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Quantitative motor unit action potential analysis of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles in adult Royal Dutch sport horses

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Summary

Reasons for performing study: Reference values for quantitative electromyography (QEMG) in shoulder and hindlimb muscles of horses are limited.
Objectives: To determine normative data on QEMG analysis of supraspinatus (SS), infraspinatus (IS), deltoideus (DT) and biceps femoris (BF) muscles.
Study design: Experimental observational study and retrospective case series.
Methods: Seven adult healthy Royal Dutch sport horses underwent quantitative motor unit action potential analysis of each muscle using commercial electromyography equipment. Measurements were made according to published methods. One-way ANOVA was used to compare quantitative motor unit action potential variables between muscles, with *post hoc* testing according to Bonferroni, with significance set at $P<0.05$. The QEMG and clinical information from horses with lower motor neuron disorders (n = 7) or myopathy (n = 4) were summarised retrospectively.
Results: The 95% confidence intervals of duration, amplitude, phases, turns, area and size index of quantitative motor unit action potential were 8.7–10.4 ms, 651–867 μV, 3.2–3.7, 3.7–4.7, 1054–1457 μV·ms and 1.1–1.5 for SS, 9.6–11.0 ms, 779–1082 μV, 3.3–3.7, 3.8–4.7, 1349–2204 μV·ms and 1.4–1.9 for IS, 6.0–9.1 ms, 370–691 μV, 2.9–3.7, 2.8–4.5, 380–1374 μV·ms and 0.3–1.3 for DT and 5.7–7.8 ms, 265–385 μV, 2.7–3.2, 2.6–3.1, 296–484 μV·ms and 0.2–0.5 for BF, respectively. Mean duration, amplitude, number of phases and turns, area and size index were significantly ($P<0.01$) higher in SS and IS than in DT and BF muscles. In addition, 4 of 7 normal horses had >15% polyphasic motor unit action potentials in SS and IS muscles.
Conclusions: Differences between muscles should be taken into account when performing QEMG in order to be able to distinguish normal horses from horses with suspected neurogenic or myogenic disorders. These normal data provide the basis for objective QEMG assessment of shoulder and hindlimb muscles. Quantitative electromyography appears to be helpful in diagnosing neuropathies and discriminating these from myopathies.

Keywords: horse; electromyography

Abbreviations

BF: Biceps femoris
DT: Deltoideus
EMG: Electromyography
IS: Infraspinatus
MUAP: Motor unit action potential
QEMG: Quantitative electromyography
SI: Size index
SS: Supraspinatus

Introduction

Electromyography (EMG) is an ancillary diagnostic technique based on recording the electrical signal generated by the motor unit, which consists of the motor neuron, including its axon, the neuromuscular endplate and the skeletal muscle fibres, during rest, voluntary muscle contraction or spontaneous activity [1,2]. Traditionally, EMG has been used in veterinary medicine to demonstrate pathological spontaneous activity to assess certain neuromuscular disorders. As an example, detection of fibrillation potentials, positive sharp waves, myotonic discharges or complex repetitive discharges of various skeletal muscles has been described in horses with equine motor neuron disease [3], hyperkalaemic periodic paralysis [4,5], myotonia [6], suprascapular nerve injury [7], experimentally induced hypocalcaemia and hypomagnesaemia [8], as well as in myopathy [9]. Quantitative EMG (QEMG) has been employed in human medicine for decades to provide further characterisation of certain neuromuscular disorders [2,10] and to study the effects of training or rehabilitation [11–13]; more recently, it has been applied to equine medicine [14–17]. Motor unit action potentials (MUAPs) are the result of voluntary or forced contraction of skeletal muscles. By not only evaluating the presence and type of insertional activity and pathological spontaneous activity, but also quantifying the resultant MUAPs (duration, amplitude and other parameters), one may characterise a disorder as neurogenic, myogenic or not resulting from changes of the motor unit [1]. More recently, the observation of abnormal QEMG patterns has been reported as a minimally invasive technique helpful in *ante mortem* diagnosis in horses suffering from equine motor neuron disease [15,16], equine grass sickness [17], equine degenerative myelopathy [15], myositis or myopathy [9,15] and botulism [18], even in the absence of spontaneous pathological changes in the EMG. The QEMG patterns in man are interpreted by taking into account the patient's age and the muscle under study [1]. In horses, it has been shown that age has an effect on QEMG parameters of MUAPs [19]. Likewise, normative values of quantitative MUAP analysis may vary between different muscles, as has been published initially for the subclavian, lateral vastus and triceps muscles [8,19–21]. Since then, reference values have been published in equine muscles for descending pectoral, splenius and brachiocephalicus muscles [22,23].

