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Original Research

Proposal and Validation of a Scale of Composite Measure Reactivity Score to Characterize the Reactivity in Horses During Handling

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ABSTRACT

This study aimed to identify the parameters related to the expression of the reactivity in horses during handling and based on that proposed and validated a scale of composite measure reactivity score to characterize horse's reactivity. To this end, the first stage (S1) proposed the scale and the second (S2) validated it. In S1, 364 Lusitano horses were evaluated, 188 were adult breeding mares (4–12 years old), and 176 were foals (males/females, aged from 2 months to 2 years). During hooves trimming, vermifuge application, palpation scores were assigned to behaviors of movement, ears and eyes position, breathing, vocalization, and urination. A response parameter called reactivity was attributed to each animal, ranging from score 1 (nonreactive/calm) to score 4 (very reactive/aggressive). The verification of the possible parameters (age, behavior), which explains the response parameter (reactivity), was taken using ordinal proportional odds model. Movement, breathing, ears and eyes position, vocalization, and age appear to explain the reactivity of horses during handling ($P < .01$). Therefore, based on these parameters, it was possible to propose two scales of composite measure reactivity score: one to characterize the mares and another the foals. On S2, the proposed scale was validated by the simultaneous application of Forced Human Approach Test, another commonly used test to evaluate the reactivity in horses, with a correlation of 0.97 ($P < .05$). The assessment of the reactivity of horses during handling by a composite measure reactivity score scale is valid, and easy to apply, without disrupting daily routine and override the impact of individual differences.

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1. Introduction

Successful relationship between humans and horses is important for the use of animals during equestrian activities such as leisure, sport, or riding. The way horses react to human results from the interaction between the animal temperament, temperament and skills of humans, and

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experience acquired by the animal through contact with humans [1]. Moreover, the performance and suitability of the horse in a equestrian modality can also be affected by its temperament [2]. A temperament trait is generally defined as individual differences in behavior that are present early in life and are relatively consistent during time and through the different situations [3].

Studies involving the temperament of horses have focused on aspects such as emotionality or emotional reactivity, learning ability, and reactions to human presence or reactivity to humans, which are most relevant traits to achieve optimal performance in those animals [4]. However, because the study of temperament is complex and there are several terminologies and different methodologies involved, the assessment of the temperament becomes difficult. Therefore, it is essential to clarify the aspect of temperament that needs to be evaluated.

In this context, this study will use the concept of reactivity, which appears as an aspect of temperament defined as behavioral expression during handling, generally attributed to fear and associated with stimuli caused by human presence [5].

In practice, the reactivity in horses has been studied by the dissociation of methodologies that assess fear through the aspect of temperament called emotional reactivity [6,7] and situations of confrontation with humans through the aspect of reactivity to humans [8,9].

These studies have used a variety of experimental techniques, such as arena tests, in which the horse is loose in a familiar environment [6]; the open field tests, in which the horse is loose in an unfamiliar environment [2]; the novel object tests in which the horse is presented a new static or moving object [10]; tests that assess the presence of a passive or active human [8]; the bridge test, which consists of a manipulator leading the animal in an unknown surface [11]; reactivity test performed during the evaluation of the conformation of the horse [12,13], and reactivity assessed in situations as feeding, riding, and turning out [14].

In the investigation of sheep and cattle reactivity, the frequently used tests are the restriction test, which punctuates the animals through scale of composite measure reactivity score, and the test of disturbance, measured when the animal is subjected to a given handling situation in the pen, such as weighing [15–17]. The application of scale of composite measure reactivity score during handling has not been used as a methodology to characterize the reactivity of horses. However, the development of a detailed methodology to assess the reactivity of equine during handling becomes necessary since the reactivity is one aspect of temper expressed by the animals during handling.

The term reactivity is specific to each individual because each animal exhibits different behavior which might be indicative of a same level of reactivity. For example, an animal that is very reactive may express this by "freezing" and other animal with the same level of reactive also may display the agitation as a manifestation of their reactivity. The combination of different behaviors within a composite score scale may override the impact of individual differences, making the animal reactivity evaluation more detailed and explanatory. Therefore, this study aimed to identify the parameters related to the expression of the reactivity of horses during handling and to propose and validate a scale of composite measure reactivity score, based on these parameters, to characterize the reactivity of horses.

2. Materials and Methods

This study was conducted in two stages, which will be detailed subsequently. The first stage was performed to identify parameters that are related to the expression of reactivity of horses during handling. In addition, during the first stage, it was also proposed a composite score scale of behavior for characterization of reactivity from parameters which can explain reactivity of horses.

The second stage a study was done to validate the scale of composite measure reactivity score in horse during handling, proposed in the first stage.

2.1. First Stage

2.1.1. Animals and Local Used

The first stage was conducted on a Lusitano horse breeding farm located in Itapira, São Paulo, Brazil, and all procedures were approved by the Ethics Committee of the University of São Paulo, Brazil, under n°. 12.1.755.74.9.

