

Chronic progressive lymphedema in the Belgian draft horse in Belgium: clinical phenotyping, prevalence and risk factor analysis

Chronisch progressief lymfoedeem bij het Belgisch trekpaard in België: klinische fenotypering, prevalentie en analyse van risicofactoren

¹K. De Keyser, ¹S. Janssens, ¹L.M. Peeters, ²F. Gasthuys, ²M. Oosterlinck, ¹N. Buys

¹KU Leuven Department of Biosystems, Livestock Genetics, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium

²Ghent University Faculty of Veterinary Medicine, Department of Surgery and Anesthesiology of Domestic Animals, Salisburylaan 133, B-9820 Merelbeke, Belgium

nadine.buys@biw.kuleuven.be

ABSTRACT

Chronic progressive lymphedema (CPL) is a well-known problem in the Belgian draft horse. However, tools for clinical diagnosis have not been standardized. Therefore, the exact disease prevalence and the possible CPL risk factors have not been investigated in the Belgian breed yet. The aim of the present study was to assess a uniform CPL clinical examination method to increase diagnostic objectivity as well as to determine the occurrence and severity of lesions. Using univariate and stepwise multivariable logistic regression models, the association of factors, such as age-gender interaction, coat color and season, with CPL occurrence was examined. In this study, it was demonstrated that CPL is highly prevalent in the Belgian draft horse: 60.66% of the horses of the total sample were affected, including a large proportion of young horses (< 3 years), whereas 85.86% was affected in a subset of older horses (≥ 3 years). In the latter, the lesions were more explicit as CPL is a chronic disorder. In some horses (i.e. 14% of the yearlings), mild clinical symptoms appeared at approximately the age of one, while distinct onset of the disease occurred from the age of three onwards. The factors age-gender interaction, coat color and season, which are significantly associated with CPL occurrence, were identified and quantified. In this study, a standardized diagnostic protocol is proposed that will enhance future data collection and furthermore will offer a foundation for quantitative genetic research. Ultimately, it will help to reduce CPL occurrence in the Belgian draft horse by means of selection.

SAMENVATTING

Het Belgisch trekpaard is belast met chronisch progressief lymfoedeem (CPL), een bekende, ongeneeslijke aandoening. Tot op heden bestaat er geen uniform diagnostisch protocol, waardoor een goede schatting van de prevalentie onbestaande is. Het doel van dit artikel was een eenduidige klinische onderzoeksmethode te ontwikkelen om de objectiviteit van de CPL-diagnose te verhogen en de prevalentie van CPL bij het Belgisch trekpaard na te gaan. Daarnaast werd aan de hand van statistische modellen het verband onderzocht tussen bepaalde factoren, zoals de interactie leeftijd-geslacht, vachtkleur en seizoen, enerzijds en het optreden van klinische symptomen anderzijds. Deze studie toont aan dat de prevalentie van CPL hoog is. In de volledige steekproef, waarbij een groot aandeel van de paarden jonger was dan drie jaar, was 60,66% aangetast. In een subset van oudere dieren (≥ 3 jaar) was dat 85,86%. Bij sommige paarden werden reeds milde symptomen gezien vanaf één jaar (14% van de jaarlingen), maar duidelijke letsels kwamen doorgaans voor vanaf de leeftijd van drie jaar. Het ontwikkelde protocol kan de CPL-diagnose en toekomstige gegevensverzameling vergemakkelijken. In de voorliggende studie werden factoren die significant geassocieerd zijn met CPL, i. e. interactie leeftijd-geslacht, vachtkleur en seizoen, geïdentificeerd en gekwantificeerd. Daarmee is de basis gelegd voor kwantitatief genetisch onderzoek met als ultiem doel CPL bij het Belgisch trekpaard te reduceren.

INTRODUCTION

Chronic progressive lymphedema (CPL) affects several draft horse breeds worldwide, including the Shire and Clydesdale (Ferraro, 2001), different German draft horse breeds (Wallraf et al., 2004), the Gybsy Cobb (Powell and Affolter, 2012), Friesians (Boema et al., 2012) and the Belgian draft horse (De Cock et al., 2003; Geburek et al., 2005). In 2013, 6,599 horses were registered in the Belgian draft horse studbook, which represents approximately 3% of the total Belgian horse population. Only 627 newborn foals were registered in 2013, which is the result of a steady decline of the annual subscriptions over the last decade. Although the Belgian draft horse is considered to be part of Belgium's cultural heritage, these decreasing numbers put the conservation of the breed on the foreground. Although owners are concerned about CPL in their breeds, the estimation of the population prevalence is hampered by the lack of a uniform and reliable routine recording of the phenotype (the Royal Society of the Belgian Draught Horse, personal communication). Clinical CPL symptoms typically occur at the level of the lower limbs, and include progressive tissue swelling, associated with deteriorating skin surface anomalies and incurable deformations (Ferraro, 2001; De Cock et al., 2003) (Figure 1A-C). Despite research during the last decade (De Cock et al., 2006a, b; De Cock et al., 2009), the high prevalence in some breeds (Wallraf et al., 2004) and its serious economic impact (Ferraro, 2003), the CPL pathogenesis has not been fully understood, and available treatment options remain symptomatic and non-curative.

