Movement asymmetries in horses presented for prepurchase or lameness examination

Aagje M. Hardeman1,2 | Agneta Egenvall3 | Filipe M. Serra Bragança2 | Marc H. W. Koene1 | Jan-Hein Swagemakers1 | Lars Roepstorff4 | Rene van Weeren2 | Anna Byström4

1Tierklinik Luesche GmbH, Luesche, Germany
2Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
3Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden
4Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence
Aagje M. Hardeman, Tierklinik Luesche GmbH, Essenerstrasse 39a, 49456 Luesche, Germany; or Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Yalelaan 112-114, NL-3584 CM Utrecht, The Netherlands.
Email: aagje.hardeman@gmail.com

Summary
Background: The increasing popularity of objective gait analysis makes application in prepurchase examinations (PPE) a logical next step. Therefore, there is a need to have more understanding of asymmetry during a PPE in horses described on clinical evaluation as subtly lame.

Objectives: The objective of this study is to objectively compare asymmetry in horses raising minor vet concerns in a PPE and in horses raising major vet concerns with that found in horses presented with subtle single-limb lameness, and to investigate the effect of age/discipline on the clinicians’ interpretation of asymmetry on the classification of minor vet concerns in a PPE.

Study Design: Clinical case-series.

Methods: Horses presented for PPE (n = 98) or subjectively evaluated as single limb low-grade (1–2/5) lame (n = 24, 13 forelimb lame, 11 hindlimb lame), from the patient population of a single clinic, were enrolled in the study provided that owners were willing to participate. Horses undergoing PPE were assigned a classification of having minor vet concerns (n = 84) or major vet concerns (n = 14) based on findings during the dynamic-orthopaedic part of the PPE. Lame horses were only included if pain-related lameness was confirmed by an objective improvement after diagnostic analgesia exceeding daily variation determined for equine symmetry parameters using optical motion capture. Clinical evaluation was performed by six different clinicians, each with ≥8 years of equine orthopaedic experience. Vertical movement symmetry was measured using optical motion capture, simultaneously with the orthopaedic examination. Data were analysed using previously described parameters and mixed model analysis and least squares means were used to calculate differences between groups.

Results: There was no effect of age or discipline on the levels of asymmetry within PPE horses raising minor vet concerns. MinDiff and RUD of the head discriminated between forelimb lame and PPE horses raising minor vet concerns; MinDiff, MaxDiff, RUD of the Pelvis, HHDswing and HHDstance did so for hindlimb lameness. Two lameness patterns
INTRODUCTION

A prepurchase examination (PPE) is generally performed to provide the buyer with information to make an informed decision as to whether a horse is likely to meet their needs. There are a number of challenges associated with a PPE. The value of horses can be very high. Standards differ internationally and expectations on gait quality may differ between clients and vets and are dependent on the envisaged equestrian discipline and level. For performing a PPE, both technical veterinary and communication skills are needed and the clinician’s advice is not only based on the instantaneous findings but also on the prospective purchaser’s expectations, intended use, and earlier medical reports. PPE protocols have been well described; however, there are no concrete guidelines for the interpretation of the dynamic-orthopaedic examination, nor are there any reference values or consensus on the acceptable level of asymmetry. Finally, PPE’s can result in legal cases.

Objective gait analysis overcomes some limitations of subjective assessment, for example, bias in interpretation of diagnostic analgesia, low agreement in determination of the lame limb, and the limitations of the human eye in detecting low grade asymmetry. However, correct interpretation of the kinematic data by the clinician is important, as asymmetries can have different causes. They may be pain-related but can also be due to neurological problems, handedness, mechanical movement restriction, or a result of biological variation. Furthermore, horses show variation in movement symmetry over time.

Data collected from horses at PPE have been used to investigate repeatability of objective asymmetry assessment and earlier research suggests that vertical peak force may be the best parameter to obtain cut-off values for clinically sound horses, but thus far no attempts have been made to compare asymmetries in horses presented for PPE versus horses presented for lameness evaluation.