The aim of this study was to perform an observational QEMG analysis of the supraspinatus (SS), infraspinatus (IS), deltoideus (DT) and biceps femoris (BF) muscles to obtain normative data to aid the diagnosis of certain neuromuscular conditions in horses. As a secondary aim, a retrospective study of horses presented to the Equine Hospital, Faculty of Veterinary Medicine, Utrecht University for orthopaedic or neurological disorders involving the shoulder muscles was performed to illustrate the clinical use of QEMG analysis.

Materials and methods

Horses

Seven adult, clinically healthy Royal Dutch Warmblood female horses were used (10.6 ± 3.9 years old, mean ± s.d. [range 7–16 years]; body weight, 569 ± 49 kg [range 485–636 kg]; height, 162 ± 5 cm [range 156–171 cm]; and rectal temperature, 37.4 ± 0.5°C [range 36.8–38.2°C]) to perform an observational QEMG study of SS, IS, DT and BF muscles. Horses belonged to the Faculty of Veterinary Medicine, Utrecht University, and were kept stabled or at pasture and in regular recreational work. Horses had normal

physical, lameness and neurological examinations and unremarkable cervical radiographs. Skeletal muscles under study were identified by palpation with the aid of anatomical landmarks.

Electromyographic examination

Procedures and materials for performing QEMG have been described previously [8,20,21]. In brief, EMG examination was performed using portable apparatus (Viking Quest EMG system)[a] and 50-mm-long, 0.45-mm-diameter (IS, SS, DT) or 100-mm-long, 0.8-mm-diameter (BF) concentric EMG needle electrodes[a] (sampling area, 0.07 mm^2). The horse stood unsedated in stocks, and a surgical pad attached to it with a girdle that connected to the preamplifier served as the earth electrode. All horses had all 4 muscles examined during a single session. Bandpass was between 5 Hz and 10 kHz. Sweep speed was 10–20 ms/division. Amplifier gain was 50–100 mV for spontaneous activity and 100–500 mV for MUAP recordings.

Standard EMG examination

Insertional activity, pathological spontaneous activity, MUAPs and satellite potentials were recorded in each muscle. The presence or absence of pathological spontaneous activity was examined outside the endplate region in the same regions in which MUAPs were obtained to detect any fibrillation potentials, positive sharp waves, complex repetitive discharges or (neuro)myotonia. Any pathological spontaneous activity was considered indicative of pathology if present in 2 or more locations.