A total of 364 animals comprising 188 adult breeding mares (4–12 year old) and 176 foals (77 males and 99 females) between the ages of 2 and 4 months (P1), 5 and 6 months (P2), 10 and 12 months (P3), 13 and 14 months (P4), and with 2 year old (P5) were observed.

The animals used in this study were maintained on pasture, grouped according to the age, and have fed in the trough once daily. The groups of mothers were separated according to the gestational state (empty, pregnant, and mares with their newborn foals). The foals were kept with their mothers until the age of 6 months, after that time, they were weaned and grouped according to sex and age.

In this property, the breeding season begins in September and extends through February. The beginning of the reproductive phase of the mares is at 4 years old. The breeding mares observed in this study were not tamed; the halter was used to facilitate handling. The foals did not use the halter.

2.1.2. Trial Period and Data Collection

The methodology used in this stage did not interfere in the daily activities of the property, and hence, the behavioral assessments of the animals were carried out during the programmed handling routine.

The observations were performed during hooves trimming (n = 176 foals), vermifuge application (n = 176 foals; 188 mares), and palpation (n = 188 mares). Each animal was observed twice for each activity, during a period of 60 days.

The animals were used to handlers and handling facilities. The professional team was consisted of two handlers responsible for vermifuge application, a veterinarian responsible for palpation, and two technicians for hooves trimming.

Table 1
Scores assigned to the animal's behavior of movement, position of ears and eyes, breathing, vocalization, and urination during management activities observed in the experimental period.

Behavioral Category	Score	Description
Movement	1	No body movement, can have absent or occasional movements of the tail, no foot movement
	2	Changes in body position, occasional tail movements, can have absent or occasional foot movement
	3	Frequent body movement, vigorous tail movements, can have occasional foot movement
	4	Continuous movement, continuous and vigorous tail movements, frequent foot movement
Position of ears and eyes	1	Ears erect or relaxed without specific attention, eyes relaxed
	2	Ears movement facing forward or backward, ears and eyes attentive
	3	Frequent ears movement (exchanges of position) can have withering ears, widening eyes
Breathing	1	No breathing audible
	2	Audible breathing and rhythmic form (no changes)
	3	Deep and audible breath, with parameter rhythm
	4	Nasal and oral breathing, with intense expiratory movements (snorting)
Vocalization	1	No vocalization
	2	Can have vocalization
	3	Frequent vocalization
Urination	1	No urination
	2	One urination
	3	Frequent urination

Mares and foals were conducted from the pasture to the location where the handling would be done. Each mare was picked up individually by the handler, using a halter and taken to the chute located indoor. The foals were held by the neck and tail, and they were handled after the handling of the mare.

The meteorological parameters of air temperature and relative humidity were recorded every 30 minutes (measured with digital hygrometer, Thermotemp, Campinas). The average temperature and average humidity during the experimental period were 26°C (±1.40) and 60% (±5.76), respectively, which was within the thermal comfort zone of horses ranging from 5°C to 30°C.

The behavioral observations of the animals were made by the visual method during 20 seconds after the beginning of each handling, by applying scores to the five behavioral parameters of the horses: movement, position of ears and eyes, breathing, vocalization, and urination [16,17] (Table 1). The observed behaviors were recorded in a spreadsheet.

When using the scale of reactivity, it is easy to separate the extremes, that is, distinguishing between a peaceful animal, possibly with score 1, and a very aggressive one, possibly with score 5. It is, however, difficult to assign intermediate scores which leads to a tendency to attribute the score 3, instead of 2 or 4 [18]. The animals in this study were ranked among 4 scores of reactivity in order to distinguish classes of high and low reactivity [19,20].

A response parameter called reactivity was attributed to the animals after the observations of behavioral categories during handling. Animals were classified based on the reactivity scores of 1–4, where a score 1 was given to a nonreactive or calm animal; score 2 was given for a slightly reactive or active animals; a score 3 was given for the reactive or restless animals and very reactive or aggressive animals received a score 4. Those given scores were based on a validated purpose scale [16,17] in which one person observes the reactivity of the animal during the initial 20 seconds of the handling.

The behavioral observations were assigned by a trained observer, and the reactivity response parameter was assigned by another trained observer. The two observers were not familiar with the animals from the property. The observers were positioned in a place where they could not be seen by the animals, avoiding any disturbance during handling. Two observers were used in order to avoid having subjective data from the behavioral evaluation (movement, position of ears and eyes, breathing, vocalization, and urination and also the composite reactivity score).

2.2. Second Stage

2.2.1. Animals and Local

This stage was conducted on a Mangalarga Marchador horse breeding farm located in Amparo, São Paulo, Brazil, and all procedures were approved by the Ethics Committee of the University of São Paulo, Brazil, under n° 12.1.755.74.9 and n° 13.1.2217.74.5.

Sixteen horses, 8 mares between the ages of 5 and 9 years and their 8 foals between the ages of 4 and 11 months, were evaluated during 8 months, one time a month.

