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Chronic progressive lymphedema in draft horses: mites or myth?

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VOORWOORD

Ze stappen, hun' bellen al klinken,
de vrome twee horsen te gaâr;
ze zwoegen, ze zweeten; en blinken
doet 't blonde gelijn van hun haar.

Ze stappen, ze stenen, ze stijven,
de stringen: en 't ronde gareel,
het spant op hun' spannende lijven:
de voerman beweegt ze aan een zeel.

De wagen komt achter, De rossen,
gelaten in 't lastig geluid
der schokkende, bokkende bossen,
gaan, stille en gestadig, vooruit.

Geen zwepe en behoort er te zinken,
geen snoer en genaakt er één haar;
zoo stappen, hun' bellen al klinken,
de vrome twee horsen, te gaâr.

Guido Gezelle: 'Twee Horsen', januari 1879

De weg die ik tijdens deze masterproef heb afgelegd, is misschien wel best te vergelijken met een trekpaard dat een zwaarbeladen oogstkar voorttrekt. Vermoeiend, een werk van lange duur, hobbelig, af en toe een struikelblok op de weg, soms even niet meer weten wat links of rechts is, maar met op het einde van de arbeid de nodige tevredenheid en dankbaarheid, het belonende schouderklopje en de trots om deze klus geklaard te hebben. Zoals Guido Gezelle hierboven beschreef, heeft een trekpaard geen extra aanmaning nodig om door te blijven gaan, hoe zwaar de arbeid ook is, en zo heb ik deze masterproef ook beleefd. Met het grootste plezier heb ik maandenlang non-stop gewerkt en heb ik heel nostalgisch Vlaanderen doorkruist. Ik heb fantastische mensen ontmoet, enorm veel bijgeleerd, tradities van lang vervlogen tijden mogen zien herleven en prachtige, machtige, Belgische trekpaarden

aanschouwd op de meest bijzondere plaatsen, gaande van de bossen op de Wijngaardberg met de boomslepende trekpaarden tot de idyllische velden waar nog steeds de oude ploeg in de grond steekt, omringd door paardenhoeven. Er zijn enorm veel mensen die ik wil bedanken voor al hun hulp, steun en medewerking in deze rit, waaraan ik dan ook met veel plezier de nodige pagina's in deze masterproef wil besteden.

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Ondertussen is dit trekpaard met haar zware kar aangekomen op de eindbestemming. Vermoeid…maar uiterst tevreden.

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SUMMARY

Chronic progressive lymphedema (CPL) is a disorder presenting with a high prevalence in the Belgian draft horse population. A genetic predisposition could be of importance in the development of this disease, however, environmental factors exert the greatest influence on severity and progression of clinical symptoms. Characterizing symptoms of CPL consist of the progressive development of skin folds on the distal limbs, nodules, excessive scaling of the skin, hyperkeratosis, fibrosis of the tissues, and are frequently complicated by the occurrence of secondary bacterial and parasitic infections of the skin. Currently, no effective treatment is available. Considering the severity of the symptoms which greatly compromises animal welfare, CPL also represents an important reason for premature euthanasia and thereby negatively affects the popularity of the already diminishing Belgian draft horse breed. Scientific research on CPL is rather limited, and to this date many uncertainties remain about its etiology, pathogenesis and possible treatment. Therefore, to gain more insight into this disorder, all current knowledge on CPL was summarized in a comprehensive review. This review is intended not only as a basis for further scientific research, but also as a guide for veterinarians and horse owners on how to recognize, understand, and (symptomatically) treat CPL in practice.

In addition, the extent to which *Chorioptes bovis* mites may influence the severity and progression of CPL was investigated. The incidence of these mites is extremely high in Belgian draft horses, however, treatment of *C. bovis* is challenging. Due to a lack of effective acaricides in horses, treatment failure and relapse are a common phenomenon. Therefore, the efficacy of a topical moxidectin solution as a treatment against *C. bovis* in Belgian draft horses was studied, and in addition, the effects of this treatment on the clinical symptoms of CPL were evaluated. Topical moxidectin proved to be very effective for the treatment of *C. bovis* in draft horses and resulted in resolution of pruritus and mange associated lesions. In addition, it was demonstrated that an effective treatment against mites also positively affects clinical symptoms of CPL. A reduction in skin folds and nodules was not obtained, however, the characterizing skin lesions of CPL and the general condition of the skin improved significantly. An effective treatment for *C. bovis* infestation is of great value for both veterinary practice and future research on the effect of mites on CPL in draft horses.

SAMENVATTING

Chronisch progressief lymfoedeem (CPL) is een aandoening met een zeer hoge prevalentie in de Belgische trekpaarden populatie. Een genetische aanleg voor CPL zou van belang kunnen zijn in het ontstaan van deze ziekte, maar omgevingsfactoren spelen de belangrijkste rol in de ernst en progressie van de symptomen. Typische symptomen bestaan uit progressieve ontwikkeling van huidplooien op de distale ledematen, nodules, een overmatig schilferende huid, hyperkeratose, fibrose van de weefsels, gecompliceerd door veelvuldig voorkomende secundaire bacteriële en parasitaire infecties van de huid. Tot op heden is er geen effectieve behandeling mogelijk. Gezien de ernst van de symptomen waardoor het dierenwelzijn sterk in het gedrang komt, beïnvloedt CPL op een negatieve manier de populariteit van het hedendaagse Belgische trekpaard en is het bovendien een belangrijke reden voor vroegtijdige euthanasie. Wetenschappelijk onderzoek naar CPL is schaars, waardoor tot op heden nog veel onzekerheden bestaan over de etiologie, pathogenese en mogelijke therapie van deze aandoening. Om meer inzicht te verwerven in deze aandoening werd daarom alle huidige wetenschappelijk kennis over CPL op een overzichtelijke manier samengebracht. Deze samenvatting dient niet alleen als soliede basis voor verder onderzoek, maar ook als leidraad voor praktiserende dierenartsen en zelfs eigenaars voor het herkennen, begrijpen en (symptomatisch) behandelen van CPL.

Daarnaast werd onderzocht in welke mate *Chorioptes bovis* mijten de ernst en progressie van CPL kunnen beïnvloeden. Deze mijten zijn uiterst prevalent bij Belgische trekpaarden, maar de behandeling van *C. bovis* is niet vanzelfsprekend. Door een gebrek aan effectieve

acariciden voor paarden hebben behandelingen in de praktijk vaak onvoldoende resultaat. De effectiviteit van een topicale moxidectine oplossing werd daarom bestudeerd, waarbij ook de effecten van deze behandeling op de klinische symptomen van CPL werden onderzocht. Topicale moxidectine bleek zeer effectief te zijn voor de behandeling van *C. bovis* bij trekpaarden en resulteerde in het verdwijnen van pruritus en schurftletsels op de huid. Daarnaast werd aangetoond dat een effectieve behandeling tegen mijten ook symptomen van CPL op een positieve manier beïnvloedt. Een reductie van de huidplooien en nodules werd niet bekomen, maar de typische huidletsels die geassocieerd worden met CPL en de algemene conditie van de huid verbeterden significant. Een effectieve behandeling van *C. bovis* infestatie is van grote waarde voor zowel de veterinaire praktijk als voor toekomstig onderzoek naar de rol van mijten in CPL bij trekpaarden.

1. GENERAL OBJECTIVES

Chronic progressive lymphedema (CPL) is a multifactorial disease that occurs in several heavy draft horse breeds, including the Belgian draft horse. In the latter, the high prevalence of this disease puts the continued existence of the breed at risk. Clinical symptoms typically manifest on the distal limbs, and include progressive soft tissue swelling and fibrosis, the development of skinfolds and nodules, and are characterized by skin surface abnormalities such as excessive scaling and ulcers (Affolter, 2013; De Keyser et al., 2014a). Since a genetic factor within this disease is suspected, several approaches have been implemented by the studbooks in an attempt to reduce the prevalence of CPL in the Belgian draft horse breed, however with limited success (François, 2018). CPL severely compromises animal welfare, requires a high amount of medical care and is a common reason for premature euthanasia, which markedly decreases the average life expectancy in the Belgian draft horse (Ferraro, 2003; Affolter, 2013). The awareness of this disease is currently widespread, but since effective treatment is non-existent, it drastically affects the popularity of the Belgian draft horse, which, in turn, further compromises the already diminishing population of this breed. Established scientific research on CPL is rather limited, notwithstanding the urgent need for answers.

Despite the severity of this condition, the etiology and pathogenesis of CPL are not yet fully understood. It is believed that both the lymphatic system and changes in dermal elastin play a major role, however, a comprehensive overview of current knowledge on the pathogenesis and main characteristics of this disease is non-existent. **Therefore, the first objective of this Master's thesis is to provide an in-depth overview of all the current knowledge on CPL in draft horses.** The current state of matter on CPL in the Belgian and other draft horse breeds provides the necessary insights for current and future research and enlightens in which ways this debilitating condition could be tackled best.

Although environmental influences are assumed to be of major importance in the progression of clinical symptoms of CPL, environmental risk factors have only been established in a limited number of studies (Ferraro, 2001; Wallraf, 2003; De Keyser et al., 2010, 2014; Geburek et al., 2005b), although mange has already been associated with CPL in various studies (De Cock et al., 2003; Walraff, 2003; Geburek et al., 2005a). But still, it remains unknown to what degree an effective treatment against mites would affect the clinical symptoms of CPL. Moreover, it is not known whether mites represent a consequence of CPL or rather a causative factor in this pathology (Ferraro, 2001; Geburek et al., 2005a). In addition, associated pruritus will cause severe discomfort to the horse, but treatment of this non-burrowing mite in feathered draft horses is challenging and treatment failure and relapse are common. **Therefore, the second objective is to evaluate the contribution of mange on the severity of CPL-lesions and to evaluate the efficacy of a new topical treatment.** Both effectiveness of a topical moxidectin preparation in the treatment of *Chorioptes bovis* and how this treatment affects the symptoms of CPL will be evaluated. An effective treatment for *C. bovis* infestation would be of great value for both veterinary practice and future research on the effect of mites on CPL in draft horses.

2. CHRONIC PROGRESSIVE LYMPHEDEMA IN BELGIAN DRAFT HORSES: A REVIEW OF THE CURRENT KNOWLEDGE

ABSTRACT

The main objective of this review was to provide a clear overview of all current knowledge on chronic progressive lymphedema (CPL) in the Belgian draft horse. This condition is characterized by the development of numerous skinfolds and nodules, hyperkeratosis, fibrosis and ulcerations on the distal limbs of affected horses. Secondary bacterial, fungal or parasitic infections can complicate and aggravate the progression of this disease. In the Belgian draft horse breed, a prevalence of up to 86% has been estimated. Because of the progressive nature and severity of the clinical signs, CPL often results in premature euthanasia. Treatment options are solely symptomatic and aim to improve the horse’s quality of life. Despite the severity of this condition, many uncertainties about its etiology, pathogenesis and subsequent possible treatment still remain to date. Established scientific research on CPL is rather limited, although there is an urgent need for strategies to tackle this disease. This review is intended not only as a basis for further scientific research, but also as a guide for veterinarians and horse owners on how to recognize, understand, and treat CPL in practice.

Keywords: *Chronic Progressive Lymphedema; Belgian draft horse; Clinical signs; Treatment; Diagnosis; Pathogenesis*

2.1. INTRODUCTION

The Belgian draft horse is a worldwide renowned breed because of its combination of strength, power, nobility and elegance. In 2018, the Belgian draft horse has been recognized as national intangible cultural heritage (VILT, 2018). The breed has deserved this recognition because of its rich history at world exhibitions, the crucial role it has played in agriculture and its importance as a Belgian export product at the beginning of the 20th century (Fig. 1). During its most glorious years (1880 – 1930) the breed counted about 278 000 animals in Belgium (Immaterieel erfgoed in Vlaanderen, 2022). Nevertheless, around 1930 the export numbers dropped drastically and following the Second World War, the arrival of the agricultural mechanization dealt the final blow to the success story of the Belgian draft horse. The post-war Marshall aid (a large-scale material support plan, aimed at the economic recovery after the war) allowed nearly all farmers to participate in the further mechanization of agriculture. From this period onwards, the number of Belgian draft horses used for breeding decreased annually by more than 10% and reached its lowest point in 1970 (Immaterieel erfgoed in Vlaanderen, 2022). Due to the loss of economic value, the breed almost disappeared. In agriculture, the Belgian draft horse had lost its functionality and was therefore reformed by enthusiasts into a hobby and recreational animal. From 1980 several associations made their appearance which were committed to the preservation and promotion of the Belgian draft horse. As a reliable, calm and majestic companion and recreational horse, the interest of the wider public gradually flourished again. Today's draft horse fulfils a role in pulling and driving competitions, old crafts such as tree dragging, traditional parades and processions, tourist events and even dressage competitions. Currently, the number of draft horses in Belgium is down to about 5000 horses with about 550 foals born annually (Trekpaard.net, 2022).