This study aims to (1) describe the magnitude and variation of movement asymmetry in horses presented for PPE and compare this to horses presented for lameness investigation in which single limb low-grade lameness was found (subjectively graded 1-2/5); (2) investigate whether measured levels of asymmetry would differ within the group of PPE horses with minor vet concerns depending on the horses' age and/or discipline, suggestive of a confounding effect related to these factors and (3) evaluate if vertical movement asymmetry can be used to differentiate PPE horses with minor vet concerns from PPE horses with major vet concerns (i.e., prompting negative advice from the clinician performing the PPE). We hypothesised that, at group level, PPE horses with minor vet concerns would show less asymmetry than PPE horses with major vet concerns and lame horses and that the magnitude of the measured asymmetry within the group PPE horses with minor concerns would vary with the horse's age and discipline, for example, that older horses would be more asymmetric than younger horses.

MATERIALS AND METHODS

2.1 Study setting

The study was designed as a case-series targeting clinical cases presented for a PPE in a single, large equine clinic. Additionally, a comparison group was created using low-grade lame horses presented to the same clinic. All data were collected at Tierklinik Lüsche, Germany, during preset study periods (May 2018 to December 2019 for the PPE horses and October to December 2019 for the lame horses). Subjective evaluation was performed by one of six veterinarians with at least 8 years of equine orthopaedic experience. The protocol used for the PPE can be found in Supplementary Item 1 (see page 13 for the protocol of the dynamic-orthopaedic part of the examination).

2.2 Horses

All horses presented for PPE during the study period were eligible and included when both owner and examining vet consented with the use of quantitative gait analysis. For the lame group, the aim was to include 20–25 horses and approximately equal numbers of...
forelimb and hindlimb cases. In all cases, quantitative data were collected by one single person. Figure 1 provides a flow chart of the selection process of both groups. Included were the following:

1. Ninety-eight horses presented for PPE, of which there were 4 ponies >1.45 m and 94 warmbloods >1.60 m, aged 2–16 years (mean 7.2), 63% dressage, 34% showjumpers and 3% other disciplines. Based on findings during the dynamic-orthopaedic part of the PPE (walk and trot on a straight line and on circles on hard and soft surface, canter on soft circles, and, in some cases, also evaluation under saddle (Supplementary Item 1, page 13), clinicians evaluated 84 horses as giving rise to no or only minor veterinary concerns, and 14 horses showing signs raising major veterinary concerns. Of these, 9 were deemed forelimb lame, 3 hindlimb lame and 2 multi-limb lame. PPE findings unrelated to the dynamic-orthopaedic evaluation were not considered.

2. 24 horses presented for lameness evaluation and found single limb 1–2/5 lame on subjective evaluation and confirmed as having a pain-related lameness by objective analysis of improvement after local analgesia above normal daily variation previously described for optical motion capture. These were all warmbloods >1.60 m, age 5–15 years (mean 9.8), 11 with a forelimb and 13 with a hindlimb lameness.

2.3 | Marker placement

Spherical reflective markers (25 mm) (Qualisys AB, Motion Capture Systems) were placed in three anatomical regions: a single head marker between the ears, three markers on the withers (one on the highest point, two markers 20 cm lateral to the central one) and a T-shaped strip with one marker at each end, so the three markers were located at the tuber sacrale and the craniodorsal aspects of both tubera coxae (Figure 2). For the lame group, markers stayed on the horse between baseline trot up and evaluation of diagnostic analgesia.

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**FIGURE 1** Flow chart showing the recruitment process of horses included in the prepurchase examination (PPE) group and the group of lame horses
2.4 | Kinematic data collection

Kinematic data were recorded using Qualisys Motion Capture software (QTM version: 2.14, build: 3180; Qualisys AB, Motion Capture Systems), connected to 20 high-speed infrared cameras (Oqus 700+; Qualisys AB, Motion Capture Systems, at 100 Hz) that were mounted in an indoor arena at the clinic. The total covered area was approximately 250 m$^2$, height covered was at least 4 m. Calibration was done according to the manufacturer’s instructions. Synchronised video recordings were obtained (Sony HDR-CX330).

2.5 | Kinematic examination protocol

Kinematic data were collected on soft surface, along the long side of the indoor arena (2 × 30 m), on which horses were trotted at their own preferred speed. The surface consisted of sand and synthetic fibre (Figure 2), which was harrowed daily. Care was taken to maximise measurement-standardisation,

15 except that some of the horses presented for PPE were trotted up by their owner/trainer.

