-Villa GJ, Ossa-López PA, *et al*. Contributions to the knowledge of hard ticks (Acari: Ixodidae) in Colombia. Ticks Tick Borne Dis 2018;9:57–66. https://doi.org/10.1016/j.ttbdis.2017.10.008.

[69] Pessoa A, Pessoa C, Neto E, Riet-Correa F. Diseases of donkeys and mules in the Brazilian semiarid. Pesquisa Veterinária Brasileira 2014;34:1210–4.

[70] Oliveira FG, Cook RF, Naves JHF, Oliveira CHS, Diniz RS, Freitas FJC, *et al*. Equine infectious anemia prevalence in feral

donkeys from Northeast Brazil. Prev Vet Med 2017;140:30–7. https://doi.org/10.1016/j.prevetmed.2017.02.015.

[71] Karatosidi D, Marsico G, Tarricone S. Modern use of donkeys. Iranian Journal of Applied Animal Science 2013;3:13–7.

[72] Walton MT, Feild CA. Use of donkeys to guard sheep and goats in Texas. Fourth Eastern Wildlife Damage Control Conference (1989), 1989.

[73] Messias TBON, Araújo E de OM, Sant'Ana AM da S, Lucena JEC, Pacheco MTB, Queiroga R de CR do E. Challenges and perspectives for exploiting donkey milk in the Brazilian Northeast. Cienc Rural 2021;52:e20210058. https://doi.org/10.1590/0103-8478cr20210058.

[74] Faccia M, Gambacorta G, Martemucci G, Natrella G, D'Alessandro AG. Technological attempts at producing cheese from donkey milk. J Dairy Res 2018;85:327–30. https://doi.org/10.1017/S0022029918000420.

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Original Article **Open Access**

Molecular Studies of Gastrointestinal Strongyle Nematodes in Migratory, Resident, and Sedentary Plains Zebras (*Equus quagga*) in Kenya

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Abstract

The molecular identity of gastrointestinal (GI) strongyle larvae recovered from faecal cultures from plains zebras in Kenya was determined using molecular tools. Internal transcribed spacer (ITS) ribosomal DNA (rDNA) extracted from the larvae were amplified using Polymerase Chain Reaction (PCR), sequenced for identification, and compared to sequences in the GenBank to determine their phylogeny. Sixteen sequences were obtained and identified as *Cyathostomum montgomeryi, Cylicostephanus minutus, Poteriostomum imparidentatum, Triodontophorus nipponicus* and *Strongylus vulgaris*. The genetic identity of *P. imparidentatum, T. nipponicus* and *S. vulgaris* from plains zebras in Kenya are reported for the first time in this study. The 16 sequences clustered into 5 clades according to the 5 genera of nematodes identified. The clade having *T. nipponicus* was placed as a sister to the Cyathostominae but was very distinct from the clade having *S. vulgaris*. The close clustering of *T. nipponicus* to the Cyathostominae supports previous suggestions that it belongs to this subfamily rather than Strongylinae. Five sequences of *C. montgomeryi* clustered closely with four sequences of *C. montgomeryi* in the GenBank isolated from zebras in Kenya. The other five sequences were evolutionary distinct. Similarly, two of the *Cy. minutus* sequences clustered with *Cy. minutus* sequences from zebras in Kenya, while the other one, was distinct. These results suggest intra-species genetic polymorphism among the *C. montgomeryi* and *Cy. minutus* isolates. The *Poteriostomum imparidentatum, T. nipponicus,* and *S. vulgaris* sequences were distinct from sequences found in the GenBank. This study contributes to the scanty but growing literature on equine strongyle genetics in zebras.

Keywords

Molecular identity; phylogeny; strongyles; plains zebras

1. Introduction

Infections by parasites are of significant concern to wildlife, as they present the most pervasive challenges to grazing herbivores [1]. Gastrointestinal (GI) helminths are among the most abundant parasites in the world [2]. These parasites are significant in regulating wildlife because of the harm they cause and can act as agents of extinction [3,4]. Studies on helminth infections have increased since the parasites are found in both wild and domestic populations. Interestingly, wildlife has been found to be a reservoir for many livestock diseases [5], and hence identifying the diverse parasites in wild populations is a key step in reducing the rate of infections across wild and domestic animals.

The Strongylidae family is the most common of the helminth parasites which consist of a diverse group of faecal-orally transmitted nematodes that chronically infect untreated equid hosts [6]. There is a rich and diverse body of research on gastrointestinal parasites of equids spanning over a century [7]. Given their existence as relatively free-ranging, zebras represent equids untouched by parasite control measures such as anthelmintics. Therefore, studies on parasites of zebras may provide foundational information for wildlife and equine parasitology [8].

Equine strongyles (Family Strongylidae) are a significant category of intestinal nematodes. They are among important helminth parasites in the *Equus* gut, and have been found in horses, donkeys, mules and zebras, and inter-species hybrids [9]. Transmission of the parasites is through ingestion of infective larvae (L_3). Most of the information available on these nematodes is from studies in horses, mainly on aspects such as prevalence, morphology, and disease control and prevention [10]. A few studies, based on morphology of adult worms, have reported on prevalence and worm burdens in zebras in Africa [8,11,12]. The equine strongyles are an important cause of illness and mortality in horses and donkeys, while the cyathostominae have shown increased resistance to anthelmintics [13,14]. The pathogenic effects of equine strongyles in zebras have not been documented, but they are potentially harmful and may have consequences on host populations dynamics. The phylogenetic relationship of species within *Equus* is well documented, confirming the close association between zebras, donkeys, and horses [15].