Figure 1. Albion d'Hor at the World Exhibition of 1923 in Milan.
Albion d'Hor, champion stallion at the 1923 World Exhibition in Milan and national Belgian champion in the same year. Albion is assumed to be the progenitor of all modern-day Belgian draft horses.
Adapted from 'Het trekpaard', by Peerlings, J., van der Weerden, T., van Hoof, W., 2008, p.38, Zutphen, Netherlands: Roodbont Uitgeverij

However, the declining population of horses has put the conservation of the breed at serious risk; the Belgian draft horse breed is in danger of extinction (KMBT, 2011). In addition to the significantly declining size of the Belgian draft horse population, a decrease in genetic variation and an increase of inbreeding rates has become the greatest threat to the future existence of this breed (Nagels, 2008; Janssens et al., 2021b). To quantify how a particular population will be affected by inbreeding, the calculation of effective population size is used. Effective population size is the size of an "ideal population" of horses (constant population size, equal sex ratio and no immigration, emigration, mutation, or selection) that would experience the effects of inbreeding to the same degree as the population under study. Populations with an effective population size (based on inbreeding rates) under 50 are at immediate risk of extinction (Franklin, 1980). This is because, in such small populations, inbreeding could quickly push the population into an extinction vortex. Janssens et al. (2021b) have calculated that currently the effective population size of the Belgian draft horse counts about 75 animals, based on the evolution of the average inbreeding rate. However, when the effective population size was calculated based on the increase in co-ancestry, it dropped to 48 animals. This vulnerable population of Belgian draft horses has been further compromised by an incurable disorder; **chronic progressive lymphedema (CPL)**. As the condition progressively aggravates, it often results in premature euthanasia, consequently markedly decreasing the average life expectancy in the Belgian draft horse (Ferraro, 2003; Affolter, 2013). De Keyser (2014) has established a prevalence of 86% in the Belgian draft horse population which demonstrates the impact of CPL on conservation of the breed.
CPL is characterised by progressive swelling of the distal limbs with associated skinfolds, hyperkeratosis, fibrosis, nodules and ulcerations on the distal limbs of the horse. Clinical signs of CPL can start to develop at an early age, progress over several years and often end in severe deformity of the distal limbs, leading to major discomfort for the horse. The etiology and pathogenesis of chronic progressive lymphedema have not yet been fully elucidated. CPL is being considered a multifactorial disorder with a genetic susceptibility (De Keyser et al., 2014a). A genetic trait is suspected since Belgian draft horses were incorporated as ancestors in draft horse breeds all over the world, and all these breeds appear now to be affected by CPL (De Keyser et al., 2014b; Mittmann et al., 2010). Moreover, CPL also seems to be more prevalent in certain bloodlines. However, additional selection against a genetic disorder will be

challenging without further limiting genetic variation in the population. Currently, CPL is incurable and supportive treatment is time consuming and is only aimed at improving the comfort of the horse's life.

Not only the Belgian draft horse is considered an affected breed. In fact, the condition has also been observed frequently in Shires and Clydesdales (Ferraro, 2001; De Cock et al., 2003), Gypsy cobs and Gypsy Vanners (De Keyser, 2014; Powell and Affolter, 2012), Friesians (Affolter et al., 2020), American Belgian (De Keyser et al., 2014b), German draft horse breeds (South German, Black Forest, Schleswig, Saxon-Thuringian, Rhenish-German, Mecklenburg) (Wallraf et al., 2004), Percheron (Affolter, 2012; De Cock et al., 2006a), Ardennes, Bréton and Boullonais (Duclos, 1972: in Wallraf, 2003; Affolter, 2013), Cheval Trait Auxois (personal communication with owners, 2021) and several cross breeds out of the previously mentioned breeds. The emerging prevalence in multiple breeds requires a practical management and therefore it is of great importance to acquire a better understanding of the etiology, pathogenesis and possible diagnostics of this disease.

2.2. TERMINOLOGY, DEFINITION AND PREVALENCE

The Belgian draft horse has been subjected to incurable abnormalities of the distal limbs for a very long time (De Keyser, 2014). Based on the chronic, progressive and incurable nature of the skin lesions on the distal limbs, the clinical presentation was called 'Chronic Pastern Dermatitis' (CPD) or 'Chronic Proliferative Pastern Dermatitis' (CPPD). Pastern dermatitis is not a single disease, but rather a cutaneous reaction pattern in the horse. A large number of possible causes of pastern dermatitis has been described. Identifying and treating the predisposing and perpetuating factors are just as important as addressing the primary underlying cause of equine pastern dermatitis. CPD is a type of equine pastern dermatitis, of which is assumed that the predisposing factor is primarily genetic and the perpetuating factors are mainly secondary infections. Only in 2003, the syndrome was redefined as 'Chronic Progressive Lymphedema (CPL), mainly because of its remarkably similar clinical and histological presentation with its human counterpart, non-filarial chronic lymphedema or elephantiasis nostras verrucosa (De Cock et al., 2003). Nowadays, both C(P)PD and CPL are commonly used in scientific literature, both referring to the same clinical presentation. The term CPL can be preferred above CPD, since associated clinical lesions are not confined to a dermatological problem only but are due to an underlying cause (De Keyser, 2014). For example, a reduced lymphatic clearance was demonstrated in the limbs of clinically affected draft horses, by means of lymphoscintigraphy (De Cock et al., 2006b). Therefore, the clinical CPL signs are assumed to occur secondary due to a malfunction of the lymphatic system.

However, chronic progressive lymphedema could also not be an accurate designation for this disorder, since the etiology and pathogenesis of this condition remain only speculative to date. Malfunction of the lymphatic system, progressive fibrosis of the tissues and a disruption of the elastin matrix in the skin all appear to be key components within this pathology, although further research is required to establish the exact chronology of these events within the pathogenesis of CPL. Lymphedema may well be a contributing factor rather than a causative one.

In this thesis however, the term CPL will be used since this designation is widespread and most commonly used in literature and practice for referring to this particular condition in draft horse breeds.

Nowadays, CPL is defined as a progressive swelling of the distal portions of the legs, which is associated with scaling, marked dermal fibrosis and the development of skinfolds and nodules (Fig. 2). Typically, secondary recurrent bacterial and parasitic infections complicate these lesions and contribute to the aggravation of the lymphedema. In CPL, a partial genetic susceptibility is suspected (De Keyser et al., 2014c; François, 2018). CPL may result in marked disfigurement of the distal extremities and, as such, often leads to lameness and premature euthanasia (Ferraro, 2003; Affolter, 2013).

Figure 2. Clinical signs of CPL on the distal legs of a Belgian draft horse.
Characterizing skinfolds and nodules are present on the distal legs, associated with scaling of the skin.

CPL has been described in several draft horse breeds. Therefore, these breeds are referred to as CPL susceptible breeds. The Belgian draft horse is one of the few susceptible breeds where research on the prevalence of CPL has been established (Table 1). However, the research of Verschooten et al. (2003) only included 2 and 3 year old stallions. For this reason, the established prevalence by De Keyser et al. (2014c) will probably be the most accurate representation of the true prevalence of CPL in the Belgian draft horse breed. In the latter study, a prevalence of 60.66% has been established based on the data of a total sample of 762 horses, including many young horses. Since CPL is a chronic and progressive condition, clinical signs are often absent in animals less than 3 years old (De Keyser et al., 2014c). When calculating CPL prevalence in horses over the age of 3 years, the prevalence increased to 85.86%. In addition, clinical signs were detected in only 14% of the 1-year-old horses. In addition, Wallraf (2003) has established the prevalence of pastern dermatitis in German draft horse breeds. Although in this study, various types of skin lesions were evaluated, in literature, these numbers are referred to as CPL prevalence in German draft horse breeds (De Keyser, 2014). Whereas no population wide studies were performed in the other susceptible breeds, disease prevalence is expected to be high (Boema et al., 2012; De Cock et al., 2003; Ferraro, 2003, 2001; Powell and Affolter, 2012).

Table 1. Prevalence of clinical CPL associated lesions in draft horses.

Breed	n	Age range (years)	Method of examination	Affected (%)	Reference
Belgian Draft (stallions)	161	2-3	Radiography analysis	82.0	Verschooten et al., 2003
Belgian Draft	762 456*	≥4 months ≥3	Clinical examination	60.66 85.86	De Keyser et al., 2014c
South German	455	≥2.5	Clinical examination	58.5	Wallraf, 2003
Black Forest	139	≥2.5	Clinical examination	47.5	Wallraf, 2003
Schleswig	100	≥2.5	Clinical examination	86.0	Wallraf, 2003
Saxon-Thuringian	83	≥2.5	Clinical examination	84.3	Wallraf, 2003
Rhenish-German	77	≥2.5	Clinical examination	96.1	Wallraf, 2003
Mecklenburg	55	≥2.5	Clinical examination	76.4	Wallraf, 2003

n = sample size, affected (%) = percentage of the sample with clinical lesions on at least 1 limb.
*same dataset but only horses ≥ 3 years were included (n = 456).
Adapted from "Clinical assessment, genetic parameters and the role of elastin in chronic progressive lymphedema in the Belgian draught horse" by K. De Keyser., 2014, (Inaugural dissertation). Faculty of Bioscience Engineering, Catholic University of Leuven, Belgium.

2.3. CPL ASSOCIATED LESIONS

2.3.1. Clinical signs

Primary lesions associated with CPL

The onset of clinical signs can start at any age, although it has been demonstrated that most animals develop clinical signs starting at the age of 2 years (De Cock et al., 2003). From the onset of the first clinical signs, no full recovery can currently be obtained and CPL lesions will worsen with time. The first signs of this disease typically manifest at the level of the horse's distal legs. In many cases the hind legs are more severely affected than the front legs (De Cock et al., 2003). In horses with CPL, the lymphatic drainage at the level of the distal legs is compromised, which will result in an increased lymphatic hydrostatic pressure in these tissues (De Keyser, 2014). The first signs of this increased pressure are visible as a swelling of the distal extremities (edema), possible hyperkeratosis and associated scaling of the skin. Characteristic of the early stage of this condition are the thickened skinfolds in the rear of the pastern region. Gradually, the clinical signs will worsen with increasing age, however, the speed of progression is largely dependent on additional influences such as hygiene, skin care, secondary infections and trauma (MacLaren, 2001). Lymphedema will have an effect on the dermis, subcutis and tissues in the distal legs, causing structural changes that can manifest in the form of fibrous nodules, thickened skinfolds and even deformation of the distal limbs (De Keyser, 2014). Initially, the thickened fibrous nodules and skinfolds are mainly located at the

distal palmar/plantar aspect of the limb (fetlock cavity) and will later on extend proximally to the carpus/tarsus. In more advanced stages, these clinical signs may also develop on the dorsal aspect of the limb. Finally, the dermal thickening can be so severe that it encloses the entire leg, typically at the level of the joints, resulting in a restriction of the horse's movement. This can cause a certain degree of malformation of the distal limbs and subsequently lameness.
The long hairs and dermal scaling in CPL are an ideal environment for *Chorioptes bovis* mites. Horses will experience severe itching, which manifests in stomping and even auto-mutilation. Mites, in turn, will damage the skin and create opportunities for other pathogens.

(Lymph)edema
As the name of the condition implies, disturbances in lymphatic drainage is considered a primary cause. Edema, also referred to as "pitting edema", may be present locally or diffusely on the distal limbs (Fig. 3). However, this is often not visible due to the heavy feathering (De Keyser, 2014). Edema is mainly characteristic of the early stage of CPL and will gradually progress to dermal fibrosis (De Keyser, 2014). It is therefore often only seen in young horses.

Figure 3. Edema in the distal limbs.
A. In the early stages of CPL, a localized onset of 'pitting' edema in the fetlock region (green arrow) can often be noticed. In this horse, slight folding of the skin of the plantar aspect of the cannon bone is also already visible. Insert, contralateral leg with absence of edema in the pastern region. **B.** Generalized edema of the distal right forelimb. Notice skin fissures on the carpus.

Skinfolds
One of the most characteristic signs of CPL are skinfolds on the distal legs (Fig. 4). Typically, in the early stages, thickening will occur in the pastern region and, in more advanced stages, these thickenings will progress to the palmar and plantar aspect of the fetlock and the cannon bone. In the most severe and final stages of CPL, fold-like thickenings will also appear dorsally, circularly enclosing the entire leg. At the joints, the dermal lesions can limit the horse's movement and even cause lameness due to the forced inappropriate alignment of the articular bones. In addition, a warm and moist environment will arise between these skinfolds and mechanical friction can damage the skin causing dermatitis (intertrigo) (Nobles and Miller, 2021).

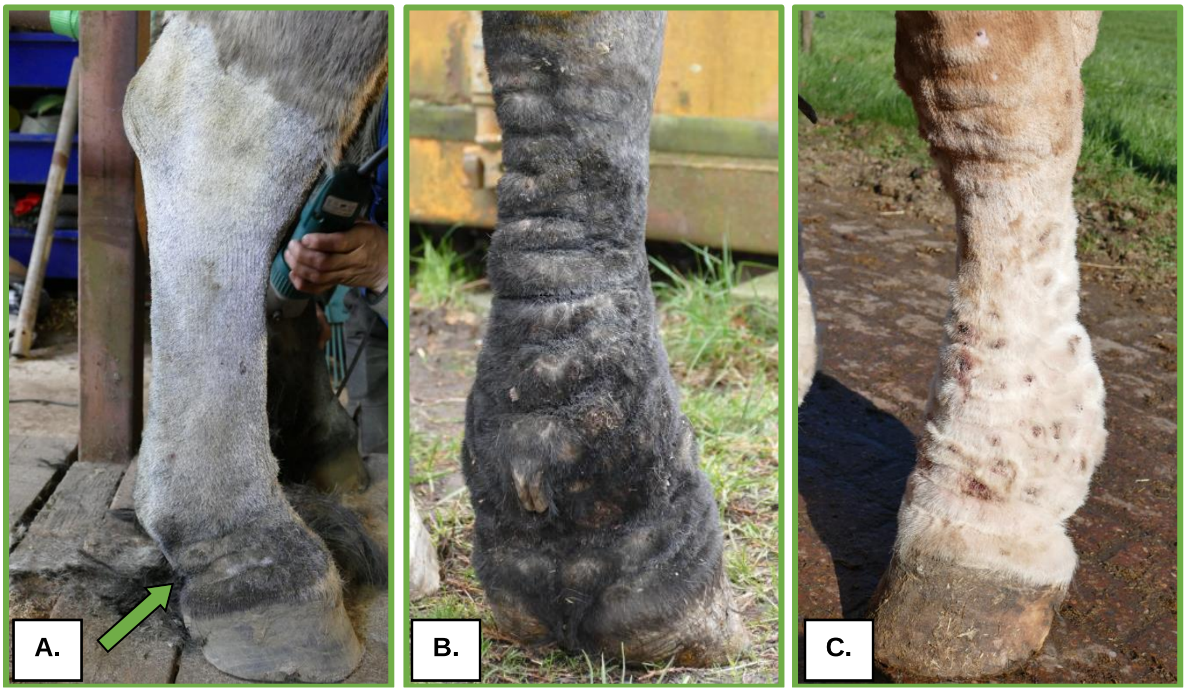

Figure 4. Skinfolds, the main feature of chronic progressive lymphedema in the Belgian draft horse.
A. Early stage lesion of CPL: one or two folds in the pastern region (green arrow). **B.** Advanced stage of CPL: expansion of the folds to the palmar aspect of the cannon bone. **C.** Final stage of CPL: skinfolds appearing dorsally.