2.6 | Kinematic data analysis

Kinematic data were analysed using custom-made Matlab scripts (Matlab, The MathWorks, Inc.). Filtering (Butterworth high pass filter, cut-off 70% of stride frequency)$^{20}$ and stride segmentation$^{21}$ were performed as described previously. Single strides were excluded if stride duration or tuber sacrale vertical range of motion (ROM) differed more than 20%, or if head vertical ROM differed more than 40% from measurement median. Speed was calculated by smoothed differentiation of the horizontal coordinates (x,y) of the marker on the tuber sacrale. Calculated parameters were: MinDiff/MaxDiff (difference between the two minima/maxima of one stride); RUD/ RDD (Range Up/Down Difference; difference in upward/downward movement between right and left halves of a stride) for head, withers and pelvis; HHDswing/HHstance (Hip Hike Difference-swing/stance; difference between the upward movement of the tuber coxae during swing/stance phase). Single parameters were calculated both in millimetres and as fraction of vertical ROM by dividing the asymmetry per stride by vertical stride ROM of that anatomical location. Additionally, single parameters were combined to pattern variables formulated in accordance with frequently seen compensatory patterns in forelimb or hindlimb lame horses$^{22,23}$ (Persson-Sjodin et al. unpublished data): forelimb lame pattern = RUD Poll + MinDiff Withers − RUD Pelvis and hindlimb lame pattern = RUD Pelvis + RUD Poll − MinDiff Withers. The terms in these formulas are set to be either positive or negative such that the included single parameters will add up if their signs follow the expected pattern. Finally, based on a study on veterinary inspection before competition,$^{24}$ an overall symmetry score was calculated to quantify whole body asymmetry. This score is based on the vector sums (VS): $VS = \sqrt{\text{MinDiff}^2 + \text{MaxDiff}^2}$, for head and pelvis, combined into an overall asymmetry measure: Overall asymmetry = $\frac{VS_{\text{head}}}{2} + VS_{\text{pelvis}}$.

2.7 | Data analysis

Open software R (3.3.1) (R-Studio) was used, including the packages lme4 (1.1–21), lmerTest (3.1–1), emmeans (1.4.3.01), gridExtra (2.3), and ggplot2 (3.2.1). Mixed model analysis was done to test PPE-horses with minor vet concerns for associations between asymmetry and age (linear effect), asymmetry and discipline (dressage and showjumping), and the two-way interaction between age and discipline. Differences in asymmetry between all groups (PPE minor vet concerns, PPE major vet concerns, forelimb lame, hindlimb lame) and differences in the stride-to-stride variation between all groups (median absolute deviation [MAD], calculated per measurement) were similarly investigated with mixed model analysis. The models including all groups were made with outcome variables both in millimetres and as fraction of vertical ROM for the same marker (e.g., tuber sacrale for RUD Pelvis). Figure 3 presents a flow chart of all statistical tests used in the study. Least squares means and contrasts were evaluated to investigate differences between groups. All asymmetry parameters were analysed as square-root transformed absolute values. Speed was tested and removed if nonsignificant. Normality of outcome variables was evaluated using Q-Q plots. Correction for multiple comparisons was not applied.

3 | RESULTS

One horse in the PPE group with minor vet concerns was excluded from the study as straight-line trot-up was not possible due to misbehaviour. Mean/median number of strides used for kinematic analysis for the horses presented for PPE was 19/20, and for the lame horses, it was 21/21. Mean speed (s.d.) was 3.6 (0.37) m/s for the horses...
presented for PPE and 3.4 (0.26) m/s for the lame horses. Speed was not significant in any model. Mean values per parameter for the PPE horses with minor vet concerns are illustrated in Figure 4.

Mixed model analysis of data from the PPE horses with minor vet concerns showed no significant effect of age or discipline on the objective asymmetry, for example, horses in this group were not more or less asymmetric if used for showjumping versus dressage, nor was there any significant interaction between age and discipline. Model outcomes are given in Data S1.

Of the 14 single parameters, significant differences between PPE horses with minor vet concerns and lame horses were observed in the MinDiff and RUD Head for forelimb lame horses, and in the MinDiff, MaxDiff and RUD Pelvis, HHDswing and HHDstance for hindlimb lame horses (Figure 5). Least square mean (mm) and standard error (SE) per parameter per group can be found in Table 1, and comparisons between groups are presented in Table 2.