Equine strongyles are classified into two sub-families, the Strongylinae (large strongyles), and the Cyathostominae (small strongyles). The large strongyles are further classified into 5 genera (*Strongylus, Oesophagodontus, Triodontophorus, Bidentostomum*, and *Craterostomum*) and 14 species. The small strongyles are classified into 14 genera (*Caballonema, Coronocyclus, Cyathostomum, Cylicocyclus, Cylicodontophorus, Cylicostephanus, Cylindropharynx, Gyalocephalus, Hsiungia, Parapoteriostomum, Petrovinema, Poteriostomum, Skrjabinodentus,* and *Tridentoinfundibulum*) and 50 species [6].

Most of the equid strongyles can infect multiple equid species [8,16,17]. Identifying equine strongyles using morphological features at some life cycle stages (eggs and larvae (L_3)) is difficult. DNA technology is an alternative approach for identifying these nematodes [18,19]. DNA technology has not been fully exploited in veterinary parasitology. Techniques, like the PCR [20] has opened opportunity for identification of nematode eggs and larvae during diagnosis of parasitic infections. While there is diverse literature on genetic identification of horse strongyles, most of this work is limited to strongyles infecting the domestic horse [9,21,22]. Despite the importance of strongyle nematodes as disease-causing agents in horses and possibly other equids, there is a scarcity of reports on species identification.

There are two species of zebras in Kenya, the plains zebra (*Equus quagga*) and the Grevy's zebra (*Equus grevyi*). The plains zebras are the most common and are found in many parts of the country while the Grevy's zebras are endangered and commonly found in the Northern part of the country. Only one report by [7] on the genetic identity of gastrointestinal nematodes in the plains and the Grevy's zebras in Kenya is available. It has been observed that each year, some of the plains zebras (migratory) in the Masai Mara National Reserve (MMNR) migrate together with wildebeest and other animals between the Serengeti National Park in Tanzania and Kenya's Masai Mara and vice versa in search of pasture and water. The resident zebras in Masai Mara remain in the reserve throughout the year, with an unlimited range (open-sedentary system) of grazing land. Other zebras are in protected fenced parks such as Lake Nakuru National Park (LNNP) and are in a closed sedentary system. In a previous study by [23] gastrointestinal nematodes eggs/larvae in these zebras were identified morphologically to genera level. The study reported that these zebras were infected by different types of helminths including nematodes, trematodes, and cestodes; the strongyle nematodes were the most abundant. The aim of the study was to determine the molecular identity and phylogenetic relationships of GI strongyle (L_3), previously isolated from the three groups of plains zebras in MMNR and LNNP.

2. Materials and Methods

2.1. Study Area and Design

The study area and design were described previously by [23]. In brief, fresh faecal samples were collected in 2014 and 2015 from pastures and resting places for the migratory and resident plains zebras in MMNR, and sedentary plains zebras in LNNP. In total, 867, 732, and 616 samples were collected from the migratory, resident, and sedentary plains zebras, respectively. During the faecal collection, sampling areas measuring 20m x 100m were marked out in the 2 conservation areas. The areas were then walked in a Z pattern, collecting faecal samples using the procedure for collection of herbage samples described in [24]. The samples were examined for helminth eggs using microscopy and those positive for nematode eggs cultured for larvae identification.

2.2. Molecular Techniques

2.2.1. Faecal Culture and Larvae Isolation

GI nematodes were identified using molecular markers, larvae from fresh faecal samples were cultured and harvested using a modified Baermann technique [25]. Composite faecal samples for each of the three groups of zebras (migratory, resident, and sedentary) were placed in different containers, moistened, and gently mixed. They were incubated at room temperature for 12 days with daily monitoring which involved moistening with water and stirring to prevent them from being invaded by fungi.

Larvae (L_3) extraction from the cultures was carried out on the 13th day. The culture jars were filled with lukewarm water, stirred, and inverted on a petri dish. The exposed area of the inverted jar was filled with clean water and left to stand for 12 hours; nematode larvae swam from the cultured faecal mixture into the clean water. The larvae suspension was examined under a microscope at 10X to 40X magnification and identified according to procedure in the MAFF manual [24]. The L_3 culture results were used as a general identification of the worm genera using key characteristics like intestinal cell number, head characteristics, and sheath tail characteristics.

The prevalence and distribution of L_3 isolated from the faecal cultures for the three groups were reported by [23]. L_3 of cyathostomes, *Strongylus* species (spp), and *Trichostrongylus* spp. were recovered, L_3 of the cyatostomes were the most abundant followed by *Strongylus* spp. For each culture per zebra group, that is, the migratory, the resident (R), and the sedentary (S), 200 (L_3) were preserved in 70% ethanol. A total of 600 individuals (L_3) were collected across all the three zebra groups.

2.2.2. DNA Extraction

Ethanol used in preserving the larvae was removed from the (L_3) using a lyophilizer and then washed in Phosphate saline buffer to completely remove ethanol residues. DNA extraction from the 600 L_3 samples was done using Invitrogen PureLink Genomic DNA (gDNA) Mini Kit Cat no. K1820-02 as per manufacturer's protocol. The samples were quantified using the NanoDrop 2000. Eluted gDNA samples were run on 1.5% Agarose Gel using 1X TBE Buffer to check for the integrity of the DNA. The samples were then standardized to 23ng/µl for PCR and stored at -20°C until use. Internal Transcriber Spacer I and II Polymerase Chain Reaction (PCR) Amplification and Sequencing