Generalized skin lesions

In some cases, the elastin-rich skin of the neck can be clinically affected and in even more rare cases the entire body will display generalized skinfolds (Fig. 5 and 6). Since a lower quantity of dermal elastin in both the neck and limbs was demonstrated in horses of a susceptible breed in comparison to a non-susceptible breed and in addition, the absence of lesions in other organs (including the elastin-rich aorta), it was hypothesized that CPL is a generalized skin disorder (De Cock et al., 2003, 2006a, 2009). In older stallions, skinfolds in the neck region appear to be a frequently seen phenomenon during studbook inspections (personal communication with Ton van der Weerden, 2022).

Figure 5. Generalized signs of CPL.
A Belgian draft horse, exhibiting generalized skinfolds.
Reprinted from "Chronic progressive lymphoedema in draught horses" by K. De Keyser et al. 2014, Equine Veterinary Journal 47(3), 260–266.

Figure 6. Skinfolds in the neck of a Belgian draft horse.
A 6 years old Belgian draft horse stallion, showing clear folding of the skin in the neck region. The manes are partially clipped in order to be able to braid them more easily during studbook inspections.
(Courtesy of T. van der Weerden, 2021)

Verrucous pastern dermatitis (dermatitis verrucosa) or wart-like nodules
Dermatitis verrucosa is a chronic hyperplastic form of pastern dermatitis, characterized by hairless, wart-like elevations and sclerosis of the skin and hypodermis on the distal limbs, mainly affecting draft horses (Fig. 7) (Geburek et al., 2005a). This clinical presentation of pastern dermatitis is often referred to as a clinical sign of CPL (or CPD) based on the similarities with the classic signs of CPL: the predilection for cold-blooded horses with abundant feathering on the legs and the progressive nature of the lesions (Geburek et al., 2005a; Poore et al., 2011; Yu, 2013). However, some notable differences can be observed: the wart-like lesions are not always present in horses with CPL (unlike the skinfolds) and if present often do not affect all four limbs (Johnsen, 2021a). It remains unclear to this day whether verrucous dermatitis is a separate clinical identity in draft horses or an atypical clinical presentation of CPL.

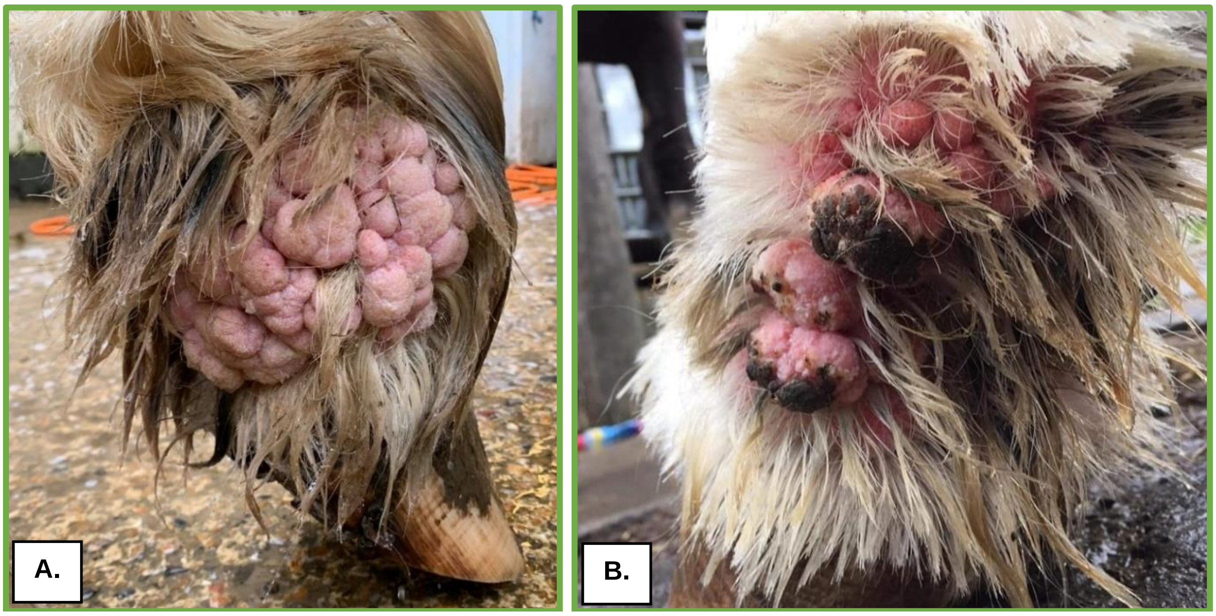

Figure 7. Verrucous pastern dermatitis.
A. Wart-like nodules in the pastern region of a Gypsy Cob *(Courtesy of B. Shaw, 2021).* **B.** The skin in these areas is very sensitive to trauma, resulting in a high risk of infection and necrosis *(Courtesy of F. May, 2021)*.

Scaling and skin fissures

At the dorsal aspect of the hock and the palmar aspect of the carpus, where often scaly scabs are present, the mechanical forces will cause tearing of the skin (Fig. 8). CPL is characterized by general hyperkeratosis of the skin and a high quantity of epidermal keratin, causing the skin to become dry, scaly and prone to mechanical damage (Affolter, 2013; De Keyser et al., 2014a; De Cock et al., 2003; Moncrieff et al., 2013). The scaly tears are often referred to as 'mallenders' (palmar aspect of the carpus) and 'sallenders' (dorsal aspect of the hock) (Johnsen, 2021b). Without proper management, these scaly scabs will become ulcerated and painful, and pose a high risk for secondary bacterial infections.

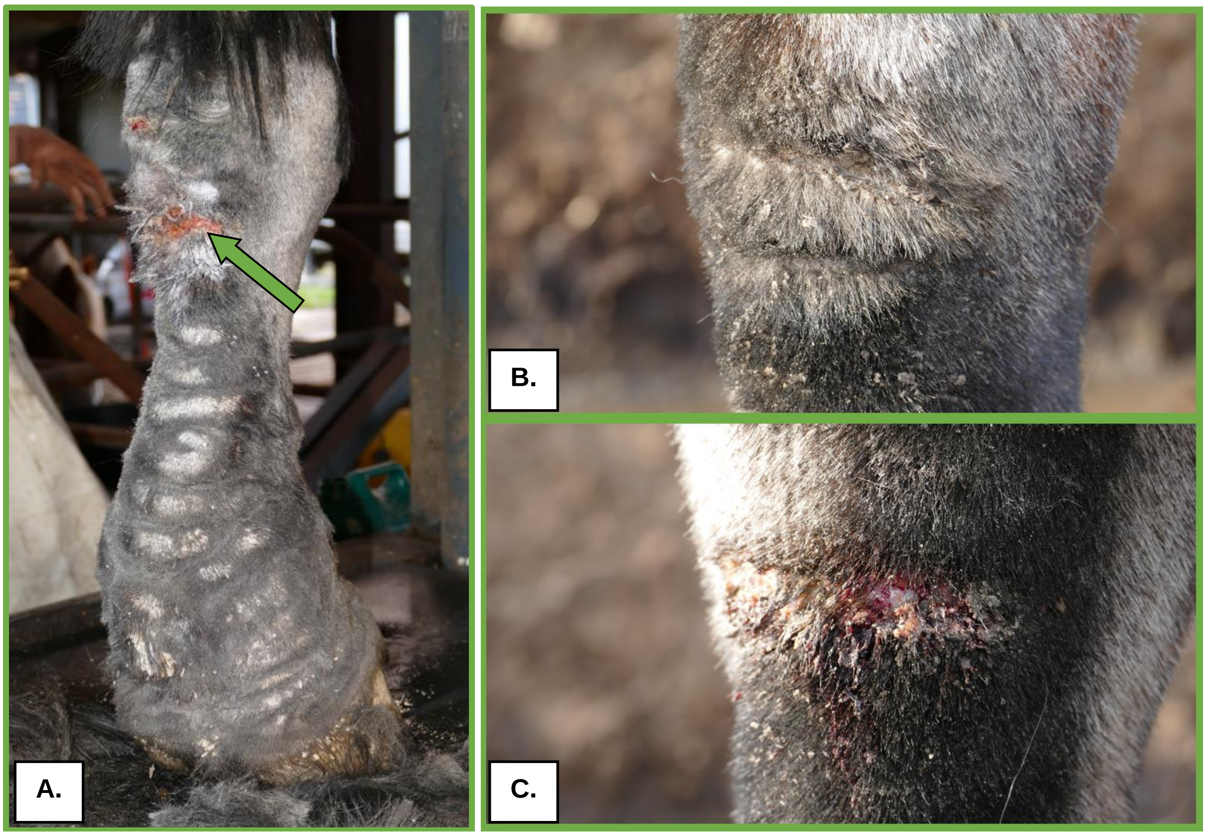

Figure 8. Skin fissures on the carpus.
A. Typical skin fissure on the palmar aspect of the carpus (green arrow), also referred to as 'mallenders'. **B.** A non-infected skin fissure with clear signs of scaling and hyperkeratosis on the carpus. **C.** If not properly treated, the skin will continue to rupture, creating a painful wound that is susceptible to secondary bacterial infections.

Poor hoof horn quality and prominence of ergots and chestnuts

Hoof quality is significantly correlated to severity of CPL lesions in draft horses (Geburek et al., 2005b), although infrequent and poor grooming does not influence the occurrence (Wallraf, 2003). In addition, the prominence of chestnuts and ergots has been shown to be significantly correlated to the severity of CPL (Fig. 9). However, it is not clear if these features are consequences of CPL rather than they are a risk factor for disease severity (De Keyser, 2014). The clinical presentation of the poor hoof horn quality is remarkably similar to that of coronary band dystrophy, which already has been described in draft horses (Menzies-Gow et al., 2002; Twitchell et al., 2014). Stannard (2000) even states that this condition is most commonly seen in draft horse breeds. Hoof Canker is a second clinical condition affecting the hoof, ergots and chestnuts, that has been frequently described in draft horses and is considered to be prevalent in these breeds as well (Nagamine et al., 2005; Redding and O'Grady, 2012; Fürst, 2012; Brandt et al., 2011). However, to date it is not known whether both conditions are related to the occurrence of CPL and whether the poor hoof quality, as often seen in horses affected by CPL, is a consequence of the presence of these two conditions or whether it has a separate etiology.

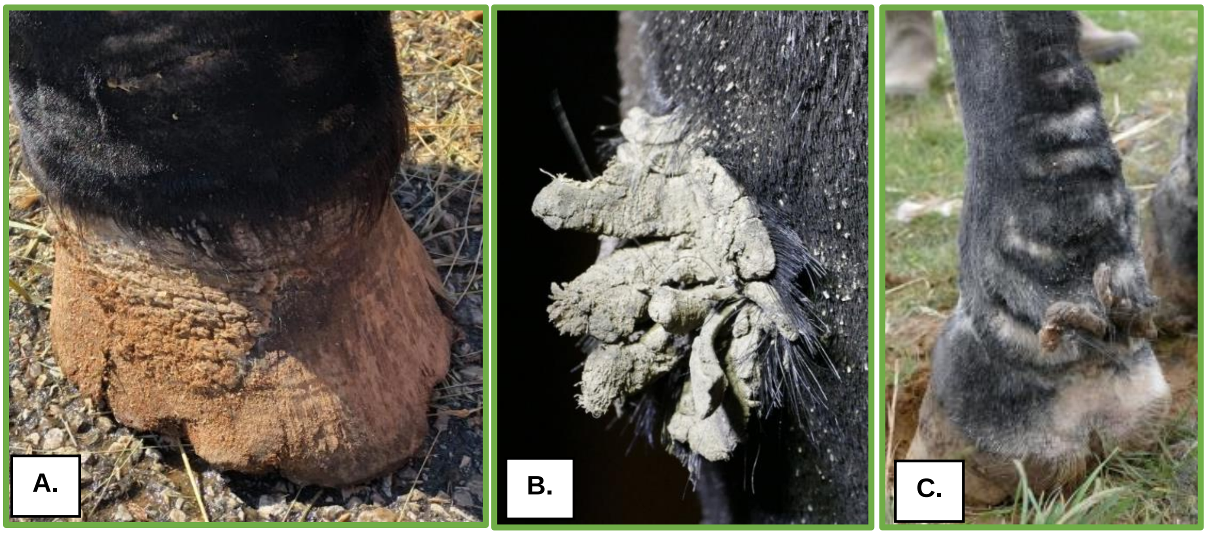

Figure 9. Poor hoof horn quality and prominent chestnuts and ergots in draft horses.
A. The hoof wall appears to be poorly keratinised, with proliferation, scaling and rings of hoof growth *(Courtesy of D. Conder Emge, 2021*). **B.** Hyperplastic and hyperkeratotic chestnut and **C.** ergot in Belgian draft horses.

<u>Secondary lesions and clinical signs associated with CPL</u>
Secondary conditions often develop as a consequence of the primary lesions of CPL. In between the skinfolds dirt and moisture will accumulate, creating opportunities for secondary bacterial or fungal infections. Secondary bacterial infections of the skin will produce foul-smelling exudate and ulcers and this will in turn attract flies, resulting in an infestation with maggots. In addition, hyperkeratotic skin provides an ideal environment for mites. Lymphatic dysfunction will also result in a lowered skin immunity, which facilitates bacterial invasion and infection (Yuan et al., 2019; Affolter, 2013).