Quantitative MUAP analysis

After insertion of the EMG needle, horses were manipulated as necessary to induce moderate muscle fibre recruitment and individual MUAPs. In order to recruit SS, IS and DT muscles, the horse was pushed from the contralateral shoulder in order to force the horse to bear weight on the leg being studied. Likewise, in order to recruit muscle fibres of the biceps femoris, the ipsilateral front leg was elevated and the weight gently shifted onto the hindleg by pulling on the withers towards the measuring site. The muscle force induced was kept at a low level to avoid inducing interference patterns that would prevent analysis of individual MUAPs. At least 3 insertions and 3 directions per insertion were made per investigated muscle. The needle was redirected several times and, by selecting sharp-sounding MUAPs while the needle was withdrawn in 3 mm increments, sampling was performed throughout the muscle. The MUAPs were selected partly in a semi-automatic way, using a trigger line that selects identical MUAPs above the chosen amplitude. The automatic selection of MUAPs was corrected manually offline, where indicated (artefacts and noise excluded). The end-point of MUAP duration was corrected by on-screen visual assessment if not automatically shown correctly [24–26]. Amplitude, duration, number of phases and number of turns were automatically calculated and obtained from at least 20 MUAPs per muscle, with a maximal rise time of 0.8 ms rise and identically firing at least 4 times. The percentage of polyphasic MUAPs was calculated, polyphasia being defined as ≥4 phases. Motor unit action potentials with a number of turns ≥5 were considered complex MUAPs [1,27]. In addition, other quantitative parameters recently described in horses, such as size index (SI) and MUAP area, were recorded and analysed [23]. The MUAP area was calculated automatically. Size index was calculated automatically as $2 \times \log(\text{amplitude}) + \text{area/amplitude}$.

Interference pattern analysis

The low-frequency filter was set at 20 Hz, the high-frequency filter at 10 kHz and the sampling frequency at least 25 Hz [24–26]. Thirty contractions at random force per segment in each muscle were recorded with standard concentric needle electrodes[a] and evaluated. Interference patterns were analysed by measuring maximal voluntary activity expressed by turns/second and cloud analysis [28,29]. The EMG software calculated these variables automatically. The muscle force induced was not literally maximal, but randomised muscle force induced after vigorous stimulation by pushing or pulling on the withers as described in the previous subsection.

Retrospective study of horses with orthopaedic or neurological disorders of the shoulder muscles

A sample of convenience of horses presented to the Equine Hospital, Faculty of Veterinary Medicine, Utrecht University for locomotion disorders involving the shoulder muscles was retrospectively identified by searching the QEMG software database in order to produce comparisons with the normative data presented and illustrate the clinical use of QEMG. Inclusion criteria were as follows: 1) a clinical diagnosis by a board-certified specialist orthopaedics/internal medicine clinician of a disorder of the lower motor neuron system or myopathy involving at least the shoulder muscles; and 2) having performed QEMG evaluation of at least the shoulder muscles. A clinical diagnosis was reached by complete orthopaedic and neurological examination, radiographs and haematology and plasma biochemistry as indicated depending on the clinical presentation. Exclusion criteria were orthopaedic disorders causing signs of lameness or other locomotion disorders.

Data analysis

Data on MUAP variables were analysed using a one-way ANOVA with repeated measures and muscle (SS, IS, ST and BF) as the independent factor. Data satisfied the assumptions of normal distribution and homoscedasticity. If the null hypothesis was rejected, *post hoc* testing according to Bonferroni was performed to isolate significant differences between muscles. The significance level was set at $P \leq 0.05$. The mean MUAP variables per horse were used for statistical analysis, as previously reported [17].

Data are described as means ± s.d. unless otherwise stated. A statistical software package (Sigmastat 3.5 software package)[b] was used for all computations.

Results

In all 4 muscles under study, insertional activity was not prolonged or absent and pathological spontaneous activity was not detected except that satellite potentials were occasionally identified.

Quantitative electromyography

Semiquantitative analysis online showed that MUAPs from SS and IS muscles appeared to have a higher amplitude and duration and a higher number of phases and turns than those of BF, and DT had intermediate MUAP features (Fig 1). The mean duration was ~40% greater and the mean amplitude was ~2- to 3-fold higher in SS and IS muscles relative to BF (Table 1). The mean duration and amplitude of DT were not statistically different from BF (Table 1). Likewise, MUAP area and size index were significantly higher (Table 1) in SS and IS relative to BF, and DT muscle presented intermediate values (Table 1). The mean ± s.d. percentage of polyphasic MUAPs was 17 ± 7, 16 ± 10, 14 ± 12 and 5 ± 5% in SS, IS, DT and BF muscles. In addition, 4 of 7 horses had >15% polyphasic MUAPs in SS and IS muscles, 3 of 7 horses had >15% polyphasic MUAPs in DT, whereas only one of 7 horses had >15% polyphasic MUAPs in the BF muscle. Complex MUAPS were occasionally recorded in SS and IS muscles.