Each gDNA extract was amplified at the region spanning internal transcriber spacer 1 (ITS1) 5.8S and ITS2 of ribosomal DNA (rDNA) using two sets of primers, first by NC5 (forward, 5' GTAGGTGAACCTGCGGAAGGATCATT-3') and NC2 (reverse, 5'-TTAGTTTCTTTTCCTCCGCT-3'). Amplification was carried out in 15µ reactions containing 1.5 µl of genomic DNA, 6 µl of 5 PRIME hotmaster mix (Hamburg, Germany), 0.75 µl of each primer (10 µM) and 6 µl of PCR-quality water. Amplification was preceded by a 2-minute polymerase activation step at 90°C, followed by 35 cycles of 45 sec each at 57°C annealing, 72°C extension and 95°C denaturation. Amplification was terminated by a final extension step at 72 °C for 5 minutes. The nested reaction was carried out using primers NC1 (forward, 5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (reverse, 5'- TTAGTTTCTTTTCCTCCGCT-3'). The PCR condition for this primer was identical to the (NC5-NC2 primer) conditions except that its annealing temperature was 55°C [26]. PCR amplicons in gels were excised and purified using QIAquick PCR purification Kit (Cat no. 28106) as per the manufacturer's protocol. The purified DNA was sequenced using same forward and reverse PCR primers at Macrogen Europe Laboratories (Amsterdam, the Netherlands).

2.3. Data Analysis

The obtained nematode ITS rDNA nucleotide sequences were viewed and manually verified using chromatogram peaks, edited, and assembled using CLC Main Workbench 6.3.8 software (CLCbio, Qiagen GmbH, Germany) and BioEdit program. Bioinformatics analysis of the sequences was done using the nucleotide Local Alignment Search Tool (BLASTn), multiple sequence alignment, and phylogenetic analysis. Multiple sequence alignment was done using Log-Expectation (MUSCLE) v3.8.31 [27]. Sixteen ITS gene consensus sequences obtained in this study and seventeen reference ITS gene sequences of closely related helminths species retrieved from the GenBank **(Table 1)** were used to reconstruct a phylogenetic tree employing the Maximum Likelihood (ML) algorithm implemented in MEGA X [28]. The best nucleotide substitution model with the lowest BIC (Bayesian Information Criterion) scores of 1724.87 was found to be Tamura 3- parameter model [29] as determined using MEGA X with 1000 bootstrap replicates [30]. The analysis involved a total of 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 84 positions in the final data set. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches.

3. Results

3.1. Molecular Detection and Prevalence of Plains Zebra Gastrointestinal Strongyles

DNA was extracted from all the larvae from faecal cultures of the three groups of plains zebra, upon amplification only 104 produced positive amplicons for sequencing. Out of the 104 amplicons sequenced only 32 were of good quality for further analysis. After cleaning and editing, only 16 sequences matched with nematodes in the GenBank. The sequences obtained were distributed among the three groups of zebras: 5 each from the migratory (MMMZ) and resident (MMRZ) plains zebras from MMNR and 6 from the sedentary (LNSZ) plains zebras from LNNP **(Table 2)**.

All the 16 identified (L_3) belonged to the family Strongylidae of which fourteen (87.5%) belonged to the subfamily Cyathostominae while the remaining two (12.5%) belonged to the subfamily Strongylinae as shown in **Table 3**.

Table 1: References for sequences of species of Strongyles and Bunostomum selected from GenBank and included in the phylogenetic tree.

Nematode Name	Host	Country	Gene	Accession No
Cyathostomum montgomeryi	Plains Zebra	Kenya	ITS	MZ435504
Cyathostomum montgomeryi	Grevy's Zebra	Kenya	ITS	MZ435572
Cyathostomum montgomeryi	Plains Zebra	Kenya	ITS	MZ435563
Cyathostomum montgomeryi	Plains Zebra	Kenya	ITS	MZ435583
Cylicostephanus minutus	Horse	China	ITS	MT382658
Cylicostephanus minutus	Horse	Germany	ITS	MH487658
Cylicostephanus minutus	Plains Zebra	Kenya	ITS	MZ435498
Cylicostephanus minutus	Plains Zebra	Kenya	ITS	MZ435536
Poteriostomum imparidentatum	Horse	China	ITS	KY495604
Poteriostomum imparidentatum	Donkey	China	ITS	KP693433
Poteriostomum imparidentatum	Horse	Australia	ITS	Y08590
Triodontophorus nipponicus	Horse	China	ITS	KR296739
Triodontophorus nipponicus	Donkey	China	ITS	KP693437
Triodontophorus nipponicus	Donkey	China	ITS	KU205013
Triodontophorus nipponicus	Donkey	China	ITS	KU205011
Strongylus vulgaris	Donkey	China	ITS	KP693439
Bunostomum phlebotomum	Cattle	Australia	mtDNA	NC_012308

Three species of the subfamily Cyathostominae were identified and distributed as *Cyathostomum montgomeryi* (10; 71.43), *Cylicostephanus minutus* (3; 21.43%), and *Poteriostomum imparidentatum* (1; 7.14%). Species of Strongylinae identified from PCR products were *Triodontophorus nipponicus* (1; 50%) and *Strongylus vulgaris* (1; 50%) **(Table 3)**.

Amplicons belonging to both subfamilies were identified from both the migratory and resident zebras from MMNR, while only sequences belonging to the Cythostominae subfamily were recovered from zebras of Lake Nakuru National Park as shown in **Table 3**.