Cellulitis and lymphangitis
In draft horses with CPL, an impaired skin barrier and skin lesions predispose a port of entry for bacteria, which might lead to an acute onset of cellulitis or lymphangitis. Cellulitis is defined as a diffuse bacterial infection of the dermis and subcutaneous tissues (Adam and Southwood, 2007), while in lymphangitis the bacteria infect the lymphatic vessels in the limb (Van Vleet and Ferrans, 2001). Lymphangitis may accompany cellulitis and vice versa (Oomen et al., 2013). In both conditions, the limb will be warm, painful and will have a ‘stovepipe’ appearance as a result of the significant swelling (Fig. 10). Since the clinical presentation of lymphangitis is almost identical to cellulitis, it may be difficult to distinguish with certainty a case of cellulitis from a case of lymphangitis based on clinical signs only. As a result, both terms are often used interchangeably in practice (Braid and Ireland, 2021).

Figure 10. Clinical appearance of cellulitis/lymphangitis in a Belgian draft horse with CPL.
A leg affected by lymphangitis may have the same 'stovepipe' appearance as cellulitis, especially when there is a diffuse swelling of the entire limb.
(Courtesy of A. Callens, 2022).

Intertrigo

In animals with advanced stages of CPL, the appositional skin of large folds is subject to the friction of movement, increased local heat, maceration from retained moisture and irritation from accumulation of debris, resulting in a superficial inflammatory dermatosis or intertrigo (Nobles and Miller, 2021; Scott and Miller, 2010). When these factors are present to a sufficient degree, dissolution of stratum corneum, exudation, and secondary bacterial infection are inevitable. Lesions include oozing, crusting, secondary bacterial and/or yeast infections, and in severe cases, necrosis (Fig. 11). A foul odor may be present. The horse may react painfully when being touched in this area. In addition to the risk for bacterial and/or fungal overgrowth, these lesions also form an ideal niche for maggot infestation.

Figure 11. Intertrigo or skinfold dermatitis in Gipsy Cobs with CPL.
The skin in the fold is ulcerated, hyperemic and covered with a serous to suppurative exudate.
(Courtesy of M. Creel, 2021 and G. Anderson, 2021)

Bacterial infections
Secondary bacterial infections occur frequently in horses affected by CPL as the skin is more sensitive to trauma, causing easy development of skin lesions. In addition, the skinfolds provide a warm, moist environment for the growth of bacteria and fungi and the feathering on the legs can facilitate moisture retention. Also, an impaired skin immunity in horses with CPL will increase their susceptibility to infections (Affolter, 2013). In horses with proliferative pastern dermatitis (CPL), *Staphylococcus aureus* is the most prevalent bacterial species isolated from skin samples (Affolter, 2013; Kaiser-Thom et al., 2021). *Malassezia pachydermatis* and *Dermatophilus congolensis* are also frequently involved in skin infections of the pastern region. However, a mixed bacterial infection is often involved in pastern dermatitis. Affected skin is irritated, red, inflamed and/or swollen, and will develop a discharge that appears 'greasy' or sticky (Fig. 12).

Figure 12. Bacterial infection of the skin in two Gypsy Cobs.
An inflamed, red and irritated skin with typical exudate as seen with a bacterial infection of the skin.
(Courtesy of S. Pabst, 2021 and B. Young, 2021)

Parasitic infestations
In draft horses, a remarkably high prevalence of mites has been described in several studies, ranging from 50% up to 95% (Cremers, 1985; Geburek et al., 2005a,b; De Cock et al., 2003; Rüfenacht et al., 2010). These mites, identified as *Chorioptes bovis*, cause pruritus, scaling and crusting of the distal limbs of horses (Fig. 13). Draft horses are especially predisposed to this condition (Moriello et al., 1998). The heavy feathering of the pasterns provides an ideal environment for mite multiplication (Paterson and Coumbe, 2009). The feathering traps skin scales and epidermal debris which provides a rich and necessary food supply for mites. It also protects them against temperature extremes. As a result of the mite infestation, the horse will be restless, frequently stamping its legs and eventually demonstrate auto-mutilation. Clinical signs are usually more severe during the winter as mite activity and egg production are both increased at lower temperatures (Wall and Shearer, 1997).
An additional problem that is regularly seen in severely affected draft horses, especially during the summer, is an infestation with maggots (Fig. 14). Flies (e.g., *Lucilia* sericata) are attracted to the warm, moist environment between the prominent skinfolds after which fly larvae will develop and feed on dead tissue and wound debris.

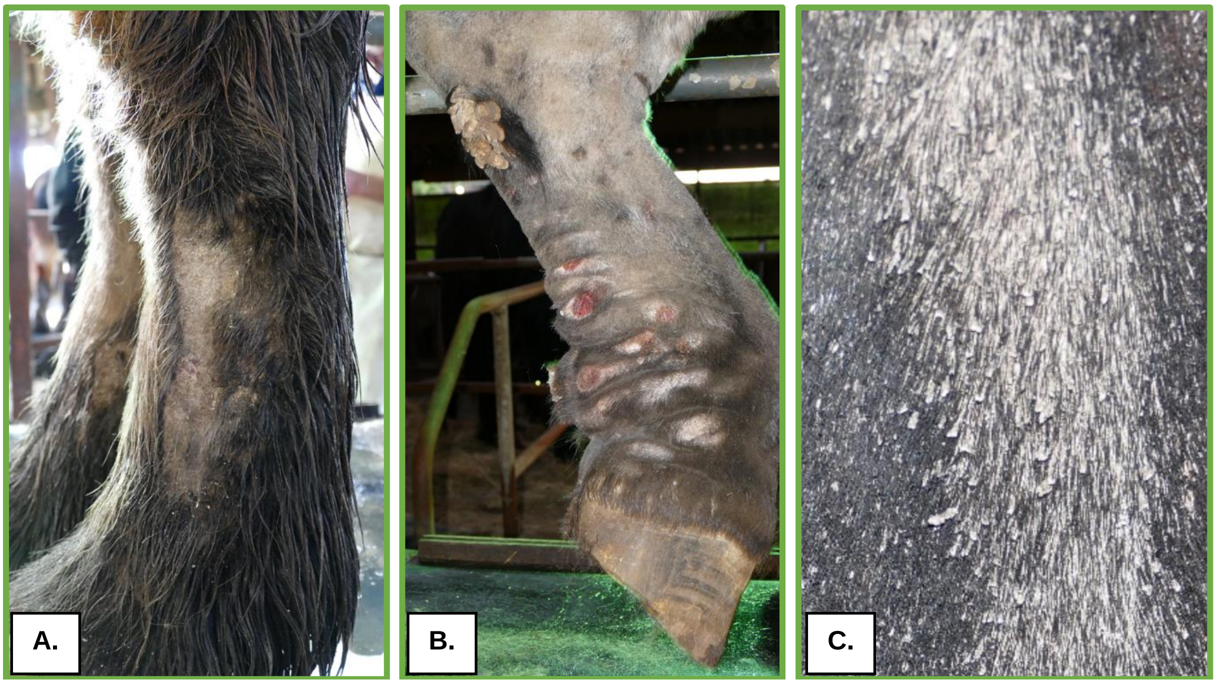

Figure 13. Clinical signs of mange.
A. Alopecia is a characteristic feature of a mite infestation, often visible on the lateral or medial side of the cannon bone. **B.** Because of the severe itching, the horse will rub the inside of its legs against each other, resulting in wounds on the medial side of both the front and hind limbs. **C.** An extremely scaly patch of skin as a result of a severe mite infestation. Note the typical white debris surrounding the hair shafts.

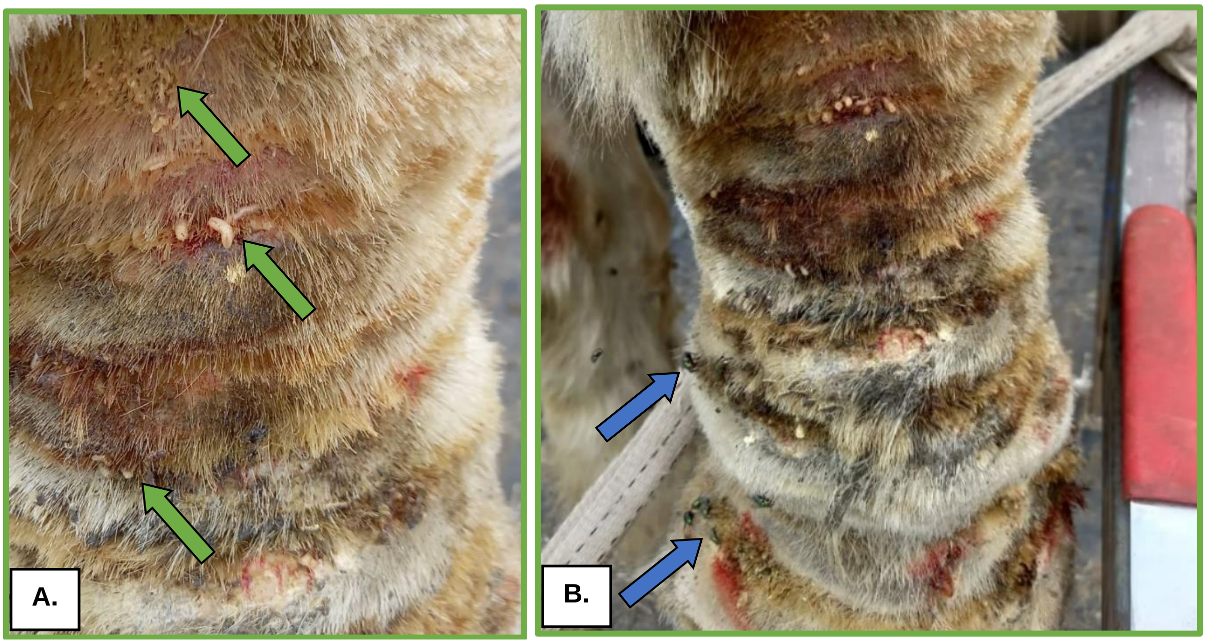

Figure 14. Numerous maggots invading the skin of a Belgian draft horse with severe CPL.
A. Fly larvae or maggots (green arrows) will invade the damaged skin of CPL-affected horses. **B.** Especially during summer, flies (blue arrows) will deposit their eggs on or near a wounds or ulcers.
(Courtesy of E. Wuijts, 2021)

2.3.2. Histopathology

A limited number of studies have been conducted examining the histological changes in CPL. Lesions were found in the vasculature, dermis and epidermis.

Lymphatic vessels

The most significant histological changes were present in the lymphatics vessels of the skin. In mildly affected horses, the lymphatics were mildly to moderately dilated. As clinical signs aggravate, the vessels became more severely affected and appeared clearly dilated and torturous. This was usually accompanied by subendothelial accumulation of basophilic mucinous matrix and perilymphatic fibrosis (De Cock et al., 2003). The degree of fibrosis varied from thin, indistinct layers to thick, prominent layers of collagen bundles arranged circumferentially around the lymphatics (De Cock et al., 2003).

Arteries and veins

Focal subendothelial accumulations of basophilic fibrous matrix associated with the disruption of the lamina elastica interna were often present in arteries (De Cock et al., 2003). Proliferation of the middle and deep vascular plexi of the skin, with smooth muscle hyperplasia, was a characteristic feature. In advanced stages a prominent proliferation of the superficial vascular plexus was observed. In addition, vasa vasorum of the arterioles were prominent and increased in number (De Cock, 2003).

Epidermis and dermis

A main characteristic of CPL is hyperplasia of the epidermis with formation of irregular rete ridges, hyperkeratosis and vacuolar degeneration of keratinocytes (Fig. 15) (De Cock et al., 2003; Geburek, 2002). The epidermis is often ulcerated and covered with a serocellular crust. A typical perivascular dermatitis predominated by lymphocytes and eosinophils is often present (Fig. 15) (De Cock et al., 2003). In addition, variable numbers of lymphocytes in the deep dermis, occasionally forming lymphoid aggregates, were observed (De Cock et al., 2003).

Elastin network

In severely affected limbs, the elastic layer of the subepidermal dermis and elastic fibers surrounding the lymphatics of the deep dermis were markedly distorted and separated by fibrous tissue. In parallel with the clinical CPL progression, the dermal elastin quantity significantly increased. However, the elastin seemed to be assembled in a disorganized and non-functional form (De Cock et al., 2009). In contrast, the perilymphatic elastin was histologically sparse in severely affected horses (De Cock et al., 2003). These lymphatics lacked the concentric ring of elastin fibers (Fig. 16) (Affolter, 2013). Clinically normal draft horses of a susceptible breed tended to show lower elastin concentrations (based on desmosine concentration in the skin) in comparison with horses of a non-susceptible breed. However, this was statistically significant only for the mid-dermis of the neck region (De Cock et al., 2009).