Interference pattern analysis

Maximal voluntary activity differed significantly between muscles. Specifically, maximal voluntary activity expressed by turns/second was higher in DT than BF muscles (152 ± 59 vs. 76 ± 43, P = 0.03), and the amplitude of the interference pattern measured was lower in BF (257 ± 54 μV) than in the other 3 muscles (mean 451–460 ± s.d. 82–158 μV, P<0.02). By projecting interference pattern recordings of 30 muscle contractions per muscle, expressed as amplitude/turns plotted against turns/second, of all 7 horses per muscle on top of each other, a cloud of measuring points was graphed, as documented previously [23]. Each cloud was located in the lower left corner of the figure (Fig 2). The centre of gravity of measuring points was subjectively different between muscles. The normal limit in man is set at <15% of the total number of points outside the normal boundaries [28].

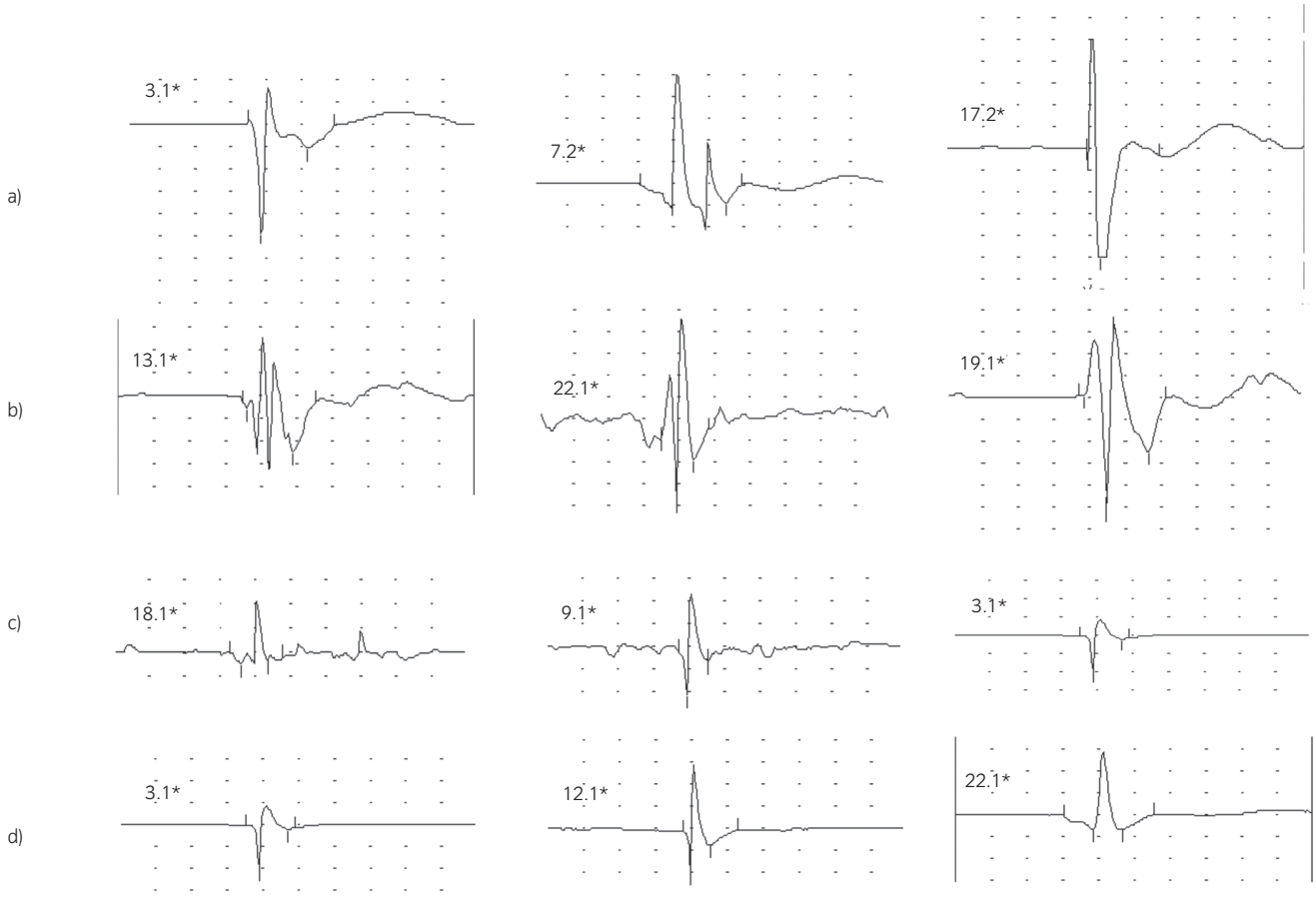

Fig 1: Representative screen captures of motor unit action potentials of supraspinatus (a), infraspinatus (b), deltoideus (c) and biceps femoris muscles (d). Each division represents 100 μV (vertical axis) and 5 ms (horizontal axis).

Clinical cases with neuropathy or myopathy of the shoulder muscles or biceps femoris

Horses with neurological disorders involving the lower motor neurons had increased values of one or more of the following QEMG parameters: amplitude, duration, phases, turns, size index and percentage of polyphasia (Table 2) relative to those in normal horses (Table 1). These included horses with clinical evidence of suprascular nerve injury, locomotion disturbances characterised by decreased protraction of one or both forelimbs not caused by orthopaedic lesions, and cases of equine motor neuron disease. Horses with myopathy had lower values of one or more of the following QEMG parameters: amplitude, duration and size index (Table 2) than those in normal horses (Table 1).