2.2.2. Trial Period and Data Collection

To validate the scale of composite measure reactivity score proposed in the first stage of this paper, a study was done to evaluate the reactivity of mares and foals by applying the methodology proposed in the first stage of present work and through the Forced Human Approach Test, which is usually used to evaluate the reactivity in horses [21].

Each pair was individually evaluated, first the mare and then the foal, during brushing, a habitual handling in the horse farm, inside a fenced area of 3 × 5 meters. During brushing, the mares were stuck by a halter and the foals were held by the neck and tail. Both remained in the pen during the handling of another.

Each evaluating was done during 20 seconds after the beginning of brushing, by applying scores to the four behavioral parameters of the horses: movement, position of ears and eyes, breathing and vocalization (Table 1). From

Table 2
Odds ratio of the univariate analysis for the score of reactivity according to the parameters of age and behavior.

Parameter	OR	95% CI
Movement	157	134.6–183.3
Breathing ≥2 versus breathing = 1	11	7.5–16.2
Position of ears and eyes	24	16.1–34.8
Vocalization ≥2 versus vocalization = 1	6.5	3.1–13.5
P1[a] versus adult mares	10	6.5–15.0
P2[b] versus adult mares	5	3.7–7.1
P3[c] versus adult mares	3	1.4–5.7
P5[d] versus adult mares	3.5	1.7–7.2

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Foals between the ages 2 and 4 months.
[b] Foals between the ages 5 and 6 months.
[c] Foals between the ages 10 and 12 months.
[d] Foals at 2 years.

these observed behaviors, the response parameter called composite measure reactivity score was attributed to the mares and to the foals (see results from stage 1) after the observations of behavioral categories during handling. Animals were classified based on the reactivity scores of 1–4, where a score 1 was given to a nonreactive or calm animal; score 2 was given for a slightly reactive or active animals; a score 3 was given for the reactive or restless animals and very reactive or aggressive animals received a score 4.

Following evaluation of reactivity during handling, each pair of mare and her foal was released individually in a fenced area of 67 × 21 meters for the application of the Forced Human Approach Test, first applied to the mare, second to the foal. The unknown human (unfamiliar) opened the fenced area door and entered the paddock and approached the horse slowly at approximately one step per second with their hands by his side. If the horse stood still at 2 meters from the person, he slowly raised his hand to allow the horse approach. If the animal sniffs his hand, he tried to touch the neck [21]. A horse was scored from 1 to 4 on the following scale:

Score 1—The person could touch the horse on the neck.

Score 2—The horse sniffed the person's hand.

Score 3—The horse stood still when the person was within a 2-m range.

Score 4—The horse moved away from the person before he/she got within a 2-m range.

Table 3
Values of the coefficients, standard deviation, and *P* values obtained for the first proportional odds model adjusted, referring to the three categories of the response parameter, movement, position of ears and eyes, and junction of ages P1 + P2.

Parameter	Coefficient	Standard Deviation	*P* Value
Reactivity score 3	−37.900	3.980	<.001
Reactivity score 4	−48.447	5.074	<.001
P1[a] + P2[b] versus adult mares	1.373	0.528	.009
Position of ears and eyes	8.501	0.974	<.001
Movement	7.676	0.775	<.001

[a] Foals between the ages 2 and 4 months.
[b] Foals between the ages 5 and 6 months.

Table 4
Values of the coefficients, standard deviation, and *P* values obtained for the second proportional odds model, referring to the four categories of the response parameter, the parameter of age, and behavioral parameters position of ears and eyes, breathing, and vocalization.

Parameter	Coefficient	Standard Deviation	*P* Value
Reactivity score 2	−7.616	0.511	<.001
Reactivity score 3	−9.901	0,572	<.001
Reactivity score 4	−12.26	0.653	<.001
P1[a] versus adult mares	1.408	0.293	<.001
P2[b] + P3[c] + P4[d] versus adult mares	0.605	0.234	.009
P5[e] versus adult mare	1.194	0.409	.003
Position of ears and eyes	3.079	0.214	<.001
Breathing ≥2 versus breathing = 1	2.796	0.342	<.001
Vocalization ≥2 versus vocalization = 1	1.043	0.455	.022

[a] Foals between the ages 2 and 4 months.
[b] Foals between the ages 5 and 6 months.
[c] Foals between the ages 10 and 12 months.
[d] Foals between the ages 13 and 14 months.
[e] Foals at 2 years.

2.3. Statistical Analysis

2.3.1. First Stage

For this statistical analysis, the ordinal logistic regression was used and the proportional odds for the ordinal model were adjusted. In this model, the odds ratio shows the risk that the parameter has to increase a unit in the scale [22]. Data were analyzed by value of optimism in the coefficient of Sommer's and Nagelkerke R^2 and area under the curve (AUC) value. In the first measure, a correlation coefficient of 0 represents no discriminating ability and a value of 1 represents perfect discrimination. AUC value is the estimated area under a receiver operating characteristic (ROC), or simply ROC curve, which is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied.