3.2. Molecular Identities of Detected Cyathostominae and Strongylinae Subfamilies

Results from the local alignment search tool (BLASTn) for all the 16 gastrointestinal Strongylidae nematode larvae ITS rDNA are shown in **Table 4**. The partial ITS rDNA sequences appear in GenBank under the accession numbers; OK235465 to OK235480. BLASTnn analysis revealed that majority, 10 (62.5 %), of the Cyathostominae were similar to *Cyathostomum montgomeryi* with matching identity between 92.26 and 100% to annotated sequences in the GenBank. The five annotated sequences for *C. montgomeryi* in the GenBank having the highest match to sequences obtained in the current study were for nematodes previously reported from zebras in Kenya. The sequences identified as *C. montgomeryi* in the current study were almost equally distributed across the migratory (n=3, isolates ME, MF26, and MO), resident (n=3, isolates R4, R5, and RX), and sedentary (n=4, S3, S4, S7, and S8).

Three (18.75%) of the sequences were similar to *Cy. minutus* with sequence identity of between 91.63 and 99.18%. The three sequences matched two annotated sequences for *Cy. minutus*, previously reported from zebras in Kenya. One sequence was similar to *Poteriostomum imparidentatum* retrieved from GenBank isolated from an adult feral horse (*Equus caballus*) from Australia with sequence identity of 96.77% **(Table 4)**. This is the first report on genetic identity of *P. imparidentatum* species from zebras in Kenya.

Two of the sequences were those of Strongylinae subfamily, one similar to *S. vulgaris* with sequence identity of 90.12% and the other similar to *T. nipponicus* with sequence identity of 96.45% **(Table 4)**. This is also the first report of *S. vulgaris* and *T. nipponicus* in zebras in Kenya.

Table 2: Distribution of 16 rDNA amplicons obtained from the polymerase chain reaction of rDNA extracts from gastrointestinal strongyle nematode larvae isolated from cultures of faecal samples from the three Zebra groups.

Zebra Group	MMMZ (Migratory)	MMRZ (Resident)	LNSZ (Sedentary)
PCR Amplicon	ME/KEN/MMNR/2015	R1/KEN/MMNR/2015	S1/KEN/LLNP/2015
	MF25/KEN/MMNR/2015	R3/KEN/MMNR/2015	S2//KEN/LLNP/2015
	MF26/KEN/MMNR/2015	R4/KEN/MMNR/2015	S3/KEN/LLNP/2015
	MO/KEN/MMNR/2015	R5/KEN/MMNR/2015	S4/KEN/LLNP/2015
	MO2/KEN/MMNR/2015	RX/KEN/MMNR/2015	S7/KEN/LLNP/2015
			S8/KEN/LLNP/2015

Table 3: Species of Cyathostominae and Strongylinae subfamilies identified from PCR amplicons of larvae ITS rDNA from cultures of the three Zebra groups.

Strongylidae nematode larvae	Distribution of nematode species among the zebra groups			Total
	MMMZ (n=5)	MMRZ (n=5)	LNSZ (n=6)	
Subfamily Cyathostominae				
Cyathostomum montgomeryi	3	3	4	10
Cylicostephanus minutus	0	1	2	3
Poteriostomum imparidentatum	1	0	0	1
Subfamily Strongylinae				
Triodontophorus nipponicus	1	0	0	1
Strongylus vulgaris	0	1	0	1

3.3. Phylogenetic Analysis of Zebra Strongyles

Results of the phylogenetic analysis of the strongyle (L_3) rDNA sequences from the plains zebras and those in the GenBank are shown in **Figure 1**. Overall, the 16 sequences clustered into 5 clades, according to the 5 genera of nematode larvae identified. The five clades were (A) having *C. montgomeryi,* (B) having *Cy. minutus,* (C) having *P. imparidentatum,* (D) having *T. nipponicus* and (E) having S. *vulgaris* **(Figure 1)**. The clade having *T. nipponicus* (Clade D), which is classified as a Strongylinae was placed as a sister clade to the one having the true Cyathostominae (*C. montgomeryi, Cy. minutus* and *P. imparidentatum*). The clade having *T. nipponicus* appeared evolutionary distinct from the only other rDNA sequences from a Strongylinae, *S. vulgaris.*

Five of the ten sequences of *C. montgomeryi* from this study clustered closely with four sequences of *C. montgomeryi* in the GenBank. The four sequences were from adult worms previously sampled from zebras in Central Kenya. The other five sequences appeared distinct, but distantly related to those in the GenBank. Two of the three *Cy. minutus* sequences from the current study clustered with two *Cy. minutus* sequences in the GenBank, from the same study in central Kenya. One of the sequences of *Cy. minutus* from the current study appeared distinct (bootstrap value of 96) from the other two sequences and was a sister to a clade having sequences from horses in China (MT382658.1) and Germany (MH487658.1). *Poteriostomum imparidentatum* singleton obtained in this study was distinct from *P. imparidentatum* isolates obtained from the GenBank. The sequences in the GenBank were KY495604.1 and Y08590.1 from horses in China and Australia, respectively, and KP693433.1 from wild donkeys in China.

The *T. nipponicus* sequence identified in this study was distantly related to sequences in the GenBank from horses and wild donkeys from China. Our single isolate of *S. vulgaris* was distinct but related to *S. vulgaris* sequence (KP693439.1), found in the GenBank and isolated from a wild horse in China. No closely related sequences for any previous isolates of *P. imparidentatum, T. nipponicus* or *S. vulgaris* from zebras, donkeys, or horses in Kenya were found in the GenBank.