Discussion

Our main conclusions were as follows: 1) SS and IS muscles have similar quantitative EMG features as in previously published data from the triceps muscles but different from other studied skeletal muscles in horses [8,19–22]; 2) the MUAP parameters of SS and IS muscles (i.e. increased MUAP duration and amplitude) observed in these normal horses would be suggestive of neurogenic disorders if muscle-specific normal data were not available and decisions were made by comparing with values from other skeletal muscles [14], thereby ignoring the muscle-specific values; and 3) the MUAPs of the biceps femoris muscle differ greatly from those of the lateral vastus muscle, especially in amplitude [21], again highlighting the need for muscle-specific values from healthy animals.

Electromyography has long been in use as an ancillary diagnostic method to characterise certain neurological and muscular disorders in man [1]. Electromyographic examination involves assessment of insertional activity during needle placement, spontaneous activity following needle insertion without active muscle contraction and motor unit action potentials during recruitment of muscle fibres by voluntary contraction. Quantitative assessment of MUAPs has been demonstrated in man to provide more information than simply detection of prolonged insertional activity or spontaneous pathological activity [2,10]. In horses, EMG examination has been performed for decades [30]. However, unlike human neurophysiology, quantitative assessment of MUAPs has been used rarely [14] despite an increase in the number of publications on the use of QEMG in the last 10 years [9,15–17,22]. In horses, MUAP analysis has been shown to enable distinction between normal, neuropathy or myopathy [9,15–17], but MUAP parameters are also influenced by age [19] and type of muscle [8,21,22], as well as neuromuscular disorders [18].