For both head and pelvis, the RUD was significantly different between PPE horses with minor vet concerns and forelimb or hindlimb lame horses, whereas the RDD was not (Figure 6, Table 2). A substantial proportion of the hindlimb lame horses showed an asymmetry in the MinDiff and RUD of the withers, which was significantly different compared to the PPE horses with minor vet concerns, PPE horses with major vet concerns and forelimb lame horses (Table 2).

The majority of the parameters remained significant, or not, after correction for ROM (92 out of 96). However, the ‘forelimb lame pattern’ became significant for differentiating PPE horses with minor vet concerns from PPE horses with major vet concerns, when corrected for ROM (Table 2).

For the overall symmetry score, all PPE horses with major vet concerns scored in the upper half of the asymmetry range measured for all PPE horses (Figure 7). Hindlimb lame horses had significantly different overall symmetry scores compared to PPE horses with minor vet concerns, PPE horses with major vet concerns and forelimb lame horses, but scores in PPE horses with minor vet concerns and PPE horses with major vet concerns were not different (Table 2).

Two parameter combinations were significantly different between PPE horses with minor vet concerns and both forelimb, and hindlimb lame horses: the ‘forelimb lame pattern’ and the ‘hindlimb lame pattern’ (Figure 8, Table 2). The ‘hindlimb lame pattern’ was also significantly different in hindlimb lame horses compared to forelimb lame horses and compared to PPE horses with major vet concerns. Least square mean values (mm) and standard errors (SE) are given in Table 1.

PPE horses with minor vet concerns had numerically higher stride-to-stride variability (MAD) compared to lame horses in 12 out of 14 parameters, but this was not significant (p values: 0.11-0.78, mean 0.33). Model outcomes can be found in Data S2. Horses presented for PPE tend to spread more along the MAD (y)-axis (Figure 9).

4 | DISCUSSION

This is the first study to document objectively measured asymmetry in a large group of horses undergoing PPE in which minor vet concerns were identified. Documented purchase prices ranged from €5,000 to €1,200,000. Most horses were dressage or showjumping warmbloods, intended to be used at intermediate to elite level. Within this relatively homogeneous group, there was still a large variation in asymmetry. We hypothesised that part of this large variation would be explainable from the horse’s age and discipline, because these are factors that are generally seen as important in decision-making among equine veterinarians, and indeed also recognised as such at the clinic where the study was performed. However, contrary to our hypothesis, no statistically significant effect of age or discipline was found on the measured vertical movement asymmetry variables in this study.

Data from the PPE horses with minor vet concerns can serve as reference values for similar populations. When comparing the PPE horses with minor vet concerns to the PPE horses with major vet concerns, it proved difficult to separate these two groups based on vertical asymmetry parameters. None of the 14 single parameters was significantly different between the horses that received positive versus negative purchase advice after the PPE. It should be recognised that the latter group was small (n = 14). Further, there may have been a selection bias limiting inclusion of horses in this group since both clients and veterinarians had a say in the decision whether or not objective analysis was performed in addition to the routine PPE. In cases where subjectively perceived unacceptable asymmetry had been observed on initial clinical examination, the option of adding objective gait analysis may have
been declined to avoid increasing costs or considered unnecessary because the purchasers had already decided to withdraw.

It has recently been suggested that asymmetry patterns may be more powerful in identifying lameness than single parameters.\textsuperscript{22} We therefore formulated combinations of single parameters based on earlier research describing asymmetry patterns in lame horses.\textsuperscript{10,22} These combined parameters included the difference in the direction of the withers asymmetry, being ipsilateral in relation to head asymmetry in case of primary forelimb lameness and being contralateral in relation to head asymmetry in case of primary hindlimb lameness.\textsuperscript{23} (Persson-Sjödin et al. unpublished data). The forelimb lame pattern was indeed able to differentiate PPE horses with minor vet concerns.