Assessments of CPL prevalence are sparse. In a study of Wallraf et al. (2004), the disease prevalence averaged 65.1% (ranging from 47.5 to 96.1%) in a sample of German draft horses older than 2.5 years ($n = 912$, 6 different breeds). In a study with 161 Belgian draft horse stallions aged two to three years, the prevalence was 82% (Verschooten et al., 2003). Verschooten et al. (2003) collected data at Ghent University, Faculty of Veterinary Medicine, during eight successive years (1995-2002), by means of clinical examination and radiographic analysis of the four lower limbs in all stallions presented for their final approval to enter the breeding program as an official stud.

Clinical symptoms vary with age (progressive character) and gender (Wallraf et al., 2004; De Keyser et al., 2011) and might be affected by coat color, the geographical location of sampling and by random environmental conditions, such as season of sampling. However, these last three factors have not been examined yet. As certain breeding lines seem more affected than others, it is expected that there is a genetic component to CPL susceptibility. The aim of the present study was to develop a standardized clinical examination tool for a uniform CPL diagnosis in draft horses. A detailed and uniform assessment of CPL (separately per limb and categorized by severity of lesions) will provide a better insight into the current state of the disease.

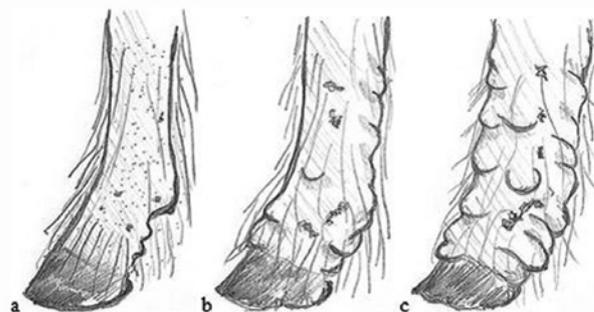


Figure 1. Progression of clinical chronic progressive lymphedema symptoms at the level of the lower limbs. A. (CPL score = 1) mild symptoms in the early disease stage include slight limb swelling with hyperkeratosis and scaling, one to two small skin folds in the rear pastern region. B. (CPL score = 2 to 3) progression of lesions, with a more severe limb swelling and formation of multiple skin folds and fibrotic nodules. Small ulcerations coalesce into bigger ulcerative areas. C. (CPL-score=3 to 4) massive limb swelling and extreme deformation due to skin folds and nodules encircling the entire lower limb. Multiple large wounds/ulcers. (skin scales were not drawn in figures B and C).

MATERIALS AND METHODS

Clinical phenotyping and traits

CPL associated lesions have been reported in several breeds (Shire, Clydesdale and German breeds) (Ferraro, 2001; De Cock et al., 2003; Wallraf, 2003). Histopathological data combined with the authors' clinical observations in preliminary field trials of the present study, resulted in a list of possible CPL associated lesions at the lower limb level (Table 1). Categorization (from 0 to 4) indicates clinical severity and defines the magnitude of the affected limb region, using the fetlock as a reference. Disease severity is determined by the presence and severity of long-term features, such as swelling and deformation and/or by skin and hair anomalies which are listed separately in Table 1. Based on this scoring system, the four limbs of each horse included in this study, were clinically examined and given a 'clinical score per limb' (from 0 to 4). To obtain a full impression of CPL severity per horse, the four scores were added up, resulting in a 'CPL score per horse' (from 0 to 16).

Population

Data collection was performed at official draft horse contests in Belgium throughout two years (2009-2011). Since at such contests, predominantly younger horses are presented, additional stable visits were performed (in Flanders, Belgium), in order to obtain a representative sample regarding age and location. The geographical location of sampling was chosen at random, and at each location, the authors

Table 1. Overview of CPL-associated lower-limb lesions in draught horses, categorizing affected limbs based on the severity of lesions and the dimensions of the affected region.