4. Discussion

This study reports for the first time the molecular diversity in migratory, resident, and sedentary plains zebras in Kenya. Overall, the study revealed that the majority of the sequenced reference samples belonged to the subfamily Cyathostominae and were identified as *Cyathostomum montgomeryi*, *Cylicostephanus minutus,* and *Poteriostomum imparidentatum.* The rest belonged to the subfamily Strongylinae and were *Triodontophorus nipponicus* and *Strongylus vulgaris*. Only one previous report which looked at the genetic identity of GI nematodes from zebras in Central Kenya is currently available in the literature [7]. The study sequenced ninety-one amplicons from adult worms expelled in faeces from plains and Grevy's zebras and identified four genera of equine strongyles from the subfamily Cyathostominae (*Parapoteriostomum* spp, *Cylindropharynx brevicauda, C. intermedia, C. longicauda, Cyathostomum montgomeryi* and *Cylicostephanus minutus*) and one genus from the subfamily Strongylinae (*Craterostomum acuticaudatum*). Our study also reported the occurrence of *C. montgomeryi* and *Cy. minutus* in zebras in Kenya, as was reported by [7].

Table 4: Sixteen Strongylidae infective larvae ITS rDNA sequences from plains zebras, identified using Local Alignment Search Tool (BLASTn), with their corresponding accession numbers, matching sequences, accession numbers of the highest match and percentage identity.

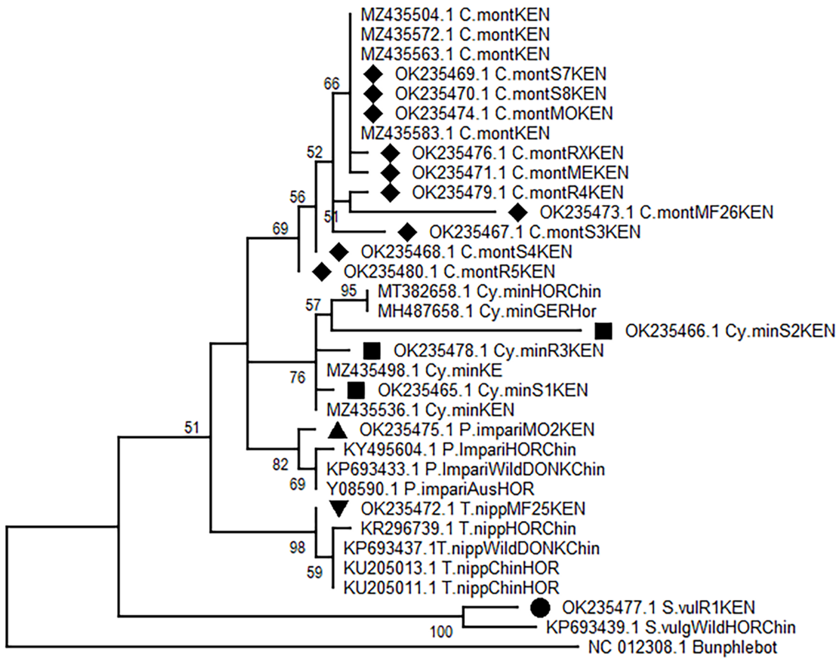
Isolate	Our accession number	Matching sequence	Accession no. of highest match	E-value	Identity (%)
MF26	OK235473	*Cyathostomum montgomeryi*	MZ435563.1	0.0	94.60
ME	OK235471	*Cyathostomum montgomeryi*	MZ435563.1	0.0	99.56
MO	OK235474	*Cyathostomum montgomeryi*	MZ435572.1	0.0	99.69
RX	OK235476	*Cyathostomum montgomeryi*	MZ435583.1	0.0	96.02
R5	OK235480	*Cyathostomum montgomeryi*	MZ435563.1	0.0	97.13
R4	OK235479	*Cyathostomum montgomeryi*	MZ435497.1	0.0	92.29
S4	OK235468	*Cyathostomum montgomeryi*	MZ435583.1	0.0	98.96
S7	OK235469	*Cyathostomum montgomeryi*	MZ435553.1	0.0	99.71
S8	OK235470	*Cyathostomum montgomeryi*	MZ435563.1	0.0	100
S3	OK235467	*Cyathostomum montgomeryi*	MZ435563.1	0.0	95.69
R3	OK235478	*Cylicostephanus minutus*	MZ435498.1	0.0	97.61
S1	OK235465	*Cylicostephanus minutus*	MZ435536.1	0.0	99.18
S2	OK235466	*Cylicostephanus minutus*	MZ435536.1	0.0	91.63
MO2	OK235475	*Poteriostomum imparidentatum*	Y08590.1	0.0	96.77
R1	OK235477	*Strongylus vulgaris*	KP693439.1	0.0	90.12
MF25	OK235472	*Triodontophorus nipponicus*	KU205013.1	0.0	96.45

Results from our study indicated that *C. montgomeryi* was the most abundant equine strongyle, [7] reported the predominance of *Parapoteriostomum* spp. Previous reports [31,32] suggest that *C. montgomeryi* is restricted to *Equus* species in Africa. Results from our study support this observation as no matching sequences for this parasite could be found in the GenBank from studies in equines from other parts of the world. The number of amplicons examined in the study by [7] was much larger than the sixteen amplicons examined in our study. They also analyzed adult worms collected from plains zebras, Grevy's zebras, and a Grevy's x plains hybrid zebra at Mpala in Laikipia County. The differences in species of zebras examined, the type of samples used, and the numbers of amplicons analyzed could account for differences in nematode genera identified in this study and that by [7].

The ten ITS gene sequences identified as *C. montgomeryi* in our study had varying species identity (92.26 to 100%) with the five annotated sequences for the same parasite, reported from zebras in Kenya by [7]. Similarly, the three ITS gene sequences identified as *Cy. minutus* in our study had varying species identity (91.63 to 99.18%) with the two annotated sequences for *Cy. minutus* reported from zebras in Kenya by [7]. This data suggests that there is intra-species genetic polymorphism of *C. montgomeryi* and *Cy. minutus* in zebras in Kenya.