FIGURE 4 Boxplots of the PPE horses with minor vet concerns ($n = 83$) for selected single parameters; MinDiff Head, RUD Head, MinDiff Pelvis, MaxDiff Pelvis, RUD Pelvis, HHDswing, HHDstance (single parameters are defined as MinDiff/MaxDiff [difference between the two minima/maxima of one stride]; RUD/RDD [Range Up/Down Difference; difference in upward/downward movement between right and left halves of a stride]; HHDswing/HHDstance [Hip Hike Difference-swing/stance; difference between the upward movement of the tuber coxae during swing/stance phase]), the forelimb lame pattern ($= RUD Poll + MinDiff Withers - RUD Pelvis$), the hindlimb lame pattern ($= RUD Pelvis + RUD Poll - MinDiff Withers$) and the overall symmetry score ($VS = \sqrt{MinDiff^2 + MaxDiff^2}$, Overall asymmetry $= \sqrt{\text{MaxDiff}^2 + \text{MaxDiff}^2}$). All given in mm
from PPE horses with major vet concerns when corrected for ROM. Of the 14 PPE horses with major vet concerns, 9 were forelimb lame. Of the other 5 horses, 3 were hindlimb lame and 2 had a multi-limb lameness, thus the sample size is too small to expect highly significant results. The overall symmetry score, was intended as a tool to differentiate fit from unfit-to-compete, but did not discriminate between PPE horses with minor vet concerns and PPE horses with major vet concerns. All PPE horses with major vet concerns scored within a relatively small range, overlapping with the upper half of the measured asymmetry (mm) range of the PPE horses with minor vet concerns.

Horses diagnosed with single limb lameness were significantly more asymmetric than PPE horses with minor vet concerns and
parameter combinations significantly differentiated both forelimb and hindlimb lame horses from PPE horses with minor vet concerns at group level. However, there was still a large overlap. Thus far, thresholds for distinguishing sound from lame horses on an individual level have only been suggested for single parameters, using data from inertial measurement units.\textsuperscript{26,27} No previous study has compared all 14 parameters evaluated in the current study, but earlier studies have suggested that the MinDiff and RUD Pelvis, HHDswing and the maximal vertical acceleration of the head are among the most useful parameters for identifying lameness.\textsuperscript{23,28,29} This was corroborated in our study where MinDiff and RUD Head for forelimb lame horses and MinDiff, MaxDiff and RUD Pelvis, HHDswing and HHDstance for hindlimb lame horses were significantly different from PPE horses with minor vet concerns.

The MinDiff and RUD Withers were significantly different for hindlimb lame horses versus PPE horses with minor vet concerns, PPE horses with major vet concerns and forelimb lame horses but did not differentiate forelimb lame from PPE horses with minor vet concerns. This is in agreement with earlier research, which concluded that the withers fail to detect subtle forelimb lameness\textsuperscript{28} and with the reported lower asymmetry of the withers in forelimb versus hindlimb lameness.\textsuperscript{23} In our dataset, most hindlimb lame horses were subjectively classified as 2/5 lame and most forelimb lame horses as 1/5. This may also have contributed to the MinDiff and RUD Withers being significant for hindlimb-, but not for forelimb lame, versus PPE horses with minor vet concerns. Another possible explanation is that pelvis and withers are mechanically stronger connected than head and withers. Although withers asymmetry is not an optimal parameter for differentiating between sound and (forelimb) lame, the parameter is important for differentiating between primary forelimb versus primary hindlimb lameness\textsuperscript{23,29} (Persson-Sjödin et al., unpublished data).

The RDD was not significantly different in PPE horses with minor vet concerns versus lame horses whereas the RUD was, in consensus with previous literature.\textsuperscript{29} Biomechanically, this is possibly due to the fact that a RUD suggests MinDiff (impact) and MaxDiff (push-off) asymmetry assigned to the same limb, whereas a RDD suggests MinDiff and MaxDiff asymmetry assigned to contralateral limbs. These gait parameters have been visualised previously.\textsuperscript{10} Correcting for vertical ROM did not lead to marked improvement in differentiating PPE horses with minor vet concerns from lame horses, though differentiation of PPE horses with minor vet concerns from PPE horses with major vet concerns became statistically significant after correcting for ROM. This suggests correcting for ROM can improve differentiation, even in our relatively homogeneous study population. Another benefit of correcting for ROM may be a better comparison of gait parameters between different gait analysis systems.\textsuperscript{20,30} This could thereby lead to reference values usable for multiple systems.