Severity	CPL	Skin surface and hairs	Swelling and deformation
Mild Below fetlock	0	Hairs are supple Slight skin thickening Scaling	Normal limb diameter* Normal limb diameter Soft, compressible swelling 1-2 SF (P/PL)
	1	Hyperkeratosis Hairs supple (Hock: hairs upright) Moderate skin thickening Hyperkeratosis, scaling	Normal limb diameter Moderate hard, diffuse swelling >2 SF and NOD (D/P/PL)
Moderate Until fetlock	2	Wounds, ulcera Hairs less supple (Hock: hairs upright + exudate) Severe skin thickening Hyperkeratosis, scaling	Increased limb diameter Hard, diffuse swelling SF and NOD (D/P/PL) Mechanical impairment
		Wounds, ulcera, exudate Greasy skin + bad odour Hairs rough + broken Hock: hairs upright + exudate + wound	
Severe Above fetlock	3	Severe skin thickening Hyperkeratosis, scaling Wounds, ulcera, exudate Greasy skin + bad odour Hairs rough + broken Hock: hairs upright + exudate + wound	Increased limb diameter Hard, diffuse swelling SF and NOD surrounding limb Severe mechanical impairment General loss of condition 'Final stage'
		Severe skin thickening Hyperkeratosis, scaling Wounds, ulcera, exudate Greasy skin + bad odour Hairs rough + broken Hock: hairs upright + exudate + wound	
Extreme Above fetlock	4	Severe skin thickening Hyperkeratosis, scaling Wounds, ulcera, exudate Greasy skin + bad odour Hairs rough + broken Hock: hairs upright + exudate + wound	

SF = skin folds, NOD = nodules, D = dorsal, P = palmar, PL = plantar. * A normal limb diameter ranges from 25 to 30 cm for forelimbs (measured directly below the carpus) and from 33 to 38 cm for hind limbs (measured directly under the hock) (De Cock et al., 2003).

attempted to examine all horses present. Pedigree data (25,376 records) were provided by the Royal Society of the Belgian Draught Horse (Brussels, Belgium) and additional information about e.g. sire and dam, date of birth and coat color was retrieved afterwards, based on the horse's studbook number (provided by the studbook).

Statistical analysis

Statistical analysis was performed using SAS 9.3 software (Sas Institute Inc., Cary, USA). The MEANS and FREQ procedure were used to obtain descriptive statistics and to assess CPL prevalence. The CPL scores of the horses that were repeatedly measured, were averaged. Since especially younger horses were included in the present study, prevalence was assessed for the total sample and, analogous to Wallraf et al. (2004), also for a subset of horses older than three years of age. To identify risk factors, CPL was treated as a binary response: present/yes (CPL score > 0) or absent/no (CPL score = 0). Factors possibly associated with the presence of CPL lesions (i.e. the interaction effect age-gender, coat color, location and season of examination) were analyzed using the LOGISTIC procedure in univariate and stepwise forward multivariable models. The Akaike information criterion (AIC) was used for model selection (Akaike, 1974).

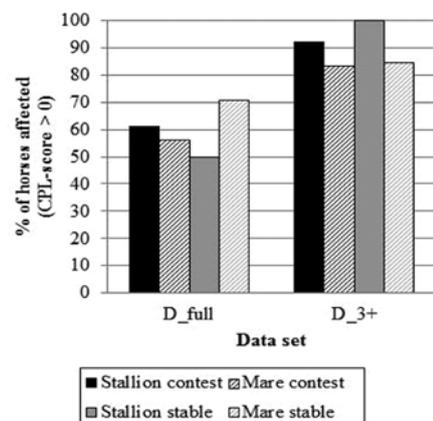


Figure 2. Percentage of affected horses (CPL score > 0) per gender and location, for the total sample (n = 980) and for a subset of horses of at least three years old (n = 456).

RESULTS

Clinical phenotyping, sample and descriptive statistics

Table 1 provides an overview of possible CPL associated lower limb conditions in draft horses, leading to a standardized classification of affected limbs in five CPL scores: 0 = not, 1 = mildly, 2 = moderately, 3 = severely and 4 = extremely affected.



Figure 3. Average severity of lower limb lesions (CPL score) in Belgian draft horse mares and stallions (range 0-16) per age (years). Stallions of ≥ 8 years and mares of ≥ 10 years were grouped. Contest and stable stallions were grouped together because of a limited number of stallions sampled at stable visits.