The analysis by ordinal models must be preceded by a comparison between each parameter analyzed with a response parameter. Through this analysis, known as univariate analysis, one can select the factors that will be introduced in the regression model [23].

Table 5
Odds ratio scores reactivity according to the behavioral parameters of position of the ears and eyes, breathing, vocalization, and parameters of age, for the second proportional odds model.

Parameter	OR	95% CI
P1[a] × adult mares	4	2.3–7.3
P2[b] + P3[c] + P4[d] × adult mares	2	1.2–2.9
P5[e] × adult mares	3.5	1.5–7.4
Breathing ≥2 versus breathing = 1	16	8.4–32.0
Vocalization ≥2 versus vocalization = 1	3	1.2–6.9
Position of ears and eyes	22	14.3–33.1

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Foals between the ages 2 and 4 months.
[b] Foals between the ages 5 and 6 months.
[c] Foals between the ages 10 and 12 months.
[d] Foals between the ages 13 and 14 months.
[e] Foals at 2 years.

Table 6
Scale of composite measure reactivity score for characterize the reactivity of the mares, according to the scores of movement, breathing, the position of the ears and eyes and vocalization.

Reactivity Score	Scores of Behavioral Category			
	MOV	BRE	PEE	VOC
1: Nonreactive or calm animal	1	1	1	1
	1	1	2	1 or 2
	1	2	2	1
	1	1	1	2
	2	1	1	1
2: Slightly reactive or active animal	1	1	3	1
	2	1	2	1 or 2
	2	2	2 or 3	1
3: Reactive or restless animal	1	2	3	1
	2	1	3	1
	3	1	2	1 or 2
	3	2	2	1
	4	2	2	1
4: Very reactive or aggressive animal	3	1	3	1
	3	2	3	1 or 2
	4	1	3	1 or 2
	4	2	3	1
	4	3	2	1

Abbreviations: BRE, breathing; MOV, movement; PEE, position of ears and eye; VOC, vocalization.

The verification of the possible parameters associated with reactivity response parameter was performed using univariate and multivariate analyses with proportional odds by employing the following parameters: (1) the dependent parameter, with values of score 1 to nonreactive or calm animals, score 2 for a slightly reactive or active animals, score 3 for reactive or restless animals and score 4 for very reactive or aggressive animals; (2) categorical independent parameters of behavior (according to behavior scores described in the methodology: movement, position of ears and eyes, breathing, vocalization, and urination), and age (adult mares, P1, P2, P3, P4, and P5).

Table 7
Scale of composite measure reactivity score for characterize the reactivity of the foals, according to the scores of movement, breathing, the position of the ears and eyes, and vocalization.

Reactivity Score	Scores of Behavioral Category			
	MOV	BRE	PEE	VOC
1: Nonreactive or calm animal	1	1	1	1 or 2
	1	1	2	1 or 2
	2	1	1	1
2: Slightly reactive or active animal	1	1	3	1 or 2
	2	1	2	1 or 2 or 3
	2	2	2	1
3: Reactive or restless animal	2	1	3	1 or 2
	2	4	2	1
	3	1	2	1 or 2 or 3
	3	2	2	1
	4	1	2	2
4: Very reactive or aggressive animal	3	1	3	1 or 2 or 3
	3	2	3	2
	3	4	2	1
	4	1	2	3
	4	1	3	1
	4	2	2	1
	4	2	3	1 or 2

Abbreviations: BRE, breathing; MOV, movement; PEE, position of ears and eye; VOC, vocalization.

Two models having ordinal proportional odds were adjusted from parameters considered to be associated with reactivity response parameter after the univariate analysis. The proportional odds assumption of these models was validated by cross-validation of the model by bootstrap. For the first model, three categories for the response parameter reactivity were considered: the first category was the junction of reactivity scores 1 and 2, the second category was reactivity score 3, and the third category was reactivity score 4. Furthermore, the parameters of age P1, P2, and adult mares and behavioral parameters of movement and position of the ears and eyes were analyzed.

In the second model, the four categories for the response parameter reactivity were considered: the first category was score 1, the second category was score 2, the third category was score 3, and the fourth category was score 4. Furthermore, all parameters of age and behavioral parameters breathing, position of ears and eyes, and vocalization were evaluated. In this model, the behavioral parameter movement was not consider because it absorbed the effect of other parameters and was strongly associated with the parameter reactivity, leading to an almost complete separation in terms of a contingency table.

2.3.2. Analysis to Propose the Scale of Composite Measure Reactivity Score

From the statistical analysis used for phase 1, parameters regarding the expression of reactivity of horses during handling were identified.

The significate parameters to explain the reactivity response parameter were computed with all the different combinations between the scores of observed behaviors during the handling in the experimental period. Therefore, based on these combinations and reactivity response parameter, a scale of composite measure reactivity score was presented to characterize horse's reactivity in the present study.

2.3.3. Second Stage

Spearman correlation was performed between the reactivity scores obtained in the evaluation during brushing and the reactivity scores in Forced Human Approach Test. The significance level used in the analysis was 1%, and the experimental results were presented with coefficients and standard deviation.