There was no significant correlation between level of asymmetry and stride-to-stride variation, though variation was numerically higher for PPE horses with minor vet concerns compared to lame

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
 & \textbf{PPE minor vet concerns} & & \textbf{PPE major vet concerns} & & \textbf{Forelimb lame} & & \textbf{Hindlimb lame} \\
 & \textbf{Mean (mm)} & \textbf{SE} & \textbf{Mean (mm)} & \textbf{SE} & \textbf{Mean (mm)} & \textbf{SE} & \textbf{Mean (mm)} & \textbf{SE} \\
\hline
\textbf{MinDiff Head} & 13.3 & 0.6 & 15.5 & 1.55 & 22.2 & 2.08 & 13.3 & 1.46 \\
\textbf{MaxDiff Head} & 13.0 & 0.55 & 15.4 & 1.45 & 14.4 & 1.58 & 11.1 & 1.26 \\
\textbf{RDD Head} & 17.5 & 0.79 & 17.0 & 1.87 & 18.2 & 2.17 & 12.9 & 1.66 \\
\textbf{RUD Head} & 19.3 & 0.82 & 24.6 & 2.22 & 33.8 & 2.92 & 22.1 & 2.15 \\
\textbf{MinDiff Withers} & 6.0 & 0.34 & 5.3 & 0.76 & 6.5 & 0.94 & 12.4 & 1.2 \\
\textbf{MaxDiff Withers} & 7.6 & 0.41 & 8.6 & 1.05 & 6.9 & 1.05 & 7.3 & 0.99 \\
\textbf{RDD Withers} & 8.1 & 0.44 & 9.3 & 1.12 & 9.8 & 1.3 & 8.1 & 1.09 \\
\textbf{RUD Withers} & 11.0 & 0.64 & 11.4 & 1.56 & 7.9 & 1.47 & 18.4 & 2.05 \\
\textbf{MinDiff Pelvis} & 6.9 & 0.45 & 8.5 & 1.2 & 6.0 & 1.14 & 17.9 & 1.79 \\
\textbf{MaxDiff Pelvis} & 7.0 & 0.44 & 8.2 & 1.14 & 8.5 & 1.31 & 18.6 & 1.78 \\
\textbf{RDD Pelvis} & 9.3 & 0.53 & 12.7 & 1.48 & 11.0 & 1.55 & 10.7 & 1.4 \\
\textbf{RUD Pelvis} & 10.6 & 0.66 & 11.0 & 1.62 & 10.5 & 1.78 & 36.6 & 3.04 \\
\textbf{HHD Swing} & 12.7 & 0.82 & 12.9 & 2.0 & 13.4 & 2.29 & 41.5 & 3.69 \\
\textbf{HHD Stance} & 11.0 & 0.59 & 11.7 & 1.48 & 10.1 & 1.54 & 32.7 & 2.53 \\
\textbf{Forelimb lame pattern} & 25.2 & 1.12 & 33.3 & 3.09 & 35.5 & 3.58 & 41.2 & 3.52 \\
\textbf{Hindlimb lame pattern} & 23.6 & 1.38 & 23.3 & 3.31 & 35.8 & 4.61 & 58.3 & 5.39 \\
\hline
\textbf{Overall symmetry} & 24.1 & 0.78 & 28.9 & 2.07 & 29.2 & 2.34 & 40.4 & 2.52 \\
\hline
\end{tabular}
\caption{Least square mean (mm, absolute values) and standard error (SE) per parameter, per group: PPE horses with minor vet concerns (n = 83), PPE horses with major vet concerns (n = 14), forelimb lame (n = 11) and hindlimb lame (n = 13).}
\end{table}

Note: See Figure 4 for the definitions/formula of the single and combined parameters.
### Table 2: P values per parameter of the differences (contrasts) between groups