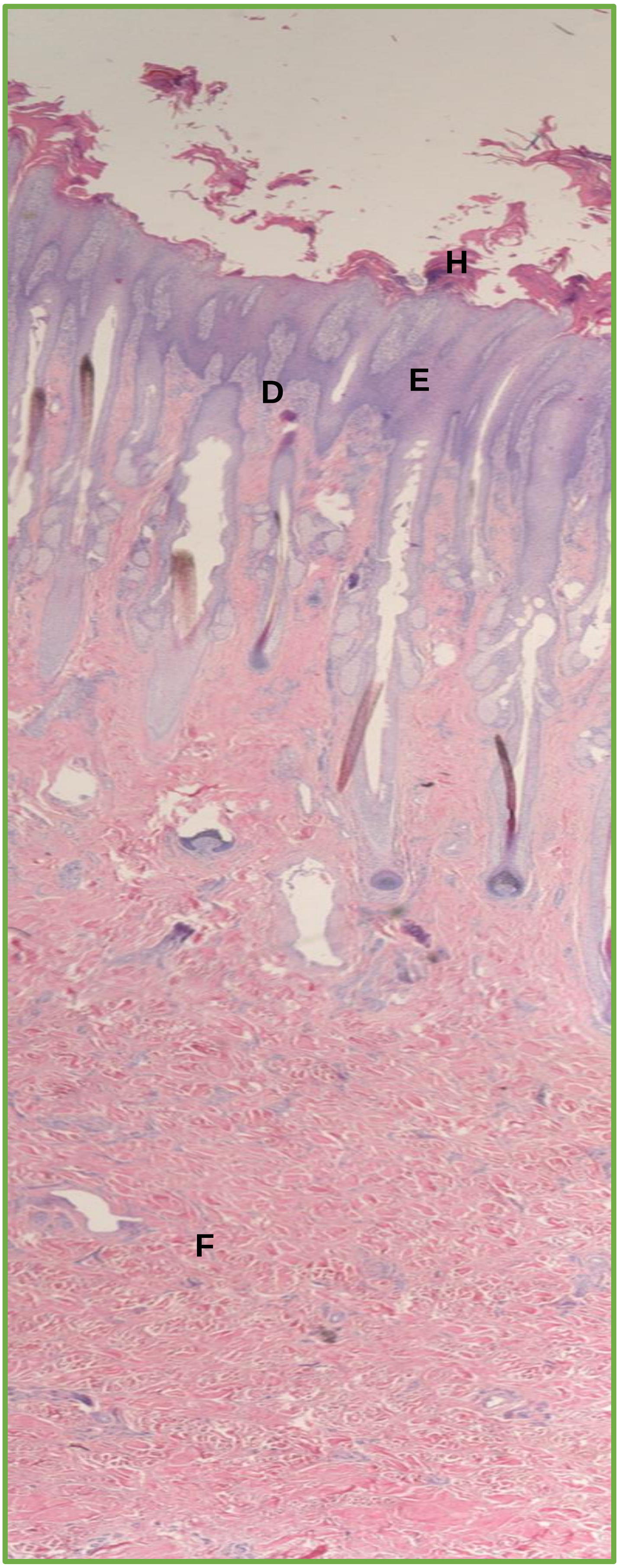

Figure 15. Histology of the skin from the pastern region of a CPL-affected Belgian draft horse. H&E staining. The skin of a CPL affected draft horse is characterized by thick layers of hyperkeratosis (**H**), hyperplasia of the epidermis (**E**), and perivascular dermatitis predominated by lymphocytes (**D**). In the dermis variable degrees of fibrosis are present (**F**).

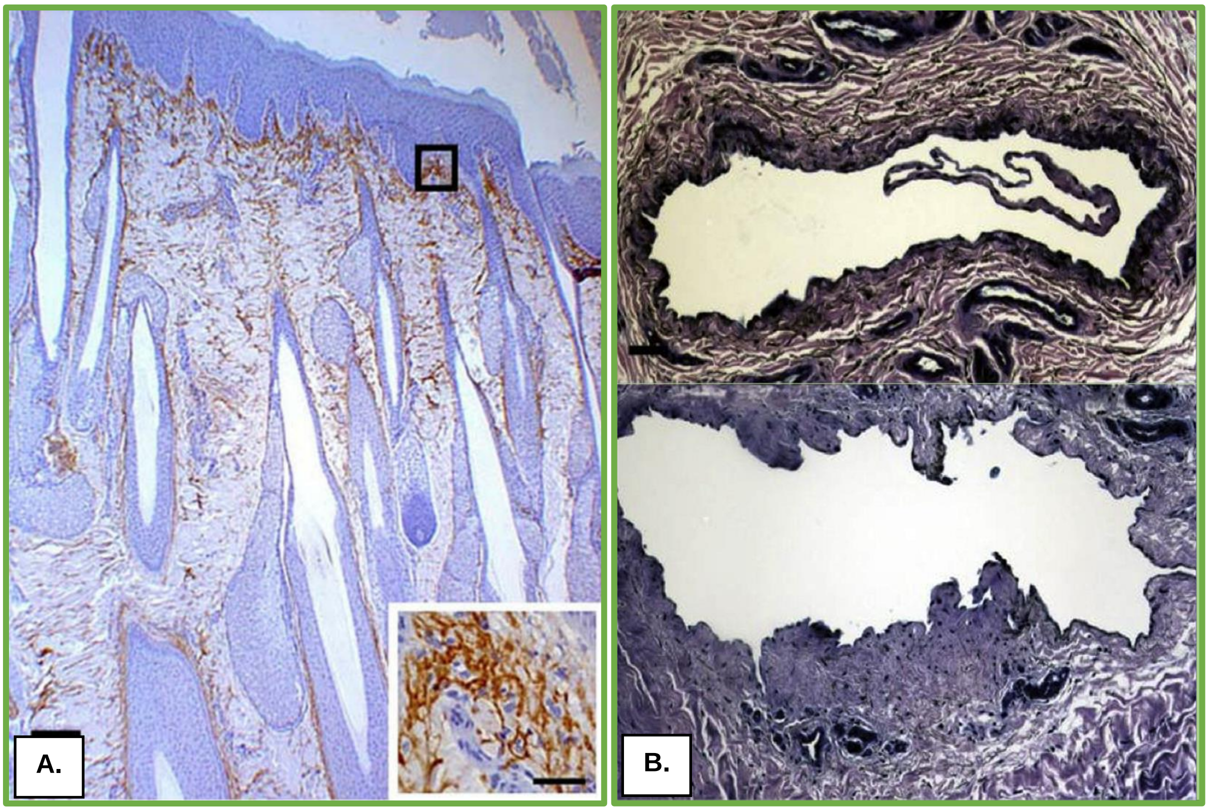

Figure 16. Alterations in the elastin network of affected draft horses.
A. Immunolabelling of dermal elastin in the distal limb of a severely affected draft horse. Marked accumulation of elastin is present, seen as a broad subepidermal band of clumped and densely labelled material (see inset). IHC. **B.** Lymph vessels of the deep soft tissues in a normal (top) and an affected (bottom) draft horse. Notice the dense band of elastic fibers (black) supporting the normal lymphatics (top) compared with the thin disorganized elastic fibers surrounding the affected lymphatic (bottom). Acid orcein-Giemsa.
Reprinted from "Progressive Swelling, Hyperkeratosis, and Fibrosis of Distal Limbs in Clydesdales, Shires, and Belgian Draft Horses, Suggestive of Primary Lymphedema" by H. De Cock et al., 2003, Lymphatic Research and Biology, 1(3), 191–199, and "Quantitative and qualitative evaluation of dermal elastin of draught horses with chronic progressive lymphoedema" by H. De Cock et al., 2009, J. Comp. Pathol. 140, 132–139.

2.4. PATHOGENESIS: TWO DIVERGING HYPOTHESES

Despite numerous attempts to unravel the etiology and genetic origin of CPL, the causative factor and subsequent pathogenesis of this disease remain unknown (De Cock et al., 2003, 2006a; De Cock et al., 2009; De Keyser et al., 2014a,c; Geburek et al., 2005b; Mittmann et al., 2010; Mömke and Distl, 2007; Van Brantegem et al., 2007b; Young et al., 2007; François, 2018). However, based on the observations made in various studies, two diverging hypotheses have been proposed.

2.4.1. Failure of the lymphatic system

Quantitative and qualitative dermal and perilymphatic elastin abnormalities have been associated with CPL in draft horses (De Cock et al., 2003, 2006a). In addition, the presence of anti-elastine antibodies in affected horses also indicated a pathological degradation of elastic fibers and tissue damage, resulting in a failing elastic skin network (Van Brantegem et al., 2007b). These lower quantities of dermal elastin and dysfunction of the elastin matrix might lead to an improper lymphatic elastic support, causing a reduced lymphatic drainage (De Cock et al., 2003, 2006a; De Cock et al., 2009). The elastin network fulfils an important role in facilitating lymph clearance since elastic fibres interconnect lymph vessels and several dermal structures, forming a 'path of low resistance' and promoting the uptake of fluid and molecules

into the initial lymphatics (Meyer et al., 1994). In clinically affected draft horses, a delayed lymphatic clearance has been demonstrated and the extent of this delay was found to be correlated with the severity of the clinical signs (De Cock et al., 2006b). The final stage of lymphedema is characterized by a complete lymph stasis as a consequence of intrinsic lymphatic dysfunction, with severe vessel dilatation and tortuosity, and tissue fibrosis (De Cock et al., 2003). Therefore, CPL in draft horses is considered to be a primary type of lymphedema with an underlying genetic trait. Moreover, no secondary lymphedema causes (e.g., lymphatic obstruction, filariasis, traumatic injury) have yet been demonstrated in affected breeds (De Cock et al., 2003, 2006a, 2009; De Keyser, 2014). Lymphedema can promote extracellular matrix deposition and fibrosis of the tissues, as seen in affected draft horses (Rockson, 2013; De Cock et al., 2003). In this hypothesis, secondary infections occurring in affected horses are a consequence of the impaired circulation of lymph fluid and lymph drainage which results in an impaired skin barrier function, a compromised function of the skin immune system and accumulation of peripheral tissue antigens (Grada and Phillips, 2017; François, 2018). Based on these observations, a first hypothesis was formulated that considers a failure in the lymphatic elastic system as the initiating factor of this disease.
Under this hypothesis, genetic research has been established on the evaluation of potential candidate genes, such as *Forkhead box protein C2* (FOXC2), associated with primary lymphedema (lymphedema-distichiasis syndrome) in humans and the *elastin* gene (ELN), but no aberrations have yet been found (Young et al., 2007; De Keyser., 2014).

2.4.2. Inflammatory dermatitis

Other studies suggest that CPL might start as an inflammatory dermatitis, with edema and lesions developing from scaling into hyperkeratotic and hyperplastic lesions. In this case, the edema in the distal legs of the affected horses is considered to be a consequence of bacterial infection, mange infestation, vasculitis, contact with chemical irritants, or any other agent (Geburek et al., 2005a; Mittmann, 2009; Mömke and Distl, 2007). These initial causes can trigger an inflammatory response which has been reported to drive lymphangiogenesis, resulting in a dysfunctional formation of lymph vessels and causing inflammation-induced contractile dysfunction (Liao and von der Weid, 2014). This in turn can cause a decreased drainage of lymph fluid, lipids and immune cells, resulting in a persistent inflammation that hampers skin regeneration and thus triggers an inflammatory (auto)immune response (Mittmann et al., 2010). In addition, the secretion of elastase from neutrophils is a known process in a variety of chronic inflammatory diseases, again resulting in pathological degradation of elastin in the skin (Mittmann et al., 2010). In addition, eosinophils also have the capability of producing or stimulating a variety of cytokines (e.g., IL-4, IL-5) which in turn will stimulate growth factors such as vascular endothelial growth factor (VEGF) and enzymes such as elastase (Horiuchi and Weller, 1997). From this perspective, a second hypothesis was formulated that considers CPL as an overregulated inflammatory (auto)immune response (De Cock et al., 2003, 2006a; De Cock et al., 2009; Geburek et al., 2005b; Mittmann, 2009; Mittmann et al., 2010; Mömke and Distl, 2007; Wallraf, 2003; François, 2018). Mittmann et al. (2010) identified several candidate genes under the hypothesis of an overregulated inflammatory autoimmune response, two of which were confirmed to lie within regions associated with CPL (*ubiquitin protein ligase* E3A and CD109 on ECA1 and ECA10 respectively) (François, 2018). In addition, François (2018) was able to identify several processes involved in the immune response, as well as the overrepresentation of several general cellular processes in CPL affected draft horses. However, it was not possible to identify specific biological processes (François, 2018).

2.4.3. An indistinct timeline and a vicious cycle

A dysfunction of the lymphatic system, fibrosis, inflammation, and an aberrant elastin network are the four factors that appear to be associated with CPL and provide the basis for established hypotheses on the pathogenesis. However, the timeline of these events is not unambiguous. For example, lymphedema can cause inflammation and fibrosis, but on the contrary, chronic inflammation can also cause a malfunctioning lymphatic system. As such, a vicious circle can be created whereby these factors might permanently reinforce each other (Fig. 17). Because of this, no initiating or triggering factor for this condition has been identified to date. Consequently, several diverging hypotheses have been formulated regarding the cause of this disorder. However, it is possible that current hypotheses are not independent of each other, but each may explain part of the pathogenesis.

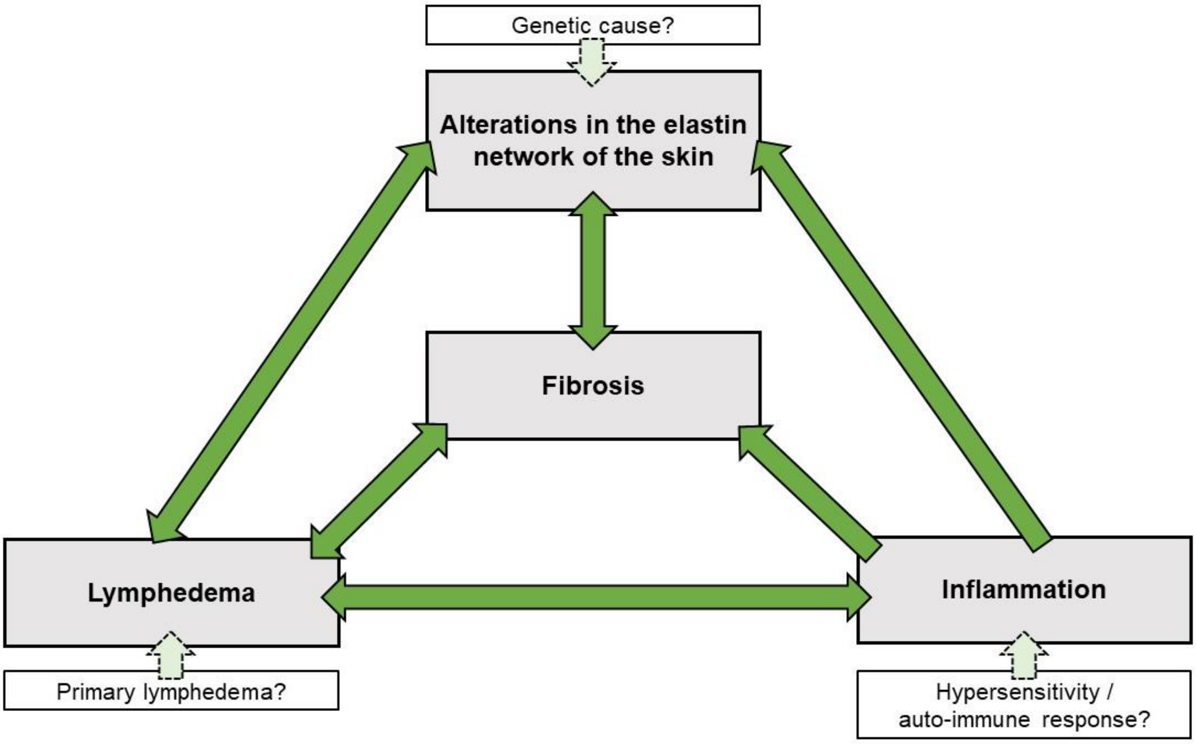

Figure 17. the vicious cycle of CPL.
All four factors associated with CPL are interconnected and will reinforce each other, with the initiating or causative factor in this cycle still remaining speculative.