3. Results

3.1. First Stage

3.1.1. Parameters of Equine Behavior Related to the Expression of Activity During Handling

In univariate analysis, the four parameters of behavior (movement, position of ears and eyes, breathing, and vocalization) were associated with the response parameter. However, the parameter urination did not reveal significance to explain the reactivity of animals in this study.

For the parameter movement, it was found a correlation coefficient gama Goodman equal to 0.993 ($P < .01$), correlation coefficient Tau-b Kendall of 0.876 ($P < .01$), and Sommer's D coefficient of 0.838 ($P < .01$), showing that there was a strong association between movement and the response parameter ($P < .01$).

The ordinal model, with the parameter movement as explanatory reactivity, had an excellent discrimination, presenting an AUC of 0.919, Nagelkerke coefficient of determination of 0.811 ($P < .01$), and a coefficient of Brier of 0.044 ($P < .01$).

From this model, it was found that for each unit increased in the score of the movement, there was a 157 times higher possibility of increasing the score of reactivity (95% confidence interval [CI] = 134.6, 183.3, Table 2).

The univariate analysis also revealed that for the parameter breathing, an animal with a score greater than 2 was 11 times more likely to have higher reactivity than an animal with a score of 1 (95% CI = 7.5, 16.2). In the parameter position of ears and eyes, with every score unit increased, the animal had about 24 times greater possibility to present higher reactivity (95% CI = 16.1, 34.8). Furthermore, for the parameter vocalization, an animal with a score greater than 2 was 6.5 times more likely to be more reactive than an animal with a score of 1 (95% CI = 3.1, 13.5, Table 2).

With regard to the age of the animals, foals had a greater chance of being more reactive than the adult animal. The possibility of being reactive was about 10 times higher for P1 foals (95% CI = 6.5, 15.0); about five times higher for P2 foals (95% CI = 3.7, 7.1); about three times higher for P3 foals (95% CI = 1.4, 5.7); and about 3.5 times higher for 2-year foals (P5) (95% CI = 1.7, 7.2, Table 2). Regarding to P4 foals, there were no significant differences ($P > .01$).

In validation of the proportional odds presupposition in the first model, a value lower than 0.001 was obtained for the optimism of the coefficient of Sommer's ($P < .01$), and a value of 0.0046 was obtained for the optimism of the Nagelkerke R^2 ($P < .01$). The adjusted model presented coefficient R^2 of 0.938 ($P < .01$), with a capacitance value of the discriminant model AUC of 0.994, considered as perfect discrimination. The coefficients of the parameters for adjusted model are shown in Table 3.

The first model revealed that higher the scores of position of ears and eyes, higher the scores of movement and younger the animals, greater the reactivity (foal presented four times higher possibility to be more reactive than an adult animal [95% CI = 1.4, 11.1]).

In validation of the proportional odds presupposition in the second model, a value lower than 0.0049 was obtained for the optimism when using the coefficient of Sommer's ($P < .01$), and a value of 0.0135 was obtained for the optimism of the Nagelkerke R^2 ($P < .01$). The adjusted model presented coefficient R^2 of 0.528, with AUC of 0.842, considered as very good. The coefficients of the parameters for the adjusted model are shown in Table 4.

The second model reported that P1 foals were four times more likely to be more reactive than an adult animal (95% CI = 2.3, 7.3); for P2, P3, and P4 foals, the possibility decreased close to the twofold (95% CI = 1.2, 2.9); and for P5 animals, the chance to be more reactive than an adult increased to 3.5 times (95% CI = 1.5, 7.4, Table 5).

According to the second model, regarding to behavioral parameters, an animal with breathing score of 2 or more was about 16 times more likely to be more reactive than an animal with breathing index of 1 (95% CI = 8.4, 32.0); animals with vocalization index of 2 or more had three times higher chance of being more reactive than animals with vocalization index of 1 (95% CI = 1.2, 6.9), and with each unit increased in the score for position of ears and eyes, there was a 22 times higher chance of increasing the reactivity score (95% CI = 14.3, 33.1, Table 5).

The proportional odds models showed that behavioral parameters of movement and position of ears and eyes, breathing and vocalization, and age were significant in explaining the reactivity ($P < .01$). Thus, all different combinations among the scores of behaviors observed during the period of trial were computed for both mares and for foals.

3.1.2. Proposal of a Scale of Composite Measure Reactivity Score

According to the results mentioned in the previous section, it was found that the movement, position of ears and eyes, breathing, and vocalization were the explanatory behavior parameters of the reactivity of horses. Furthermore, it was also found that the age of the animal altered the reactivity.

From these results, it was decided to propose two scales from these explanatory parameters of reactivity: one characterizes the reactivity of the mares (Table 6) and the other, the reactivity of the foals (Table 7).

These scales have been proposed through the different combinations of scores of behaviors: movement, position of ears and eyes, breathing, and vocalization identified as explanatory reactivity of mares and foals during the first phase of the study. For mares, 24 different combinations of scores of behavior were found (Table 6), and for foals, 29 different combinations were used (Table 7) to propose the two scale of composite measure reactivity score.