In the present study, SS and IS had MUAPs characterised by high amplitude, duration and percentage of polyphasia compared with the biceps femoris and with previously reported values for other equine muscles [21,22]. The MUAP amplitude is physiologically increased by muscle fibre hypertrophy (i.e. increased muscle fibre cross-sectional area) and pathologically increased by collateral reinnervation of muscle fibres in neuropathy [10,15,16]. In quadrupeds, the shoulder muscles contract during the weightbearing phase of every stride to stabilise the scapulohumeral joint. The SS and IS muscles are innervated by the suprascapular nerve, and its perineural anaesthesia results in marked shoulder instability, with lateral luxation of the proximal humerus during weightbearing [31]. Repetitive loading of SS and IS muscle fibres leads to hypertrophy and increased cross-sectional area, which in turn results in increased MUAP amplitude. Increased muscle fibre cross-sectional area in SS and IS muscles relative to other appendicular muscles has been shown in small primates [32] and dogs [33], but has not been demonstrated in

TABLE 1: Quantitative electomyographic parameters of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles with significance (P values) compared with biceps femoris muscle

	Muscle	Mean ± s.d.	P value	95% Confidence interval
Amplitude (μV)	Supraspinatus	759 ± 117	<0.001	651–867
	Infraspinatus	931 ± 163	<0.001	779–1082
	Deltoideus	530 ± 173	0.1	370–691
	Biceps femoris	339 ± 81	–	265–385
Duration (ms)	Supraspinatus	9.6 ± 0.9	0.001	8.7–10.4
	Infraspinatus	10.3 ± 0.8	<0.001	9.6–11.0
	Deltoideus	7.5 ± 1.7	>0.9	6.0–9.1
	Biceps femoris	6.8 ± 1.1	–	5.7–7.8
Number of phases	Supraspinatus	3.5 ± 0.3	0.02	3.2–3.7
	Infraspinatus	3.5 ± 0.3	0.02	3.3–3.7
	Deltoideus	3.3 ± 0.4	0.2	2.9–3.7
	Biceps femoris	2.9 ± 0.3	–	2.7–3.2
Number of turns	Supraspinatus	4.2 ± 0.6	0.002	3.7–4.7
	Infraspinatus	4.3 ± 0.5	0.001	3.8–4.7
	Deltoideus	3.6 ± 0.9	0.2	2.8–4.5
	Biceps femoris	2.8 ± 0.3	–	2.6–3.1
Area (μV·ms)	Supraspinatus	1256 ± 218	0.001	1054–1457
	Infraspinatus	1777 ± 462	<0.001	1349–2204
	Deltoideus	877 ± 537	0.1	380–1374
	Biceps femoris	390 ± 102	–	296–484
Size index	Supraspinatus	1.3 ± 0.2	<0.001	1.1–1.5
	Infraspinatus	1.7 ± 0.3	<0.001	1.4–1.9
	Deltoideus	0.8 ± 0.6	0.1	0.3–1.3
	Biceps femoris	0.4 ± 0.2	–	0.2–0.5

horses. An alternative explanation for the increased MUAP amplitude and duration in SS and IS muscles observed in the present study might be subclinical denervation-renervation. In a study of 14 healthy draught horses, 9 had histological evidence of chronic focal neuropathy of the suprascapular nerve at the site of reflection over the cranial edge of the wing of the scapula [34]. The present study lacks morphometric data, which can be considered a weakness of this study. However, subclinical neuropathy of the suprascapular nerve is unlikely in the present study because horses had normal findings on physical, lameness and neurological examination and unremarkable cervical radiographs.