2.5. FACTORS ASSOCIATED WITH OCCURRENCE AND SEVERITY OF CPL

CPL is assumed to be a multifactorial disorder (Wallraf, 2003; De Keyser et al., 2010; François, 2018). This implicates that variation in both disease susceptibility and development of clinical signs are determined by an interplay of two factors: the genetic variance of an individual (genotype) and environmental influences. The genotype-environmental interaction is defined as a different effect of a certain genotype on disease risk in animals with different environmental exposures (De Keyser, 2014). Environmental risk factors for CPL occurrence were established in a limited number of studies (Ferraro, 2001; Wallraf, 2003; De Keyser et al., 2010, 2014; Geburek, 2005b) and an interaction effect of genotype and environment for CPL susceptibility has been suggested (De Keyser, 2014).

2.5.1. Age

Aging is significantly correlated to disease severity (Geburek et al., 2005b; De Keyser, 2014). CPL is a progressive disease and therefore clinical signs will deteriorate with increasing age. De Keyser et al. (2014c) illustrated that only 14% of the Belgian draft horse yearlings showed mild clinical signs, while 85.86% was affected in a subset of horses older than 3 years. Severity of clinical signs, especially skinfold thickness, increases for both mares and stallions from the age of 3 onwards (Fig. 18) (De Keyser, 2014).

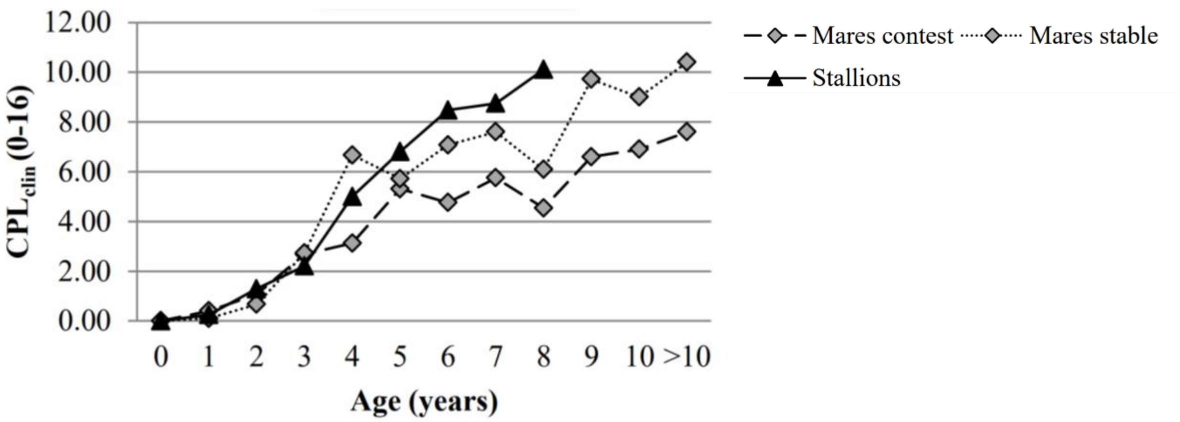

Figure 18. Clinical CPL severity/horse (CPLclin) in Belgian draft horse mares and stallions per age and location.
Reprinted from "Clinical assessment, genetic parameters and the role of elastin in chronic progressive lymphedema in the Belgian draught horse" by K. De Keyser, 2014, (Inaugural dissertation), Faculty of Bioscience Engineering, Catholic University of Leuven, Belgium.

2.5.2. Secondary infections

CPL affected draft horses are more prone to secondary infections (bacterial, fungal or parasitic) as the local skin immunity is impaired (Knottenbelt, 2009). It is therefore not surprising that the different causes (e.g. bacterial and/or fungal infection or parasites) of pastern dermatitis are also associated with secondary dermal infections in CPL. Vice versa, those secondary infections might aggravate CPL progression and even complicate CPL diagnosis (De Cock et al., 2003; Walraff, 2003; Geburek et al., 2005a). A secondary infection that is frequently diagnosed in CPL affected draft horses is chorioptic mange (Geburek et al., 2005a). *Chorioptes bovis* infestation may affect the progression of CPL in draft horses, manifesting with edema, lichenification and excessive skinfolds that can progress to verruciform lesions (Rüfenacht et al., 2010). Tackling of these secondary infections can thus not be neglected in the supportive treatment in CPL affected horses (Powell and Affolter, 2012).

2.5.3. Gender

Sex is significantly associated with the occurrence of lesions, since disease prevalence in stallions was significantly higher than in mares (Wallraf, 2003), although sex was not correlated with disease severity in one study (Geburek et al., 2005b). De Cock et al. (2003) and Verschooten et al. (2003) however stated that in general stallions are more severely affected than mares. Lesions appear sooner in mares compared to stallions, although the evolution of lesions will proceed more rapidly in stallions (De Keyser, 2014).

2.5.4. White markings

The presence of skin without pigment (white markings) has been correlated with clinical severity of CPL lesions in one study (Ferraro, 2001). Nevertheless, Geburek et al. (2005b) failed to confirm this correlation. A study by Federici et al. (2015) has demonstrated that horses with more pronounced white markings have an increased risk to suffer from pastern dermatitis, sunburns and hoof horn abnormalities. However, chronic progressive lymphedema includes only one clinical identity within the overarching concept of pastern dermatitis and a specific correlation between CPL and white markings has not been demonstrated.

2.5.5. Housing

Horses kept in outside pens on rubber flooring are significantly less severely affected in comparison to those kept on sand or soil. Poor stable hygiene significantly increases CPL severity (Geburek et al., 2005b). This could be explained mainly by the link between poor hygiene and the prevalence of pathogens which can cause secondary infections (Yu, 2013).

2.5.6. Intended use of the horse

In a study by Wallraf (2003), in horses kept for breeding and the production of meat and/or milk, lesions were significantly more prevalent than in horses used for riding and working (traditional agriculture). Although, in subsequent studies, the usage for breeding and the quantity of labor were not significantly correlated with the severity of the disease (Geburek et al., 2005b).

2.5.7. Diet

Feeding regime (silage) in winter was shown to increase CPL prevalence in German draft horses (Wallraf, 2003). De Keyser (2014) states that stallions are frequently given an energy-rich diet in preparation for the annual stallion selections. This might increase the clinical disease severity in stallions in comparison with mares as demonstrated by De Cock et al. (2003) and Verschooten et al. (2003). However, this difference in clinical severity might be attributed to the gender and hormonal differences. Further research into the influence of nutritional parameters is required to determine the exact effect of diet on the course of CPL.

2.5.8. Cannon bone, feathering, ergots, chestnuts, bulges and hoof quality

Cannon bone circumference, prominence of feathering, ergots, chestnuts and bulges in the fetlock region are significantly correlated to disease severity. It is not clear if these features are consequences of CPL or that they are a risk factor for disease severity (De Keyser, 2014). Hoof quality is also significantly correlated to severity (Geburek et al., 2005b), although infrequent and poor grooming does not influence the occurrence (Wallraf, 2003). Wallraf (2003) also demonstrated in German draft horse breeds that limb hair characteristics (e.g. hair implantation density) is correlated to clinical disease prevalence.

2.6. DIAGNOSIS

2.6.1. Clinical presentation in a susceptible breed and palpation

The observation of the unique clinical presentation (e.g., skinfolds, nodules on the distal legs) in a susceptible breed is currently the most frequently used method to establish the diagnosis of CPL. However, a final diagnosis cannot be made based on a visual clinical examination only. Additional analyses should be carried out to confirm the diagnosis by excluding other causes of pastern dermatitis such as fungi, bacterial infection or even UV damage to the skin. Since clinical signs in the early stages of CPL may be rather subtle, palpation is necessary for diagnosis. In addition, clipping of the feathering is strongly recommended as this improves palpation and clinical inspection.

2.6.2. Histology

Most of the diagnostic morphologic lesions are typically observed in the deep dermis and subcutis. Therefore, regular skin punch biopsies (sampling the superficial dermis) may not always be useful because diagnostic changes from the deep dermis and subcutis may not be represented (Affolter, 2013).

A double-punch biopsy technique is often more rewarding to visualize the changes of lymphatic vessels and vasculature. A double-punch biopsy can be obtained by using an 8 mm punch through superficial and mid-dermal epidermis, followed by a 6 mm punch through the previous 8 mm biopsy site to harvest the deep dermis and subcutis (Affolter, 2013). However, in CPL affected horses, a delayed wound healing and an increased risk of secondary infection may be cited as contraindications for a double-punch biopsy.

2.6.3. Indirect Lymphangiography

Indirect lymphangiography is a non-invasive technique in which a contrast medium is injected intradermally followed by radiography to visualize the morphology of the lymphatic vessels (Fig. 19). In the case of direct lymphangiography, the contrast medium is injected directly into the lymphatic vessels. Major disadvantages of the latter are the more invasive nature of this procedure due to the skin incisions and the fact that the peripheral lymphatics of the distal extremities are very difficult to identify (De Cock et al., 2003).

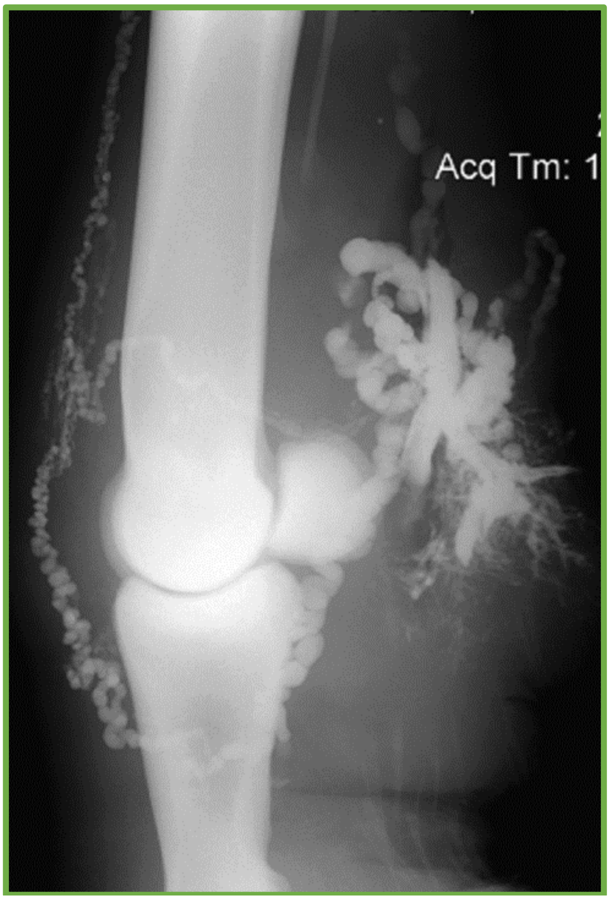

Figure 19. Lymphangiogram of a severely affected front leg of a draft horse affected by CPL. Notice the extremely dilated and tortuous lymph vessels of the distal extremity, particularly in the distal aspect of the fetlock.

Reprinted from "Progressive Swelling, Hyperkeratosis, and Fibrosis of Distal Limbs in Clydesdales, Shires, and Belgian Draft Horses, Suggestive of Primary Lymphedema" by H. De Cock et al., 2003, Lymphatic Research and Biology, 1(3), 191–199.

2.6.4. Lymphoscintigraphy

Lymphoscintigraphy is a diagnostic imaging modality that uses radiotracers to identify lymph node extent of neoplasm as well as lymphatic system flow issues (Ranzenberger and Pai, 2021). This technique has already been performed in horses with CPL to visualize the presence and severity of lymphatic stasis (Fig. 20) (De Cock et al., 2006b). However, lymphoscintigraphy has not been used for routine diagnosis or screening. In humans, lymphoscintigraphy is the method of choice to distinguish lymphedema from other causes of distal limb swelling (The international society of lymphology, 2009). Diagnostic techniques used in human medicine are often too expensive or impractical for routine diagnosis in draft horses (De Keyser, 2014).