3.2. Second Stage

3.2.1. Validation of the Scale of Composite Measure Reactivity Score

High value of the Spearman correlation coefficient (0.97; $P < .05$) was found for mare and foal between the reactivity score by the proposal methodology (handling test) and the Forced Human Approach Test score, methodology commonly employed used in horses (Table 8).

Table 8
Spearman correlation coefficient (rs) between reactivity score of the proposal methodology (scale of composite measure reactivity score during handling test) and reactivity score during the Forced Human Approach Test (methodology commonly used in horses) for mare and foal.

Reactivity Assessment Tests/Animal	Forced Human Approach Test/Mare	Forced Human Approach Test/Foal	Handling Test/Mare
Handling test/mare	0.97*	0.97*	—
Handling test/foal	0.97*	0.97*	1
Forced Human Approach Test/foal	0.86*	—	0.97*

*$P < .001$

4. Discussion

The proportional odds models showed that higher scores of movement and position of ears and eyes, breathing and vocalization scores of at least 2 and P1 animals (3–4 months old) are associated with higher scores of reactivity.

In the present study, younger horses (P1) showed higher reactivity. In an earlier study, the age also influenced the reactivity of lambs (5–6 months of age animals showed lower reactivity than the 3–4 months old ones) [24]. The reaction of young horses in a familiar environment is as intense as their reaction in an unfamiliar environment, and this influenced their reactions to humans and their behavior in general [21]. However, beyond age, the experiences were also found to affect the reactivity, and those aspects were often confused. In reality, these factors act simultaneously on the reactivity of animals, but in an independent manner [25].

The reactivity of foals in the present study was higher than that of adult mares, and this propensity of young animals to be more reactive could probably be due to the lack of previous experience in the handling [26]. Thus, in tests involving humans and animals, the expression of reactivity may be altered as a result of habituation to humans and daily handling. This change in reactivity can also be explained by the psychological development that horses undergo with age [21].

Adult breeding mares are often submitted to reproductive handling. The lower reactivity showed by these animals can be related to the fact that the mares are habituated to those activities. In agreement with our results, other authors reported that Nelore bulls were adapted to subsequent experiments of weighing showing lower reactivity scores [17].

Cattle subjected to different reactivity tests by principal component analysis showed that the movement of the body, tail, and head were important to define the score of agitation in the trunk of containment [27]. In the assessment of the behavior in young dairy cattle, on three occasions, it was demonstrated that the locomotion was the first major component to explain the reactivity [28]. The importance of agitation in the evaluation of reactivity through three methods was also observed in beef cattle [29]. In the present study, there was also an excellent association between the movement and reactivity of horses.

However, the parameter movement can be contradictory to explain the fear caused by handling or handlers because the inhibition of the movement or immobility can also be viewed as expressing a state of fear [30]. Therefore, the horse can remain static facing the new stimulus, but may show increased heart rate, indicating the same adverse reaction [31]. Weaned foals were observed during an auction, and the freezing behavior was used to assess the fear of animals during this situation [32]. In the present study, freezing is when movement has score 1, indicating the absence of movement. However, to actually indicate freezing trigged by fear, it should be accompanied by other high scores (e.g., score 3 for position of ears and eyes) showing high reactivity. The relaxed posture is when the score is 1 for both movement and position of ears and eyes. Therefore, in this case, the animal is relaxed and shows no reactivity.

Furthermore, in the present study, the position of ears and eyes proved to be indicative of reactivity in horses. In studies with horses submitted to unknown stimulus or isolation, the position of ears and eyes was also used to predict the reactivity of animals [6,31]. Thus, the position of the upper eyelid and eye movement, as well as the movement of the ears, may indicate reactivity. For example, high frequency of eye movement may indicate anxiety, while the absence of eye movement may indicate discomfort or pain [33].

Vocalization was found to be quantitatively related to reactivity [34]. High vocalization may be an indicative of fear; horses exposed to a threatening situation use the expression of this behavior with a warning purpose inside a group of animals. As previously reported, increased reactivity can lead to increased vocalization, which is a feature of social communication between mares and foals, mares and stallions, or when horses of the same social group are separated [35].

In the present study, as mares and foals were handled separately, vocalization may be related to communication between these animals. Moreover, increased vocalization score associated with increased reactivity may be due to the fear during handling or fear associated to the presence of the handler.

Breathing scores of 2 or higher were most likely related to higher scores of reactivity. The highest breathing scores may be associated with the presence of highest vocalization and movement scores [36] or physiological responses by activating the basic sympathetic nervous system in the presence of potentially aversive stimuli [34].