Official contests were visited on 22 dates: 3 in winter, 0 in spring, 4 in summer and 15 in autumn; 27 stable visits were performed on 17 dates: 4 in winter, 4 in spring, 9 in summer and 10 in autumn. A total of 762 horses was examined by means of the scoring system (Table 1), and some of the horses were examined 2 to 5 times to enable the assessment of random environmental effects in future quantitative genetic analyses. This resulted in a total of 980 records (comprising 18% of repeated measurements) with pedigree information. The average time lapse between examinations (SD) was 169 days (139).

The majority of records were collected at official contests (81.22%), with an average number of 36.27 ± 25.15 (range 7 to 110) per event, whereas on average, 6.81 ± 5.64 (range 1 to 24) records were collected per stable visit. The most common coat color was ‘bay roan’ (64% of the records); ‘bay’, ‘black roan’, ‘black’ and ‘chestnut’ accounted for respectively 16%, 10%, 5% and 5% of the sample. At official contests, the CPL scores were significantly ($p < 0.001$) higher in stallions than in mares, while the opposite was noticed at stable visits. However, this difference was not significant ($p = 0.20$) (Table 2). The average and maximum ages of the horses of the present study sample were low: 3.87 ± 3.02 and 17.48 years, respectively. On average, mares were older than stallions, but this difference was only significant for the horses at the stables ($p = 0.04$).

CPL prevalence and severity

In the present study, the clinical CPL prevalence was high, showing a CPL score > 0 in 60.66% of the horses. At official contests, more than half of the sample (57.75%) was affected (61.08% of the stallions and 56.09% of the mares) (Figure 2). At stable visits however, the percentage of the horses showing lesions was even higher, 68.55% (50.00% of the stallions and 70.92% of the mares). As CPL is a progressive disorder, it is important to mention that the average age of unaffected horses was low: for stallions 1.67 ± 0.91 years old (range 0.32 to 10.51) and for mares 2.41 ± 1.91 years old (range 0.33 to 16.02). Considering the occurrence of clear lesions from the age of three years onwards in the full set of data (Figure 3), the CPL prevalence was also calculated for horses older than three years. Horses of this subset that might have been ‘subclinical’ at young age, most likely had (already) developed clinical signs of CPL (increased certainty of the phenotype). The percentage of clinically affected horses had increased to 85.86% compared to the prevalence calculated on the full dataset (Figure 2): 85.84% were affected at the time of the contests (91.95% of the stallions and 83.33% of the mares), and 85.84% of the horses were infected at the time of the stable visits (all of the stallions and 84.91% of the mares).

Concerning disease severity, the initial lesions were detected in 1-year-old horses (Figure 3), but only in a low percentage (14%) and with an average CPL score (SD) of 0.33 ± 0.89 (range 0 to 4). The severity increased for both mares and stallions from the age of three onwards. The lesions in the mares older than three years, which were examined at the stable visits, were worse than the lesions of the mares at the events. From the age of five onwards, the stallions examined at the contests were more severely affected and showed quicker deterioration than mares. The stallions examined at the contests and at the stable visits were grouped together because of the limited number of stallions per age.

Multivariable logistic regression

The factors age and gender, coat color, location and season of sampling had a p-value lower than 0.05

Table 2. Descriptive statistics of CPL sampled in Belgian draft horses.

Location	Gender	Parameter	n	Mean	SD	Min.	Max.
Contests	CPL-score	Mares	538	2.49	3.21	0.00	12.00
		Stallions	258	3.39	4.00	0.00	16.00
	Age	Mares	538	3.51	2.64	0.33	17.48
		Stallions	258	3.27	2.14	0.32	11.62
Stable visits	CPL-score	Mares	164	5.34	5.14	0.00	16.00
		Stallions	20	3.75	5.23	0.00	16.00
	Age	Mares	164	5.98	4.13	0.46	17.31
		Stallions	20	3.97	4.07	0.81	13.27

CPL-score = sum of CPL-scores (4 limbs) per horse, n = number of records, SD = standard deviation of the mean.

Table 3. Univariate and stepwise multivariable logistic regression models.

Factor Level	¹ CPL (%)	² Odds ratio (95% C.I.)	³ Univariate regression	⁴ Model 1 (1309)	Model 2 (1285)	Model 3 (1275)	Model 4 (1273)
Age & gender			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Coat color			0.006			0.001	0.001
Chestnut	53.06	1					
Bay	66.67	1.77 (0.92-3.40)					
Bay roan	53.10	1.00 (0.56-1.79)					
Black	72.34	2.31 (0.99-5.41)					
Black roan	53.47	1.02 (0.51-2.01)					
Location			0.004			0.949	
Stable	65.76	1					
Contest	54.14	0.62 (0.44-0.86)					
Season			< 0.001		< 0.001	< 0.001	< 0.001
Winter	69.74	1					
Spring	72.50	1.14 (0.54-2.44)					
Summer	53.07	0.49 (0.34-0.71)					
Autumn	51.07	0.45 (0.32-0.65)					

¹The percentage of records with clinically affected horses (CPL-score > 0).