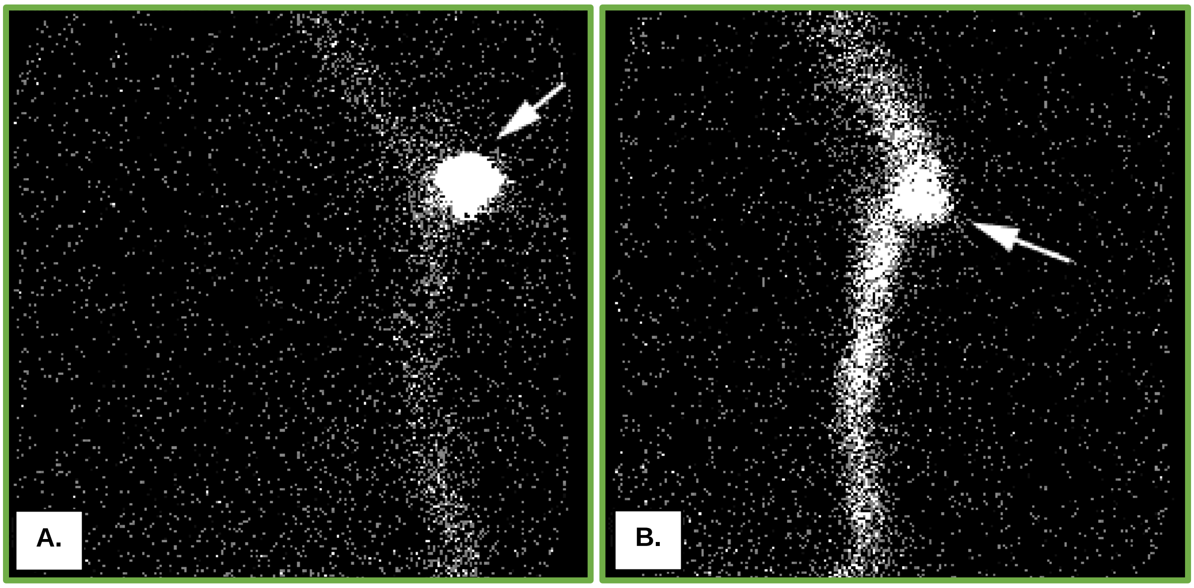

Figure 20. Lymphoscintigraphy of the left front leg in a draft horse with mild lesions of CPL.
A. Lymphoscintigraphy of the distal left front leg of a clinically non-affected draft horse. Note the weak to moderate staining of the contrast medium 30 mins post injection. **B.** Lymphoscintigraphy of the distal left front leg of a draft horse with mild lesions. Note the intense staining of the contrast medium 45 mins post injection. The focal area of activity (arrows) represents an external activity source placed at the level of the accessory carpal bone as anatomical reference point.
Reprinted from "Lymphoscintigraphy of draught horses with chronic progressive lymphoedema" by H. De Cock et al., 2006, Equine Vet. J. 38, 148–151.

2.6.5. Radiographic Imaging

Radiographic imaging was introduced as a part of the official Belgian draft horse stallion approval process and allows early detection of skin thickening, skinfolds and nodules (De Keyser, 2014). Radiography can fulfil an important added value in visualizing skinfolds and nodules whenever palpation is hampered by the presence of abundant feathering on the limbs (Fig. 21).

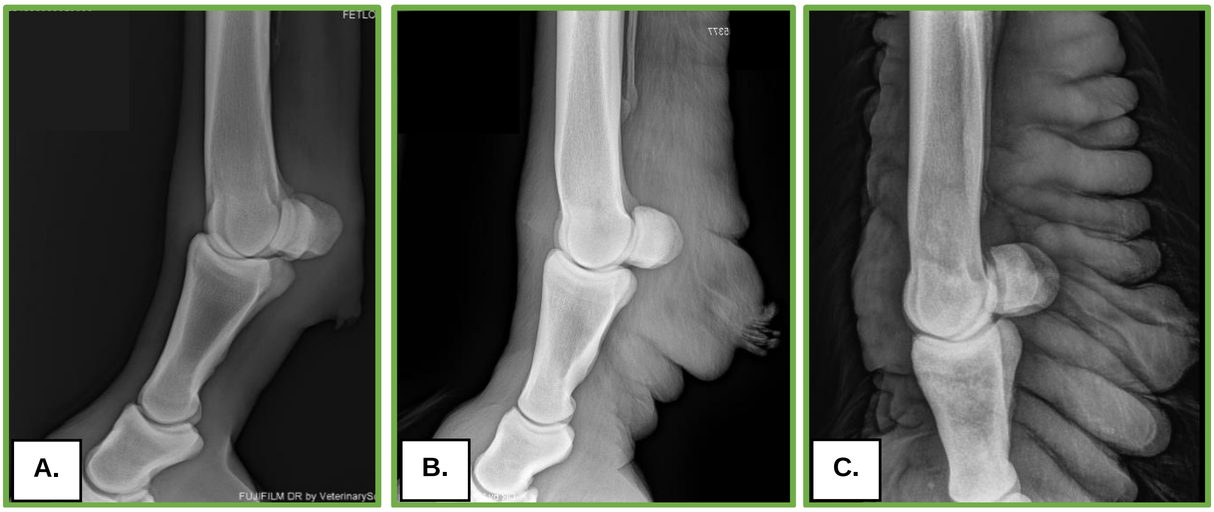

Figure 21. Radiographic images of the distal legs in CPL affected horses.
A. A non-affected warmblood horse with no skinfolds *(Courtesy of F. Brys, 2019)*. **B.** A moderately affected Gypsy cob mare with clear skinfolds in the pastern region and some slight folding of the skin plantar to the cannon bone *(Courtesy of E. Evrard, 2021).* **C.** A severely affected draft horse with pronounced, thick skinfolds on the overall distal limb *(Courtesy of Boschhoven paard, 2019)*.

2.6.6. Anti-elastin antibodies ELISA

Breakdown of elastin generates elastin derived peptides (EDP) that can be recognized by immunocompetent cells, resulting in the production of blood anti-elastin antibodies (AEAb). Since an elastin breakdown in CPL susceptible breeds is suggested (De Cock et al., 2006a, 2009), a correlation between this disease and the production of AEAb was suspected. Van Brantegem et al. (2007b) demonstrated that significantly higher AEAb levels in clinically affected draft horses were present in comparison to non-affected horses of a susceptible breed and Belgian Warmblood horses. This indicates that the AEAb-ELISA can be used as an additional diagnostic tool. Moreover, the AEAb level increased with disease severity in different clinical groups. Therefore, this ELISA was proposed as a diagnostic aid for CPL in combination with a clinical examination (Van Brantegem et al., 2007a).
However, De Keyser et al. (2015) demonstrated a very low diagnostic accuracy of AEAb with the reported ELISA for the diagnosis of CPL, due to a very large overlap between the various clinical groups and an association of AEAb levels and test lab and date of blood sampling. Therefore, this methodology is highly suggestive to be of no use as a diagnostic aid in individual clinically examined CPL susceptible draft horses (De Keyser et al., 2015).

2.6.7. Molecular assays

Even though CPL is considered a hereditary disease, to date no genetic test is available to diagnose this condition. None of the elaborated studies have identified a specific genetic marker and/or mutation correlated with CPL. Different candidate genes were suggested but further functional research is needed to identify the genetic cause underlying CPL (Lindgren et al., 2020)

2.7. SYMPTOMATIC TREATMENT AND MANAGEMENT

Currently, no curative treatment for CPL is available. Careful management and supportive therapy can improve the quality of life of affected horses. However, these treatments are labor-intensive and must be carried out during the whole lifespan of the horse to minimize discomfort, slow the progress of the disease, avoid recurrent infections and ensure quality of life. Symptomatic treatments focus primarily on wound and skin care, prevention of secondary infections and additionally on controlling tissue edema.

2.7.1. Hygiene

The typical long hairs of the pastern in draft horses (feathers) and the skinfolds associated with CPL, provide a warm and moist environment of the skin which favors growth of fungi and bacteria. Clipping of this feathering is essential for cleaning and applying topical treatment. Basic cleaning can be done with lukewarm water and a disinfectant soap, after thoroughly brushing out flakes of skin and taking into account the deep skinfolds. When excessive crusting and scaling is present, a keratinolytic shampoo can be used. Drying the legs thoroughly appears to be essential to prevent accumulation of moisture. This can be done with a towel or blow dryer to access the deep skinfolds.
Hydrotherapy not only cleans the legs, it will also further enhance blood circulation (Petrofsky et al., 2010). The use of cold water reduces the skin surface temperature and inflammation. Salt water can be used since it has a natural disinfectant and bactericidal effect (Wijnker et al., 2006). In addition, a hypertonic solution can reduce inflammation because of the established osmotic gradient onto the skin (Collins et al., 2021). However, salt water could also interfere with wound healing and activate inflammatory processes (Fan et al., 2006).

2.7.2. Secondary infections

Bacterial and fungal infections

In the presence of secondary bacterial infections, it is important to apply correct antibiotic or antifungal therapy (topical or systemic). Since *Staphylococcus aureus* is most frequently isolated, an antibiotic with a gram positive spectrum should be administered. The antibiotics commonly used as treatment for bacterial skin infections in the horse are trimethoprim-potentiated sulphonamides (oral) (White and Yu, 2006; Sauvé, 2021). In case of fungal infections caused by *Malassezia pachydermatis*, azole antifungals (e.g., miconazole) (systemic or topical) are recommended. In order to prevent frequent use of antibiotics, honey ointment can be a very effective and alternative low-cost product for the treatment of wound infections (Tasleem et al., 2011). Zinc ointment may stimulate ulcer healing by enhancing re-epithelialization, decreasing inflammation and bacterial growth (Agren, 1990) and can therefore also be used on a regular basis for wound and skin care.

Parasitic infections

Currently, there is no registered pharmaceutical product available for the treatment of mange in horses in Europe (Vetcompendium, 2022). This results in the regular off-label use of veterinary drugs for other target animal species with varying results. The cascade system offers the attending veterinarian the possibility (taking into account the legislation on food-producing and non-food-producing horses) to deviate from the strict use of veterinary drugs registered in the respective country to use a veterinary drug registered for a similar condition in another animal species, or even for a different indication (Belgian Royal Decree of 14 December 2006, art. 230 and 231).

In literature, numerous studies have evaluated the use and efficacy of different (veterinary) products for treatment of mange in horses. Heile and Schein (2005) have demonstrated the efficacy of a 0.05% phoxim solution for the eradication of *C. bovis* in horses. The solution should be used to wash the horse's whole body twice, with an interval of one week. Deltamethrin 7.5% (Heil and Schein, 2005) resulted in the occurrence of multiple adverse side effects (e.g. discoloration of the coat, severe itching and dermatitis). This treatment was poured over the horse's back twice with a one week interval. In draft horses, Rüfenacht et al. (2010) did not obtain a reduction in mite counts nor in pruritus and skinfold severity, although crust grades did decrease after dual (3 weeks interval) oral moxidectin administration in combination with a full environmental application (surfaces of the stable, grooming place, paddock and horse transporter) of 4-chloro-3-methylphenol and propoxur for treatment of *C. bovis* infestation. Similarly, Goldinger-Müller (2008) did not achieve a reduction in pruritus, lesions or an eradication of mites with oral moxidectin and environmental therapy (treatment of all stable surfaces with chlorocresol 2% solution and propoxur 1%). Rendle et al. (2007) examined the effect of a subcutaneous injection of 0.3 mg/kg doramectin in one group of horses, while a second group was treated with a 0.25% fipronil topical spray on the distal limbs. In both groups, a significant reduction in the number of mites was observed (one case in each group still displayed mites on skin samples) but no significant reduction of the mange associated lesions could be obtained. A 5% lime-sulphur solution has been proven effective as a treatment in a study by Paterson and Coumbe (2009) for *C. bovis.* This treatment was applied four times at one-week intervals, after the horses' distal legs had been shaved and washed with a shampoo. At the end of the trial (4 weeks of therapy) no mites were detected in any of the horses and lesions were significantly reduced. Amitraz, which is often used as an ectoparasitic in farm animals, should never be administered to horses because of the toxic side effects (Westermann et al., 2004; Auer et al., 1984).

In addition, *C. bovis* has been shown to be capable of surviving off the host for up to 69 days (Liebisch et al., 1985) and therefore it is important to apply repeated, regular treatment of both the animal and the environment (e.g., stable, grooming places, horse transporters).

2.7.3. Exercise

Adequate exercise is essential for the enhancement and support of lymph flow, since muscular activity acts as a pump for the adjacent lymph vessels. Therefore, it is recommended that horses with CPL will not be housed on a permanent basis. Ideally, a turnout, pasture or the possibility for free range exercise should be provided as much as possible in order to slow down the progression of lymphedema by enhancing the extrinsic pumping mechanism (Schmid-Schönbein, 1990; Scallan et al., 2016).

2.7.4. Support of lymph drainage

Manual lymph drainage (MLD) was introduced by Berens v. Rautenfeld and Rötting in 1999 and is today frequently used for therapeutic purposes in lymphedema in horses. This technique is based on manual external manipulation of the lymphatic vessels to stimulate the movement of interstitial accumulated proteins and water to the circulation. The purpose is to move lymph in a 'transterritorial' way from the affected areas to areas with an adequate functioning lymphatic system (Affolter, 2013). This manual therapy can be further supported by the use of compression bandages (Fig. 22) and is then referred to as combined decongestive therapy (Affolter, 2013). These bandages differ from classic stable bandages by creating a gradual pressure distribution (highest pressure distally). Furthermore, during movement (light exercise), they have a massaging effect stimulating lymph drainage (Affolter, 2013). Powell and Affolter (2012) demonstrated a reduction of edema and subsequent limb volume in five affected draft horses with a combination of exercise, repeated MLD and compression bandage therapy. However, the use of these bandages increased the risk of mite proliferation.

Figure 22. Compression bandages

A. Compression bandages as used in the study by Powell and Affolter (2012). *Reprinted from "Combined decongestive therapy including equine manual lymph drainage to assist management of chronic progressive lymphoedema in draught horses" by H. Powell and V.K. Affolter, 2012, Equine Veterinary Education 24(2), 81–89.* **B.** Commercial compression bandages (by Equi-Lymph Equine Compression) *(Courtesy of A. Barker, 2021).*

2.7.5. Surgical intervention

In a Belgian draft horse gelding, resection of several warty-like nodules by dissection and electro-cauterization had good postoperative results (Fig. 23) (Poore et al., 2012). After a 24-month period, no significant regrowth or complications occurred. In practice, electrical induced heat (diathermy) is a second surgical technique that also has been applied in order to reduce the number of warty-like nodules in a Gypsy Cob (Fig. 24) (personal communication with Anna Torstensson, 2022). However, nodular regrowth was observed within seven months after surgery (personal communication with Anna Torstensson, 2022). A third reported technique consists of epidermal shaving, where the hyperkeratotic epidermis and a large portion of nodules and skinfolds are removed (Vlaminck et al., 2008). In a Belgian draft horse, this procedure managed to reduce distal limb diameter, but required a rigorous postoperative supportive treatment, including compression bandaging, exercise, wound care and analgesia, to assure a long-term effect. Due to the intensive postoperative supportive treatment, long-term revalidation of the horse and the risk for the development of exuberant granulation tissue in the distal extremities of horses, surgical intervention is not highly recommended (Affolter, 2013; Vlaminck et al., 2008).