The reactivity is specific to each individual because each animal exhibits different behavior which might be indicative of a same level of reactivity. For this reason, the measurement of physiological parameters as a tool to enhance the behavior evaluation is also used [31]. However, the combination of different reactivity scores proposed by the present study may be used as a strategy to overcome individual differences. In methodologies that use behavioral observation, the use of the combination of different behaviors rather than using only one behavioral parameter may better reflect the reactivity of the horse [37]. For example, as previously reported, the movement parameter, when used alone, can be contradictory to explain the fear caused by handling or by handler. In the present study, mares showing the score 1 for movement (described in the methodology as static animal) could be classified as slightly reactive animals (reactivity score 1) or reactive animals (reactivity score 3) in the scale of composite measure reactivity score. In such cases, other behaviors are indicative of reactivity also in the mares, for example, those classified with reactivity score 2, the score 3 of behavioral category of position of ears and eyes showed the reactivity of animals. In the case mares that were classified with reactivity score 3, the breathing scores equal to 2, together with the position of ears and eyes score equal to 3 that showed the reactivity of the animal. In this way, the score 1 of behavioral category movement together with other behavioral scores indicates the case in which fear or inhibition causes immobility of movement.

There are some studies that assessed the reactivity of horses by using a behavior score, ranging from 0 to 3, with an increasing level of arousal [12,13] and also used different scales to assess horses' behaviors during the temperament test [38]. The difference between those researches and the present study is that they used various types of testing procedures, whereas this study used common handling in a horse farm.

Both Forced Human Approach Test and scale of composite measure reactivity score proposed in this study evaluate the reactivity in horses using a 1 to 4 scale. The first one is a methodology commonly used in horses, and the second one is an adapted methodology commonly used in sheep and cattle which this study proposes to use in horses. Both tests assess the response to human presence; however, this study was based on methodologies that evaluate reactivity during common handling, without changing the normal routine in a horse farm. While previously described methodologies performed tests in arenas or using unknown objects, the aim of the present study was to find a practical tool to investigate reactivity.

According to our findings, the two reactivity assessment tests applied in mares and foals showed the same reactivity classification (score), which validates the proposed scale of composite measure because both test designed to assess the reactivity of the equine in respect of the human presence.

5. Conclusion

This study proposed and validated the scale of composite measure score of behavior during daily handling as a methodology to evaluate horse reactivity. This method makes its development faster and easier, instead of having to perform tests already suitable for the specie that requires time and physical structure. By combining the scores of the most relevant behaviors to explain the reactivity of horses, it is possible to override the impact of individual differences which makes the objective of using the scoring scale stronger.