| Comparisons                        | PPE minor vet concerns vs. Hindlimb lame | PPE major vet concerns vs. Forelimb lame | PPE minor vet concerns vs. PPE major vet concerns | Hindlimb lame vs. Forelimb lame | Hindlimb lame vs. PPE major vet concerns | Forelimb lame vs. PPE major vet concerns |
|------------------------------------|-------------------------------------------|------------------------------------------|---------------------------------------------------|---------------------------------|------------------------------------------|------------------------------------------|
| Non-corrected for ROM              |                                            |                                          |                                                   |                                 |                                          |                                          |
| MinDiff Head                       | >0.9                                      | <0.001                                   | 0.5                                               | 0.002                           | 0.7                                      | 0.05                                     |
| MaxDiff Head                       | 0.5                                       | 0.8                                      | 0.4                                               | 0.3                             | 0.1                                      | >0.9                                     |
| RDD Head                           | 0.09                                      | >0.9                                     | >0.9                                              | 0.2                             | 0.4                                      | >0.9                                     |
| RUD Head                           | 0.6                                       | <0.001                                   | 0.1                                               | 0.007                           | 0.8                                      | 0.06                                     |
| MinDiff Withers                    | <0.001                                    | >0.9                                     | 0.8                                               | <0.001                          | <0.001                                   | 0.7                                      |
| MaxDiff Withers                    | >0.9                                      | >0.9                                     | 0.8                                               | >0.9                            | 0.8                                      | 0.6                                      |
| RDD Withers                        | >0.9                                      | 0.6                                      | 0.8                                               | 0.8                             | 0.9                                      | >0.9                                     |
| RUD Withers                        | 0.001                                     | 0.3                                      | >0.9                                              | <0.001                          | 0.03                                     | 0.4                                      |
| MinDiff Pelvis                     | <0.001                                    | >0.9                                     | 0.5                                               | <0.001                          | <0.001                                   | 0.4                                      |
| MaxDiff Pelvis                     | <0.001                                    | 0.7                                      | 0.8                                               | <0.001                          | <0.001                                   | >0.9                                     |
| RDD Pelvis                         | 0.8                                       | 0.7                                      | 0.1                                               | >0.9                            | 0.8                                      | 0.9                                      |
| RUD Pelvis                         | <0.001                                    | >0.9                                     | >0.9                                              | <0.001                          | <0.001                                   | >0.9                                     |
| HHD Swing                          | <0.001                                    | 0.02                                     | 0.06                                              | 0.7                             | 0.3                                      | >0.9                                     |
| HHD Stance                         | <0.001                                    | 0.03                                     | >0.9                                              | 0.01                            | <0.001                                   | 0.1                                      |
| Overall symmetry                   | <0.001                                    | 0.2                                      | 0.1                                               | 0.008                           | 0.003                                     | >0.9                                     |

| Corrected for ROM                 |                                            |                                          |                                                   |                                 |                                          |                                          |
| MinDiff Head                       | 0.2                                       | <0.001                                   | 0.6                                               | 0.1                             | >0.9                                     | 0.02                                     |
| MaxDiff Head                       | 0.8                                       | 0.7                                      | 0.4                                               | >0.9                            | >0.9                                     | >0.9                                     |
| RDD Head                           | >0.9                                      | >0.9                                     | >0.9                                              | 0.9                             | >0.9                                     | 0.9                                      |
| RUD Head                           | 0.004                                     | <0.001                                   | 0.1                                               | 0.4                             | 0.8                                      | 0.06                                     |
| MinDiff Withers                    | <0.001                                    | 0.9                                      | >0.9                                              | <0.001                          | <0.001                                   | 0.8                                      |
| MaxDiff Withers                    | >0.9                                      | >0.9                                     | 0.7                                               | >0.9                            | >0.9                                     | 0.7                                      |
| RDD Withers                        | 0.9                                       | 0.5                                      | 0.6                                               | >0.9                            | >0.9                                     | >0.9                                     |
| RUD Withers                        | <0.001                                    | 0.4                                      | >0.9                                              | <0.001                          | 0.01                                     | 0.4                                      |
| MinDiff Pelvis                     | <0.001                                    | >0.9                                     | 0.5                                               | <0.001                          | <0.001                                   | 0.5                                      |
| MaxDiff Pelvis                     | <0.001                                    | 0.6                                      | 0.8                                               | <0.001                          | <0.001                                   | >0.9                                     |
| RDD Pelvis                         | 0.7                                       | 0.6                                      | 0.1                                               | >0.9                            | 0.8                                      | >0.9                                     |
| RUD Pelvis                         | <0.001                                    | >0.9                                     | >0.9                                              | <0.001                          | <0.001                                   | >0.9                                     |
| HHD Swing                          | <0.001                                    | >0.9                                     | >0.9                                              | <0.001                          | <0.001                                   | >0.9                                     |
| HHD Stance                         | <0.001                                    | >0.9                                     | 0.9                                               | <0.001                          | <0.001                                   | >0.9                                     |
| Overall symmetry                   | <0.001                                    | 0.004                                    | 0.004                                             | >0.9                            | >0.9                                     | 0.8                                      |