The study is the first to report, the genetic identity of *Poteriostomum imparidentatum, Triodontophorus nipponicus,* and *Strongylus vulgaris* from plains zebras in Kenya. Equine strongyles in the genera *Poteriostomum* and *Triodontophorus* have been reported previously in Burchell's zebras in South Africa [8,33], while *P. imparidentatum* and *Strongylus vulgaris* have been reported in plains and Grevy's zebras in Ukraine [34]. The three studies were based on morphological identification of adult worms from zebras [8,33,34]. Our study confirms the presence of these parasites in plains zebras in Kenya, as has been reported in other parts of the world, and provides information on their genetic identity.

The phylogenetic analysis placed the Cyathostominae (*Cyathostomum, Poteriostomum,* and *Cylicostephanus*) in sister clades, indicating that they were all closely related. This differs from the results of [10], who examined the phylogenetic relationships of twelve protein-coding genes from twenty Strongyloidea nematodes from various hosts and reported that *Poteriostomum* spp was a sister to a clade having species of *Cyathostomum, Cylicostephanus, Triodontophorus,* and others. They concluded that *P. imparidentatum* was evolutionarily distant from other Cyathostominae and *Triodontophorus* spp. Results of our phylogenetic analysis indicate that *Triodontophorus* spp. was a sister to the subfamily Cyathostominae, having clades that had *Cyathostomum, Poteriostomum,* and *Cylicostephanus* species. Although our data and that from [10] indicates very close phylogenetic relationships between all four genera of nematodes (*Triodontophorus, Cyathostomum, Poteriostomum,* and *Cylicostephanus*), the differences in the placing of the parasites in the phylogenetic tree could be explained by differences in the genes used and the number of sequences analyzed in the two studies.



KEY
C.mont refers to different Isolates of *Cyathostomum montgomeryi*; Cy.min refers to different isolates of *Cylicostephanus minutus*; P. impari refers to different isolates of *Poteriostomum imparidentatum*; T. nipp refers to different isolates of *Triodontophorous nipponicus* while S. vulg refers to different isolates of *Strongylus vulgaris*. Bunphlebot refers to *Bunostomum phlebotomum*.

Figure 1: Phylogenetic analysis of ITS segment of the nucleotide sequences obtained in this study marked with different dark shapes black shapes and 17 reference ITS sequences obtained from the GenBank of the family Strongylidae including two sub-families (Cyathostominae and Strongylinae). *Bunostomum phlebotomum* (sub-family Bunostominae) was used as an outgroup.

The close relationship between *Triodontophorus* spp., which is classified in the subfamily Strongylinae and nematodes in the subfamily Cyathostominae, is an interesting finding. Similar findings have been reported by **[10]**, suggesting that *Triodontophorus* is a Cyathostominae rather than a Strongylinae. The study by **[10]**, sequenced complete mitochondrial (mt)genomes of three Cyathostominae species (*Cyathostomum catinatum*, *Cylicostephanus minutus*, and *Poteriostomum imparidentatum*) of horses. The complete mt nucleotide sequence comparison of all Strongylidae nematodes revealed that sequence identity ranged from 77.8 to 91.6%. The mt genome sequences of *Triodontophorous* species had a relatively high identity with Cyathostominae nematodes, rather than *Strongylus* species of the same subfamily (Strongylinae). Phylogenetic analyses using mtDNA data indicated that the *Triodontophorus* species clustered with cyathostominae species instead of *Strongylus* spp **[10]**, which is consistent with the current phylogenetic results. Results of a comparative study of the morphology of the larval stage four (L_4) by **[35]** also revealed more similarity between *Triodontophorus* larvae to those of Cyathostominae than to those of *Strongylus* spp. This observation further supports the suggestion that *Triodontophorus* spp. is a Cyathostominae.

The close clustering of five out of the ten sequences of *C. montgomeryi* from our study with those from the study by **[7]** indicates evolution diversity within the species. One out of the 3 sequences from *Cy. minutus* was a sister to a clade having sequences from horses in China and Germany. Unlike *C. montgomeryi*, *Cy. minutus* appears to be more widespread in equines across the world, with the parasites being phylogenetically diverse within the species. The *Cy. minutus* from horses in China and Germany however appear to be evolutionary distant from our isolates. Sequences from *P. imparidentatum, T. nipponicus,* and *S. vulgaris* from this study were evolutionary distinct from those from either horses or donkeys from other parts of the world that were obtained from the GenBank.

The taxonomic identity and phylogeny of equine strongyles detected in the three zebra groups included species of veterinary significance with little known epidemiology in Kenya. This study through the use of genetic tools was able to differentiate closely related nematode species. The eggs of these nematodes are morphologically indistinguishable and are often included in the generic category of 'strongyle eggs' which reduces the identity and richness of the strongyle nematodes. Identification of infection-causing helminth species is anticipated to progressively become important in veterinary management practices. Parasitic helminths, regardless of species, are potentially a burden to the host. The emphasis was on large and small strongyles since they are more significant in terms of effects on the host.

The relatively small sample of genotyped larvae was a consequence of two main factors that is, loss of larvae during the process of removing ethanol, and low quality of DNA that led to sequencing errors. In our opinion, the results of this study may not represent the whole species spectrum in the three zebra groups. The study used only one marker (ITS); The ITS region is known to reliably differentiate closely related nematode species **[22,26,36–40]**. This marker is also popular due to its lower level of intra-species polymorphism compared to mtDNA **[22,36–41]**. More comprehensive studies are recommended using both the ITS region and a portion of the mtDNA cytochrome oxidase 1 (CO1) gene of the mitochondrial DNA (mtDNA) since this locus undergoes rapid evolution and is good for differentiating cryptic parasite species **[40]** as well as phylogeographic groups within a single species **[40]**. However, these genetic markers are rarely used together in a single nematode species yet when used in combination they enhance identification output and provide more genetic information **[25,41]**.