Quantitative EMG parameters of DT and BF muscles are not comparable to those of other muscles previously described in horses. The 95% confidence interval of MUAP amplitude of DT (370–691 μV) was numerically higher than previously reported values for subclavius (266–344 μV) and descending pectoralis muscles (271–327 μV), similar to that of brachiocephalicus (412–483 μV), serratus ventralis cervicis (488–551 μV) and vastus lateralis (571–836 μV) and lower than that of triceps (~701–1058 μV) [21–23]. Likewise, the 95% confidence interval of MUAP duration of DT muscle (6.0–9.1 ms) was numerically higher than previously reported values for brachiocephalicus and serratus ventralis cervicis (4.3–4.7 ms) and pectoralis descendis muscles (5.3–5.8 ms), but similar to subclavius (6.4–7.4 ms), vastus lateralis (7.8–10.2 ms) and triceps muscles (7.6–10.0 ms) [21–23]. Biceps femoris had QEMG parameters (amplitude and duration) more comparable to subclavius, pectoralis descendis, serratus ventralis and brachiocephalicus and numerically lower than that of vastus lateralis and triceps.

In previous studies, the majority of data analyses were performed in age- and breed-matched controls, from which data were pooled, and natural logarithmic transformation was needed because of the skewed distribution of data [21,22]. In the present study, the mean values per horse per muscle were used for statistical analysis, and the criteria for normal distribution were met in this way without transformation. The mean values per horse for each muscle were evaluated because this mostly closely resembles the clinical situation.

The results of QEMG evaluation of MUAPs observed in clinical cases diagnosed with neuromuscular conditions in the present study illustrate the characterisation of disorders as neurogenic, myogenic or not resulting from alterations of the motor unit. It must be emphasised that EMG evaluation on its own does not provide a definitive diagnosis, but will be complementary to a thorough orthopaedic and neurological examination in selected cases [35].

The results of this study should be interpreted taking into account the relatively small number of horses, although the total number of analysed MUAPs per muscle was >140 (>20 MUAPs × 7 horses), and for interference pattern analysis this was >210 recordings (>30 MUAPs × 7 horses). Only adult horses (>18 months and <18 years old) were included; therefore, these normative data may not be directly extrapolated to all age

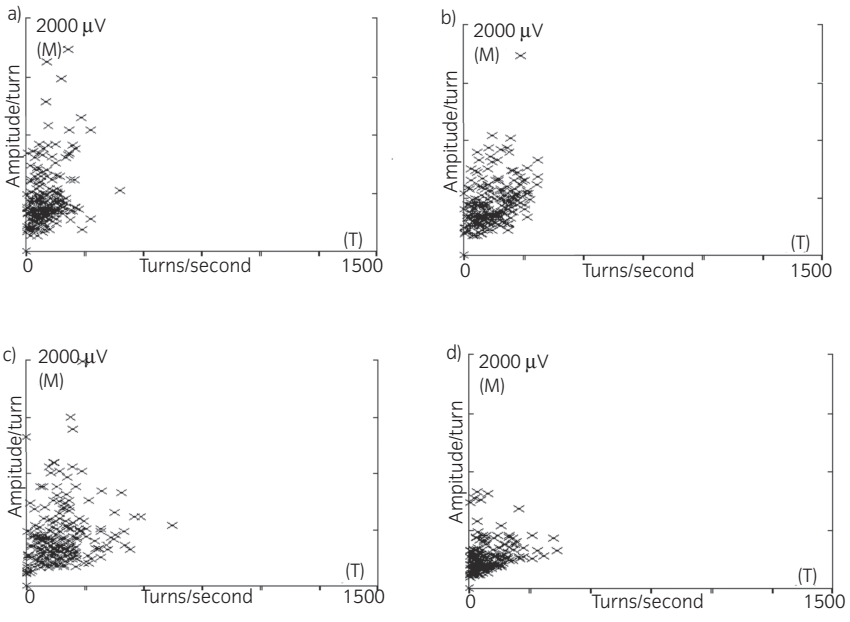

Fig 2: Interference pattern presented as cloud analysis of cumulated segments in the supraspinatus (a), infraspinatus (b), deltoideus (c), and biceps femoris muscles (d). M = mean and T = turns. Horizontal axis = number of turns per second; vertical axis = amplitude in microvolts.