Note: Groups compared in each column are given in the header (p values < 0.05 in bold). The lower part shows p values after correcting for ROM, in red p values that changed between significant and non-significant when corrected for ROM. See for the definitions/formula of the single and combined parameters Figure 4.
horses for most parameters. This contradicts earlier research which concluded that lame horses show significant lower variability overall,\(^{20}\) in the MinDiff Head and MinDiff Pelvis\(^ {31}\) and in stride-length,\(^ {32}\) the latter with high inter-individual differences, excluding the possibility to distinguish pathological and physiological variation on individual level. Nevertheless, motion variability is, also in humans, considered an important parameter for differentiating between pathological and physiological gaits.\(^ {32}\)

The substantial overlap in the measured vertical asymmetries between PPE-horses with minor vet concerns, PPE horses with major vet concerns, forelimb lame horses, and hindlimb lame horses limits the usefulness of group mean values for evaluation on an individual level. An important reason for this could be the fact that objective asymmetry data are based only on a soft surface straight line, whereas the PPEs consisted of walk/trot/canter, circles, hard surface, flexion tests and sometimes evaluation under tack. Further, only vertical movement asymmetry was used. Finally, the subjective assessment of asymmetry likely differs between veterinarians. Repeating this study with a larger number of horses, including data collection under all abovementioned conditions, numerous parameters and veterinarians, is needed to reduce overlap, but it will likely never be eliminated. Nevertheless, a horse’s level and pattern of asymmetries based on objective analyses will give clinicians additional information to aid in their decision-making. These results should not be interpreted as a black-white cut-off, neither do we expect horses to move perfectly symmetrical.\(^ {33}\) What can be achieved is establishing reference values like for any biological parameter,\(^ {33}\) which could serve as a benchmark for PPE assessment.

A record of objective data provides an individual baseline, which could be useful in case of future problems, given the low within-horse variation.\(^ {15}\) In our experience, including objective gait analysis increases interchangeability of clinical assessments with referring vets, and motivates clients to present horses to our clinic. A concern that has been raised regarding the introduction of objective gait analysis is its potential for detecting nonsignificant yet obvious deviations from perfect symmetry that might lead purchasers to try negotiating a price reduction or create problems when insuring the horse. A similar discussion has been conducted around imaging modalities.\(^ {34}\)

The study has important limitations: a priori sample size calculations were not performed as variation between individuals

**FIGURE 6** Descriptive plots of measurement mean values (mm) per horse for the RUD Head and Pelvis versus RDD Head and Pelvis. Each dot represents one horse in either the PPE horses with minor vet concerns (green), PPE horses with major vet concerns (black, symbols indicating forelimb, hindlimb or multilimb lameness), forelimb lame (red) or hindlimb lame (blue) group. See Figure 4 for the definitions of the kinematic parameters.
within groups and relevant effect size for the differences between groups were unknown. Only soft surface straight-line kinematic vertical movement asymmetry data were used, whereas the PPE included evaluation at walk and trot in straight line and on circles on hard and soft surface, at canter on soft circles and, in some cases also when ridden (Supplementary Item 1, page 13). When interpreting the results of this study, it must be considered that the PPE conclusion for each horse was based on more conditions than was measured objectively. Each horse was only evaluated by one veterinarian. Nevertheless, this is a first attempt to relate PPE outcome to quantitative analyses, and the study generated useful information regarding the application of kinematic analysis in the PPE.

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CONFLICT OF INTERESTS
No competing interests have been declared.

AUTHOR CONTRIBUTIONS
A. Hardeman contributed to the planning of the experiment, data collection, data processing, statistics and preparation of the manuscript. A. Egenvall and A. Byström contributed to data processing, statistics and preparation of the manuscript. F. Serra Bragança contributed to data processing and preparation of the manuscript. M. Koene and J. Swagemakers contributed to data collection and preparation of the manuscript. L. Roepstorff contributed to preparation of the manuscript. P.R. van Weeren contributed to planning of the experiment and preparation of the manuscript.

ETHICAL ANIMAL RESEARCH
Research ethics committee oversight not currently required by this journal: procedures were noninvasive.

INFORMED CONSENT
Owners gave consent for their animals’ inclusion in the study.

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

ORCID
Aagje M. Hardeman https://orcid.org/0000-0002-0944-2740
Agneta Egenvall https://orcid.org/0000-0002-8677-6066
Filipe M. Serra Bragança https://orcid.org/0000-0001-8514-7949
Lars Roepstorff https://orcid.org/0000-0002-4109-9284
Rene van Weeren https://orcid.org/0000-0002-6654-1817
Anna Byström https://orcid.org/0000-0002-2008-8244

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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