5. Conclusions

This study confirms the occurrence of C. *montgomeryi* and *Cy. minutus* in plains zebras in Kenya with evolution diversity within the species. This is the first report on genetic identity of *P. imparidentatum, T. nipponicus,* and S. vulgaris in the plains zebras in Kenya. These three species appeared evolutionary distinct from previously reported isolates in horses or donkeys from other parts of the world. The placing of *T. nipponicus* as a sister to the Cyathostominae further supports previous suggestions that it is a Cyathostominae rather than a Strongylinae.

Authors' Contributions

Linda G.M. Maina: Writing original draft, methodology, data collection, data analysis, funding acquisition, conceptualization. Ndichu Maingi: Drafting the manuscript, supervision, review, editing. Chege J. Ng'ang'a: Supervision, investigation, review, editing. Robert M. Waruiru and Francis Gakuya: Investigation, review, editing. Esther G. Kanduma: Formal analysis, methodology, review.

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Data Availability

The data supporting the findings of this study is available within the article.

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Conflicts of Interest

The authors declare that there are no conflicts of interest related to this paper.

Ethical Approval

The research approval for the study was obtained from the Kenya Wildlife Service (KWS) REF No: (KWS/BRM/ 5001). Faecal collection was done in adherence to the guidelines and regulations by KWS on conducting research on wildlife. All institutional and national guidelines for the care and handling of animals were observed.

References

[1] Burgess CGS, Bartley Y, Redman E, Skuce PJ, Nath M, Whitelaw F, *et al*. A survey of the trichostrongylid nematode species present on UK sheep farms and associated anthelmintic control practices. Vet Parasitol 2012;189:299–307. https://doi.org/10.1016/j.vetpar.2012.04.009.

[2] Petney TN, Andrews RH. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. Int J Parasitol 1998;28:377–93. https://doi.org/10.1016/s0020-7519(97)00189-6.

[3] Gulland FM, Fox M. Epidemiology of nematode infections of Soay sheep (Ovis aries L.) on St Kilda. Parasitology 1992;105 (Pt 3):481–92. https://doi.org/10.1017/s0031182000074667.

[4] Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, *et al*. Climate warming and disease risks for terrestrial and marine biota. Science 2002;296:2158–62. https://doi.org/10.1126/science.1063699.

[5] Michel AL, Bengis RG. The African buffalo: A villain for inter-species spread of infectious diseases in southern Africa. Onderstepoort Journal of Veterinary Research 2012;79:26–30.

[6] Lichtenfels JR, Kharchenko VA, Dvojnos GM. Illustrated identification keys to strongylid parasites (strongylidae: Nematoda) of horses, zebras and asses (Equidae). Veterinary Parasitology 2008;156:4–161. https://doi.org/10.1016/j.vetpar.2008.04.026.

[7] Tombak KJ, Hansen CB, Kinsella JM, Pansu J, Pringle RM, Rubenstein DI. The gastrointestinal nematodes of plains and Grevy's zebras: Phylogenetic relationships and host specificity. International Journal for Parasitology: Parasites and Wildlife 2021;16:228–35. https://doi.org/10.1016/j.ijppaw.2021.10.007.

[8] Krecek RC, Malan FS, Reinecke RK, de Vos V. Nematode parasites from Burchell's zebras in South Africa. J Wildl Dis 1987;23:404–11. https://doi.org/10.7589/0090-3558-23.3.404.

[9] Mitchell CJ, O'Sullivan CM, Pinloche E, Wilkinson T, Morphew RM, MCewan NR. Using next-generation sequencing to determine diversity of horse intestinal worms: identifying the equine 'nemabiome.' J Equine Sci 2019;30:1–5. https://doi.org/10.1294/jes.30.1.

[10] Gao Y, Zhang Y, Yang X, Qiu J-H, Duan H, Xu W-W, *et al.* Mitochondrial DNA evidence supports the hypothesis that Triodontophorus species belong to Cyathostominae. Frontiers in Microbiology 2017;8.

[11] Krecek RC, Reinecke RK, Kriek NJP, Horak IG, Malan FS. Helminth parasites of Cape mountain zebras from Cape Province, South Africa. Journal of Wildlife Diseases 1994;30:277–80. https://doi.org/10.7589/0090-3558-30.2.277.

[12] Wambwa EN, Ogara WO, Mudakha D. A comparative study of gastrointestinal parasites between ranched and free ranging Burchell's zebra (Equus burchelli antiquorum) in Isiolo district, Kenya. J Vet Sci 2004;5:215–20.

[13] Matthews JB. Anthelmintic resistance in equine nematodes. Int J Parasitol Drugs Drug Resist 2014;4:310–5. https://doi.org/10.1016/j.ijpddr.2014.10.003.

[14] Hamed MI, El-Allawy TA, Hassnein E. Prevalence and anthelmintic resistance of Strongyle infection of donkeys in

El-Wadi El-Gadid, Egypt. Journal of Advanced Veterinary Research 2019;9:144–50.

[15] Oakenfull EA, Clegg JB. Phylogenetic relationships within the genus Equus and the evolution of alpha and theta globin genes. J Mol Evol 1998;47:772–83. https://doi.org/10.1007/pl00006436.

[16] Matthee S, Krecek RC, McGeoch MA. A comparison of the intestinal helminth communities of Equidae in Southern Africa. J Parasitol 2004;90:1263–73. https://doi.org/10.1645/GE-3353.