²Odds ratio and 95%-confidence intervals.

³The effect of each parameter tested individually: p-values.

⁴Multivariable logistic regression: p-values (Akaike information criterion: the lower, the better).

in univariate logistic regression models and were included in the stepwise multivariable regression analysis (Table 3). Stallions had a significantly quicker CPL progression than mares, proven by the positive maximum likelihood estimate 0.09 ± 0.02 (result not displayed). CPL prevalence was found to be higher at stable visits than at official contests (approximately 66% versus 54%). Bay and black horses were more affected (around 70%) than horses with other coat colors (bay roan, black roan and chestnut, which accounted for approximately 53%). Prevalence was found highest in winter and spring time (around 70%) in comparison to the examinations in summer (53.07%) and autumn (51.07%). The lowest AIC model (best fit) combines all factors, except for the effect of location, which is not significantly associated with CPL presence (= CPL score > 0).

DISCUSSION

Selection in Belgian draft horses is based on traditional visual evaluations at official horse shows, supplemented with a veterinary clinical advice. Although horses with severe CPL associated lesions are not appreciated by the jury at official contests, there is no uniformity in the clinical evaluation, leaving the exact prevalence and severity of CPL largely unknown. This study provides a standardized examination protocol to diagnose CPL in draft horses. The scoring system enables a uniform classification (ordinal scoring of severity) of the four limbs and provides a continuous total score per horse on the one hand and an increased diagnostic objectivity on the other hand. With this

tool, the authors assessed disease prevalence in a large sample of Belgian draft horses, which has not been performed until now. To the authors' knowledge, only Wallraf (2003) and Wallraf et al. (2004) have drawn up similar reports on the prevalence and quantitative analysis of lower limb lesions in draft horses. However, in contrast with their total study population (n = 912, divided over six different German breeds), in the current study, the uniform sample included 764 horses of one single breed.

The very first (mild) CPL lesions occurred in rather young horses (from one year of age onwards), although the affected number of yearlings was limited. Distinct lesions were present from the age of three onwards, which is in accordance with the results of a study in German draft horses by Wallraf (2003). In that study, even horses younger than 2.5 years of age were omitted from the study sample, stating that no symptoms were observed at younger age. To the authors' knowledge, analogous to chronic lymphedema in humans (The International Society of Lymphology, 2009), a subclinical lymphedema state exists in susceptible horse breeds, i.e. lymphatic malfunction is already present but the clinical disease onset breaks out later. This explains why CPL prevalence in the 3-year-old horses (and older) of the present study was higher (approximately 84% in case of the mares and 96% for the stallions). In a study by Wallraf et al. (2004) with Rhenish German draft horses (older than 2.5 years), a breed with Belgian draft horse ancestral genes, prevalence averaged at 96.1%, which is higher than the average prevalence of the subset of older horses (approximately 86%) of the present study.

Most of the horses were sampled at contests, where the percentage of horses affected and their age were significantly lower than at the stable visits, whereas all the horses in the study of Wallraf et al. (2004) were examined at stable visits only. This sampling may also partially explain the high proportion of young horses in the present study. Aging was associated with an increasing disease prevalence and a decreasing number of horses (results not displayed), most likely a result of commonly performed early euthanasia of severely affected horses (Ferraro, 2003). Difference in progression was found between stallions and mares, coinciding with a generally higher clinical severity in stallions (De Cock et al., 2003). Important factors associated with CPL occurrence were identified and quantified, i.e. an effect of age-gender, coat color and season of examination. If these factors are taken into account, scores will be more comparable in cases where horses are measured in different conditions, hence facilitating selection against CPL.

In conclusion, this study provides a method for the uniform classification of CPL severity per limb in order to increase diagnostic objectivity. The results of the study indicate that chronic progressive lymphedema is a widespread problem in the Belgian draft horse, with an average disease prevalence of 60.66% in the total sample, which included a large proportion of young and subclinically affected horses. The disease prevalence in a subset of horses older than three, with more phenotypic certainty, was 85.86%. Routine recording of additional information about gender, age, season of sampling and coat color is advisable and will enable the use of statistical models to facilitate selection against CPL.

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