TABLE 2: Quantitative electomyographic parameters of supraspinatus, infraspinatus, deltoideus and biceps femoris muscles in 11 horses with suspected neurogenic or myogenic disorders

Breed	Age (years)	Diagnosis	Muscle	Amplitude (μV)	Duration (ms)	Phases	Turns	Size index	Polyphasia (%)
Neuropathies									
Trackener	5	Peripheral neuropathy, LF worse than RF	Left SS	**1079**	**10.7**	**4.0**	4.8	**1.64**	**35**
			Left IS	834	**14.1**	**4.2**	**6.3**	1.99	**60**
			Left DT	**929**	**11.9**	3.8	**5.6**	**1.65**	19
			Right SS	1087	11.7	**4.1**	**5.5**	1.65	**47**
			Right DT	**998**	**12.4**	**4.2**	**5.7**	**1.97**	39
RDSH	13	Suprascapular nerve paralysis, RF	Right SS	861	**11.7**	**4.0**	4.7	**1.62**	31
RDSH	6	UMN and LMN disorder, both forelimbs	Left SS	**947**	8.7	3.3	3.9	1.51	11
			Left IS	**1871**	9.6	**4.5**	**5.5**	**2.01**	**50**
			Left DT	**777**	7.7	3.2	3.9	1.24	8
NRPS	13	Suprascapular nerve paralysis, LF	Left IS	796	**14.2**	3.7	5.3	**2.04**	27
			Left DT	**733**	**10.3**	3.2	4.3	**1.54**	0
RDSH	14	EMND	Left BF	331	7.9	3.0	3.4	0.18	**33**
Hannoverian	14	EMND	Left BF	**805**	7.5	**5.0**	**7.2**	**0.87**	**60**
RDSH	5	EMND	Left BF	381	7.2	2.6	3.2	**0.6**	**22**
Myopathies									
Friesian	10	Generalised myopathy	Left BF	**151**	**4.5**	2.6	2.0	**0.00**	0
			Right BF	**130**	**4.9**	2.3	2.1	**0.01**	7
–	–	Myopathy, L and R	Left SS	**429**	11	3.2	3.2	**0.77**	12
			Left IS	**709**	**8.5**	2.9	3.6	**1.06**	0

Values in bold are outside the 95% confidence interval of reference ranges derived from 7 normal horses and given in Table 1. BF = biceps femoris; DT = deltoideus; EMND = equine motor neuron disease; IS = infraspinatus; LF = left forelimb; LMN = lower motor neuron; NRPS = Netherlands riding horse studbook; RDSH = Royal Dutch sport horse; RF = right forelimb; SS = supraspinatus; UMN = upper motor neuron.

categories. Breed differences and the concomitant muscle fibre composition potentially affect MUAP characteristics, and it remains questionable whether breed influence will affect normative data to an extent that may interfere with decision making on the presence of pathology or not [9,16,17]. In common with all retrospective studies, it is possible that we missed horses with the same disorders and unremarkable QEMG findings.

In conclusion, this study provides normative QEMG values for 3 shoulder muscles and biceps femoris, as well as more recently developed QEMG variables, such as SI, MUAP area and interference pattern analysis. These normative data may be helpful in diagnosing or localising neuromuscular disorders of the caudal cervical region or proximal antebrachium. However, the muscle-specific data show that SS, IS and triceps muscles in normal horses have QEMG features that would be considered suggestive of neuropathy if detected in other skeletal muscles. Muscle-specific normal data are essential for comparison with clinical cases suspected of having neuromuscular disorders.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study was approved by the Committee of Animal Welfare at the University of Utrecht. Owner informed consent for the retrospective component was not stated.

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Authorship

I. D. Wijnberg and E. Jose-Cunilleras contributed equally to the study design, execution, data analysis and interpretation, preparation of the manuscript and final approval of the manuscript.

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