[17] Tolliver SC, Lyons ET, Drudge JH. Species of small strongyles and other internal parasites recovered from donkeys at necropsy in Kentucky. Proceedings of the Helminthological Society of Washington (USA) 1985;52:260–5.

[18] Barker DC. Molecular approaches to DNA diagnosis. Parasitology 1989;99 Suppl:S125-146. https://doi.org/10.1017/s0031182000083463.

[19] Wilson SM. Nucleic acid techniques and the detection of parasitic diseases. Parasitol Today 1991;7:255–9. https://doi.org/10.1016/0169-4758(91)90243-h.

[20] Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol 1986;51 Pt 1:263–73. https://doi.org/10.1101/sqb.1986.051.01.032.

[21] Hung GC, Gasser RB, Beveridge I, Chilton NB. Species-specific amplification by PCR of ribosomal DNA from some equine strongyles. Parasitology 1999;119 (Pt 1):69–80. https://doi.org/10.1017/s0031182099004497.

[22] Gasser RB, Bott NJ, Chilton NB, Hunt P, Beveridge I. Toward practical, DNA-based diagnostic methods for parasitic nematodes of livestock--bionomic and biotechnological implications. Biotechnol Adv 2008;26:325–34. https://doi.org/10.1016/j.biotechadv.2008.03.003.

[23] Maina LGM, Maingi N, Ng'ang'a CJ, Waruiru RM, Gakuya F. Diversity, prevalence, and intensity of gastrointestinal helminth infections in migratory, resident, and sedentary plains zebras (Equus quagga) in Masai Mara National Reserve and Lake Nakuru National Park, Kenya. Vet Parasitol Reg Stud Reports 2022;33:100750. https://doi.org/10.1016/j.vprsr.2022.100750.

[24] Great Britain Ministry of Agriculture, Fisheries and Food. Manual of Veterinary parasitological laboratory techniques. H. M. Stationery Office; 1986.

[25] Archie EA, Ezenwa VO. Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. Int J Parasitol 2011;41:89–98. https://doi.org/10.1016/j.ijpara.2010.07.014.

[26] McLean ER, Kinsella JM, Chiyo P, Obanda V, Moss C, Archie EA. Genetic identification of five strongyle nematode parasites in wild african elephants (Loxodonta africana). J Wildl Dis 2012;48:707–16. https://doi.org/10.7589/0090-3558-48.3.707.

[27] Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 2004;32:1792–7. https://doi.org/10.1093/nar/gkh340.

[28] Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018;35:1547–9. https://doi.org/10.1093/molbev/msy096.

[29] Tamura K. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Mol Biol Evol 1992;9:678–87. https://doi.org/10.1093/oxfordjournals.molbev.a040752.

[30] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783–91. https://doi.org/10.2307/2408678.

[31] Lichtenfels JR. Helminths of domestic equids. Illustrated keys to genera and species with emphasis on North American forms. Proceedings of the Helminthological Society of Washington 1975;42:1–92.

[32] Kharchenko V, Lichtenfels J, Krecek R. Cyathostomum montgomeryi and its place in the Cyathostominae (Nematoda : Strongylidae). Comparative Parasitology 2001;68:97–102.

[33] Scialdo-Krecek RC. Studies on the parasites of zebras. 1. Nematodes of the Burchell's zebra in the Kruger National Park. Onderstepoort J Vet Res 1983;50:111–4.

[34] Kuzmina T, Kharchenko V, Zvegintsova N, Zhang L, Liu J. Strongylids (Nematoda: Strongylidae) in two zebra species from the "Askania-Nova" Biosphere Reserve, Ukraine: biodiversity and parasite community structure. Helminthologia 2013;50:172–80. https://doi.org/10.2478/s11687-013-0128-0.

[35] Dvojnos GM, Harcenko VA. Morphology and differential diagnostics of parasitic larvae of some Strongylidae (Nematoda) of horses. Angew Parasitol 1990;31:15–28.

[36] Chilton NB, Gasser RB, Beveridge I. Differences in a ribosomal DNA sequence of morphologically indistinguishable species within the Hypodontus macropi complex (Nematoda: Strongyloidea). International Journal for Parasitology 1995;25:647–51. https://doi.org/10.1016/0020-7519(94)00171-J.

[37] Hung G-C, Jacobs DE, Krecek RC, Gasser RB, Chilton NB. Strongylus asini (Nematoda, Strongyloidea): Genetic relationships with other strongylus species determined by ribosomal DNA. International Journal for Parasitology 1996;26:1407–11. https://doi.org/10.1016/S0020-7519(96)00136-1.

[38] Newton LA, Chilton NB, Beveridge I, Hoste H, Nansen P, Gasser RB. Genetic markers for strongylid nematodes of livestock defined by PCR-based restriction analysis of spacer rDNA. Acta Trop 1998;69:1–15. https://doi.org/10.1016/s0001-706x(97)00105-8.

[39] Gasser RB, Newton SE. Genomic and genetic research on bursate nematodes: significance, implications and prospects. International Journal for Parasitology 2000;30:509–34. https://doi.org/10.1016/S0020-7519(00)00021-7.

[40] Blouin MS. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. International Journal for Parasitology 2002;32:527–31. https://doi.org/10.1016/S0020-7519(01)00357-5.

[41] Denver DR, Wilhelm LJ, Howe DK, Gafner K, Dolan PC, Baer CF. Variation in base-substitution mutation in experimental and natural lineages of Caenorhabditis nematodes. Genome Biology and Evolution 2012;4:513–22. https://doi.org/10.1093/gbe/evs028.

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