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References

[1] Hausberger M, Roche H, Henry S, Visser EK. A review of the human–horse relationship. Appl Anim Behav Sci 2008;109:1–24.
[2] Napolitano F, De Rosa G, Braghieri A, Grasso F, Bordi A, Wemelsfelder F. The qualitative assessment of responsiveness to environmental challenge in horses and ponies. Appl Anim Behav Sci 2008;109:342–54.
[3] Bates JE. Temperament in infancy. In: Osofsky JD, editor. Handbook of infant development. New York: Wiley; 1987. p. 1101–49.
[4] Visser EK, Van Reenen CG, Blokhuis MZ, Morgan EK, Hassmén P, Rundgren TM, et al. Does horse temperament influence horse-rider cooperation? J Appl Anim Welf Sci 2008;11:267–84.
[5] Boivin X, Neindre P, Chupin JM. Establishment of cattle-human relationship. Appl Anim Behav Sci 1992;32:325–35.
[6] Lesimple AC, Fureix C, LeScolan N, Richard-Yris MA, Hausberger M. Housing conditions and breed are associated with emotionality and cognitive abilities in riding school horses. Appl Anim Behav Sci 2011;129:92–9.
[7] Von Borstel UK, Duncan IJH, Lundin MC, Keeling LJ. Fear reactions in trained and untrained horses from dressage and show-jumping. Appl Anim Behav Sci 2010;125:124–31.
[8] Górecka-Bruzda A, Jastrzębska E, Sosnowska Z, Jaworsk Z, Jeziersk T, Chruszczewski MH. Reactivity to humans and fearfulness tests: field validation in Polish Cold Blood Horses. Appl Anim Behav Sci 2011; 133:207–15.
[9] Søndergaard E, Jago J. The effect of early handling of foals on their reaction to handling, humans and novelty, and the foal-mare relationship. Appl Anim Behav Sci 2010;123:93–100.
[10] Munsters CCBM, Visser KK, Broek JVD, Oldruitenborgh-Oosterbaa MMS. The influence of challenging objects and horse-rider matching on heart rate, heart rate variability and behavioural score in riding horses. Vet J 2012;192:75–80.
[11] Von Borstel UK, Euent GP, König S, Gauly M. Equine behaviour and heart rate in temperament tests with or without rider or handler. Physiol Behav 2011;104:454–63.
[12] Rothmann J, Christensen OF, Søndergaard E, Ladewig J. Behavior observation during conformation evaluation at a field test for Danish Warmblood horses and associations with rideability and performance traits. J Equine Vet Sci 2014;34:288–93.
[13] Rothmann J, Christensen OF, Søndergaard E, Ladewig J. A note on the heritability of reactivity assessed at field tests for Danish Warmblood horses. J Equine Vet Sci 2014;34:341–3.
[14] Ellis AD, Stephenson M, Preece M, Harris P. A novel approach to systematically compare behavioural patterns between and within groups of horses. Appl Anim Behav Sci 2014;161:60–74.
[15] Gibbons JM, Lawrence AB, Haskell MG. Consistency of flight speed and response to restraint in a crush in dairy cattle. Appl Anim Behav Sci 2011;131:15–20.
[16] Piovezan U, Cyrillo JNG, Costa MJRP. Breed and selection line differences in the temperament of beef cattle. Acta Sci Anim Sci 2013; 35:207–12.
[17] Titto EAL, Titto CG, Gatto EG, Noronha CMS, Mourão GB, Nogueira Filho JCM, et al. Reactivity of Nellore steers in two feedlot housing systems and its relationship with plasmatic cortisol. Livest Sci 2010; 129:146–50.
[18] Maffei WE, Bergmann JAG, Pinotti M, Oliveira MEC, Silva CQ. Reatividade em ambiente de contenção móvel: uma nova metodologia para avaliar o temperamento bovino. Arq Bras Med Vet Zootec 2006;58:1123–31.
[19] Carneiro RLR, Queiroz SA, Dibiasi NF, Tholon P, Fries LA. Temperament score herdability estimate in Nelore Cattle. In: Proceedings of the 8th World Congress on Genetics applied to livestock Production; 2006. p. 12–7. Belo Horizonte; 2006.
[20] Conexão Delta G. Sumário de Touros: Hereford, Braford e Nelore, 2013. Brasil: GenSys, http://www.gensys.com.br; 2013 [accessed 07. 03.2014].
[21] Søndergaard E, Halekoh U. Young horses' reactions to humans in relation to handling and social environment. Appl Anim Behav Sci 2003;84:265–80.
[22] R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org/; 2013. Accessed January 24, 2015.
[23] Abreu MNS, Siqueira AL, Caiaffa WT. Ordinal logistic regression in epidemiological studies. Rev Saúde Públ 2009;43:183–94.
[24] Viérin M, Bouissou MF. Pregnancy is associated with low fear reactions in ewes. Physiol Behav 2003;72:579–87.
[25] Dodd LC, Pitchford WS, Edwards JEH, Hazel SJ. Measure of behavioural reactivity and their relationships with production traits in sheep: a review. Appl Anim Behav Sci 2012;140:1–15.
[26] Burrow HM, Dillon RD. Relationship between temperament and growth in a feedlot and commercial carcass traits of Bos indicus crossbreeds. Aust J Exp Agric 1997;37:407–11.
[27] Grignard L, Boivin X, Boissy A, Le Neindre P. Do beef cattle react consistently to different handling situations? Appl Anim Behav Sci 2001;71:263–76.
[28] Van Reenen CG, Ruis-Heutinck LFM, Van der Wer JTN, Buist WG, Jones RB, Blokhuis HJ. Behavioural reactivity of heifer calves in potentially alarming test situations: a multivariate and correlational analysis. Appl Anim Behav Sci 2004;85:11–30.
[29] Kilgour RJ, Melville GJ, Greenwood PL. Individual differences in the reaction of beef cattle to situations involving social isolation, close proximity of humans and novelty. Appl Anim Behav Sci 2006;99: 21–40.

[30] Boissy A, Bouissou MF. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Appl Anim Behav Sci 1995;46:17–31.
[31] Dziezyc J, Taylor L, Boggess MM, Scott HM. The effect of ocular blinkers on the horses' reactions to four different visual and audible stimuli: results of a crossover trial. Vet Ophthalmol 2011; 5:327–32.
[32] McGee S, Smith HV. Age, gender and coat colour do not predict reactivity in Thoroughbred (Equus caballus) foals' first experience of the auction ring [Internet], http://www.bsas.org.uk/wp-content/themes/bsas/proceedings/Pdf2004/142.pdf; 2002 [accessed 07.02. 2015].
[33] MecGreevy P. Equine behaviour a guide for veterinarians and equine scientists. 1th ed. United Kingdom: Sounder; 2004.
[34] Fraser AF, Broom DM. Farm animal behaviour and welfare. 3th ed. Wallingford: CABI: Publishing; 1997.
[35] Fraser AF. The behaviour of the horse. 1th ed. Wallingford: CABI: Publishing; 1992.
[36] Reefmann N, Kaszàs FB, Wechsler B, Gygax L. Physiological expression of emotional reactions in sheep. Physiol Behav 2009;98: 235–41.
[37] Peeters M, Verwilghen D, Sertey D, Vandenheede M. Relationships between young stallions' temperament and their behavioral reactions during standardized veterinary examinations. J Vet Behav 2012;7:311–21.
[38] Graf P, Von Borstel UK, Gauly M. Practical considerations regarding the implementation of a temperament test into horse performance tests: results of a large-scale test run. J Vet Behav 2014;9:329–40.