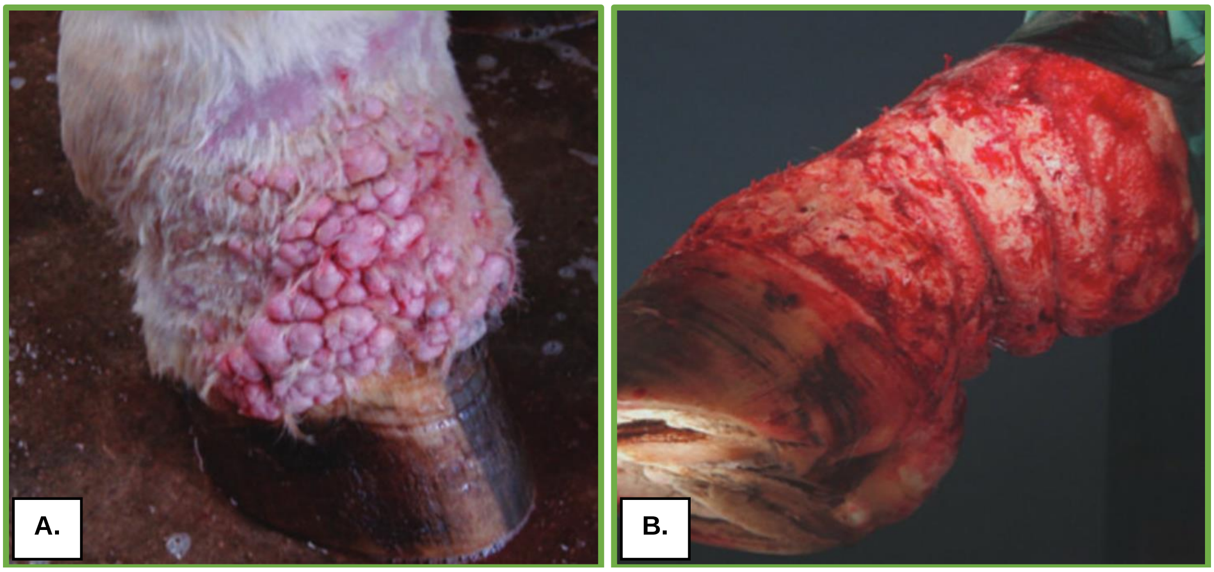

Figure 23. Dissection and electro-cauterization surgery of warty-like nodules.
A. Distal right hind limb before surgical intervention. **B.** Distal right hind limb after surgery.
Reprinted from "The clinical presentation and surgical treatment of verrucous dermatitis lesions in a draught horse" by L.A.B. Poore et al., 2012, Vet. Dermatol. 23, 71–75.

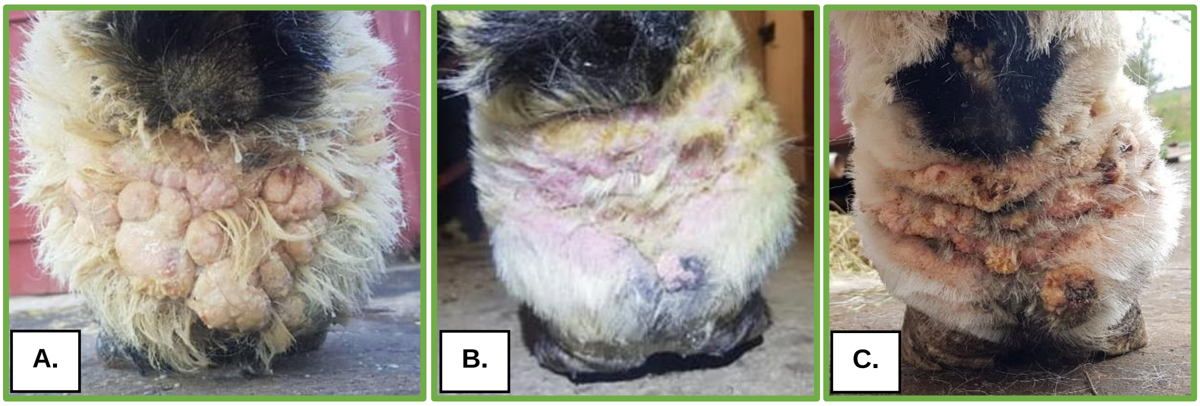

Figure 24. Diathermy surgery in a Gypsy Cob with CPL and warty-like nodules.
A. Right distal front leg before surgery. **B.** 12 days after surgery. **C.** Regrowth appeared within seven months after surgery *(Courtesy of A. Torstensson, 2022).*

2.7.6. Diet

It has been mentioned by several draft horse owners that feeding a high-sugar diet aggravates the clinical signs of CPL (personal communication with owners, 2021). Therefore, many draft horses are on a diet low in sugar and starch. However, these findings need to be confirmed in experimental studies since no research has yet been conducted on the effect of nutritional parameters on the development or aggravation of CPL lesions.

2.8. CHRONIC PROGRESSIVE LYMPHEDEMA IN BREEDING PROGRAMMES

The awareness of the necessity of far-reaching measures to protect the future of the breed is generally accepted. Given the high prevalence of CPL within the Belgian draft horse breed and the serious nature of this condition, several approaches have been implemented by the studbooks to attempt to reduce the prevalence of CPL in the Belgian draft horse breed.

2.8.1. Integration of a CPL scoring system

As a genetic susceptibility for CPL is suggested, several studbooks (e.g., KMBT (Belgium) and KVTH (Netherlands)) have incorporated a systematic assessment of clinical signs of CPL into their breeding policies. During official contests and stallion studbook approvals of the Belgian draft horse, a veterinarian assesses all four legs (each leg individually) of every horse (mainly stallions). Stallions can be presented at studbook approvals starting at the age of 30 months. Each individual leg is scored with a letter, referring to the level of presence of clinical CPL lesions. These scores are compiled for advice only and it is the jury who autonomously assigns the weight of this CPL-score in the overall final score of the horse. For stallion approval, the horse will receive a total score of 30 points (10 points per category) based on type, movement and legs. The scoring system for the legs was adapted from De Keyser et al. (2014a) (Table 2) and is based on a visual inspection and palpation of the distal leg.

Each leg is assigned a letter score in one of 5 scoring classes (AA, A, B, C, and D). In practice, the appointed veterinarians again split the A and B classes into A/A+ and B/B+ respectively. This has been proven to be a necessity in order to sufficiently differentiate the lesions. The score AA refers to perfect legs without any indication of impairment by CPL. If these letter scores are translated into a point scale, one arrives at the following point distribution: AA = 9, A+ = 8, A- = 7, B+ = 6, B- = 5, C = 4, D = 3 or less (Callebert, 2016). If the judges wish to deviate from this score, justification must be provided. Age is not considered a parameter and therefore is not taken into account by the veterinarian during the examination. However, the judge will be able to correlate this advisory score with the horse’s age in order to adapt the value of the same score across age categories when judging (Callebert, 2016). Therefore, a young horse with a leg score of B+ will be considered more severely impaired than if this score was obtained by an older horse. In the final total, this can be translated as a B+ worth 5 points or less in case of a young horse, but 6 or even 7 points for an older horse.

This series of 4 scores (one per leg), is complemented by a scoring of the hocks (one per hock). The scoring of the hocks follows the same scoring system established by De Keyser et al. (2014a) that is also used for scoring the (distal) legs. When all scores are determined, the veterinarian assigns a total score. This total score is not the arithmetic average of all 6 individual scores, but only includes the 2 lowest scores assigned to the horse. A horse that was awarded scores of B+ on both front legs, A- on both hind legs, and A- again twice on the hocks will end up with a total score of B+ (the lowest score of each individual score).

Since 2020, the Dutch studbook (Koninklijke Vereniging 'het Nederlands Trekpaard en de Haflinger', KVTH) has also implemented the systematic scoring of the quality of the legwork in studbook inspections. This score is determined in a similar way as in the Belgian studbook and also ranges from AA (perfect leg without any folds) to D (bad legwork with extreme thickening of the limb).

Table 2. CPL scoring system.

Score	Severity	Dimensions	Clinical symptoms		
			Skin lesions	**Skinfolds / Nodules**	**Soft tissue, skin and hairs**
AA	Perfect		None	- None - Normal limb diameter (25-30 cm below carpus, 33-38 cm under the hock)	- No swelling - Hairs and skin are supple
A	Mild	Below fetlock	- Sometimes slight skin thickening and scaling - (Hock: hairs upright)	- 1 to 2 skinfolds in the pastern cavity - Normal limb diameter	- Slight, mild skin thickening, impressionable
B	Moderate	Until fetlock	- Moderate skin thickening and scaling, sometimes wounds - Hock and fetlock: hairs upright with moderate lesions (- Exudate on the hock)	- 2 skinfolds in the pastern region (palmar/plantar or dorsal) - Nodules in the fetlock region - Normal limb diameter	- Moderately hard, diffuse swelling
C	Severe	Above fetlock	- Severe skin thickening and scaling, wounds - Hocks with wounds, exudate and hairs upright - Slight mechanical impairment	- Skinfolds palmar/plantar and/or dorsal - Nodules ascending - Increased limb diameter	- Hard, diffuse swelling - Hairs are rough and broken
D	Extreme		- Very severe skin thickening, scaling, wounds, blood, exudate and bad odour - Hock with open wounds and hairs upright - Severe mechanical impairment	- Skinfolds and nodules surrounding the limb - Increased limb diameter	- Hard, diffuse swelling - Hairs are rough and broken

This scoring system was developed by De Keyser (2014) and is currently being used in practice in the assessment of Belgian draft horse stallion approvals.
Adapted from "Chronic progressive lymphoedema in draught horses" by K. De Keyser et al., 2014, Equine Veterinary Journal 47(3), 260–266.

2.8.2. Selection against CPL

Heritability reflects which part of the phenotypical variation between animals is caused by differences in genetic background and which part is caused by environmental influences. It is composed of the ratio of genetic variance to phenotypic variance. The heritability can therefore have a value varying from 0 to 1, where 1 implicates that the variations between animals are caused solely by genetic differences and a value of 0 means that they are caused solely by environmental influences, without a genetic component. For CPL, heritability was estimated at 0.26 by De Keyser et al. (2014b) and 0.14 in a subsequent study by François (2018).
There are two possible methods of selection against CPL in a breed: phenotypic selection and selection based on estimated breeding values. Currently, selection against CPL in the Belgian draft horse is based on CPL phenotype. However, this has not led to a substantial improvement (Francois, 2018). Each year, approved stallions are once again presented at studbook inspections, and legwork is re-evaluated. Thus, a stallion that was inspected as a 3-year old with an AA score for his legwork may be withdrawn from stud service at a later age because of an inadequate CPL-score, since CPL is a chronic, progressive disease. As a consequence, affected animals are still frequently used in breeding programs and reducing the prevalence of this condition throughout phenotypic selection only obtains little result. In addition, only candidate stallions and horses presented at official contests are scored on their legwork.

However, mares that have not been inspected are regularly used in breeding, which complicates selective breeding. Moreover, CPL has a low to moderate heritability (14-26%), resulting in a low efficiency of phenotypic selection. This could be improved by using the estimated breeding value (EBV) for selection (De Keyser et al., 2014b). Moreover, François (2018) has demonstrated that horses with high CPL scores can still genetically be less predisposed compared to horses with lower CPL scores. This clearly demonstrates the added benefit of using EBVs instead of raw phenotypes.
Estimated breeding values are estimates of a horse's genetic merit for a particular trait and an indication of how an horse's progeny will perform. There are two main advantages of using breeding values over phenotypic selection (Janssens, 2018): (1) the breeding value is an estimate of the horse's ability, taking into account the difference in environment, nutrition, training and all other external influences as far as these are known, thus, the breeding value is corrected for environmental influences, and (2) when estimating the breeding value, not only the own performance, but also the performance of all related animals is taken into account. Therefore, more information is available and the genetic potential can be better estimated.
As a result of the current phenotypical selection against CPL in the Belgian draft horse, the average EBV of CPL in the approved breeding stallions did not show a negative trend (towards less CPL) over the years (François, 2018). Although the maximum levels of CPL scores seem to have decreased (but not the prevalence), so has the variation of the estimated breeding values, which additionally compromises selection based on EBV (François, 2018).

2.8.3. Genetic diversity at risk

Recent research by Janssens et al. (2021a) states that in 2019 and 2020, 46% of the foals registered in the Belgian draft horse studbook were sired by only a combined total of 10 stallions. On average, the co-ancestry degree between these stallions and the active (breeding) mares counted 4%. François (2018) has established a similar pedigree-based level of inbreeding in the Belgian draft horse of 3.14%. However, the genomic inbreeding level based on runs of homozygosity amounted to 13.5% (François, 2018).
For domesticated breeds, the effective population size must be above the safe lower limit of 100 animals. An effective population size of 50 animals is considered the absolute critical lower limit. Below these limits, inbreeding and co-ancestry rates will increase at excessive rates. In case of an effective population size of 100 and 50 animals, it is estimated that respectively 5% and 10% of the genetic variation will be lost in a total period of 10 generations. Janssens et al. (2021b) have calculated that currently the effective population size of the Belgian draft horse (based on data of the KMBT) counts about 75 animals (2020), when calculated based on the evolution of the average inbreeding rate. However, when the effective population size was calculated based on the increase in co-ancestry, it dropped to 48 animals (2020) (Fig. 25). The difference in calculated population size based on increasing inbreeding rates and increasing co-ancestry can be explained by breeders deliberately trying to avoid close inbreeding. The inbreeding rate of the foals will therefore not increase significantly, whereas the co-ancestry within the breed as a whole will increase at a high rate. This can be attributed to a combination of the frequent use of the same stallions (lines) and a significantly reduced number of breeders and registered foals (Janssens et al., 2021b).
Over a period of 1 generation (7 years on average), the effective population size has dropped from 142 to 48 animals (Janssens et al., 2021b). These numbers put the Belgian draft horse breed at serious risk and complicate additional selection against a genetic disorder without further limiting genetic variation in the population.

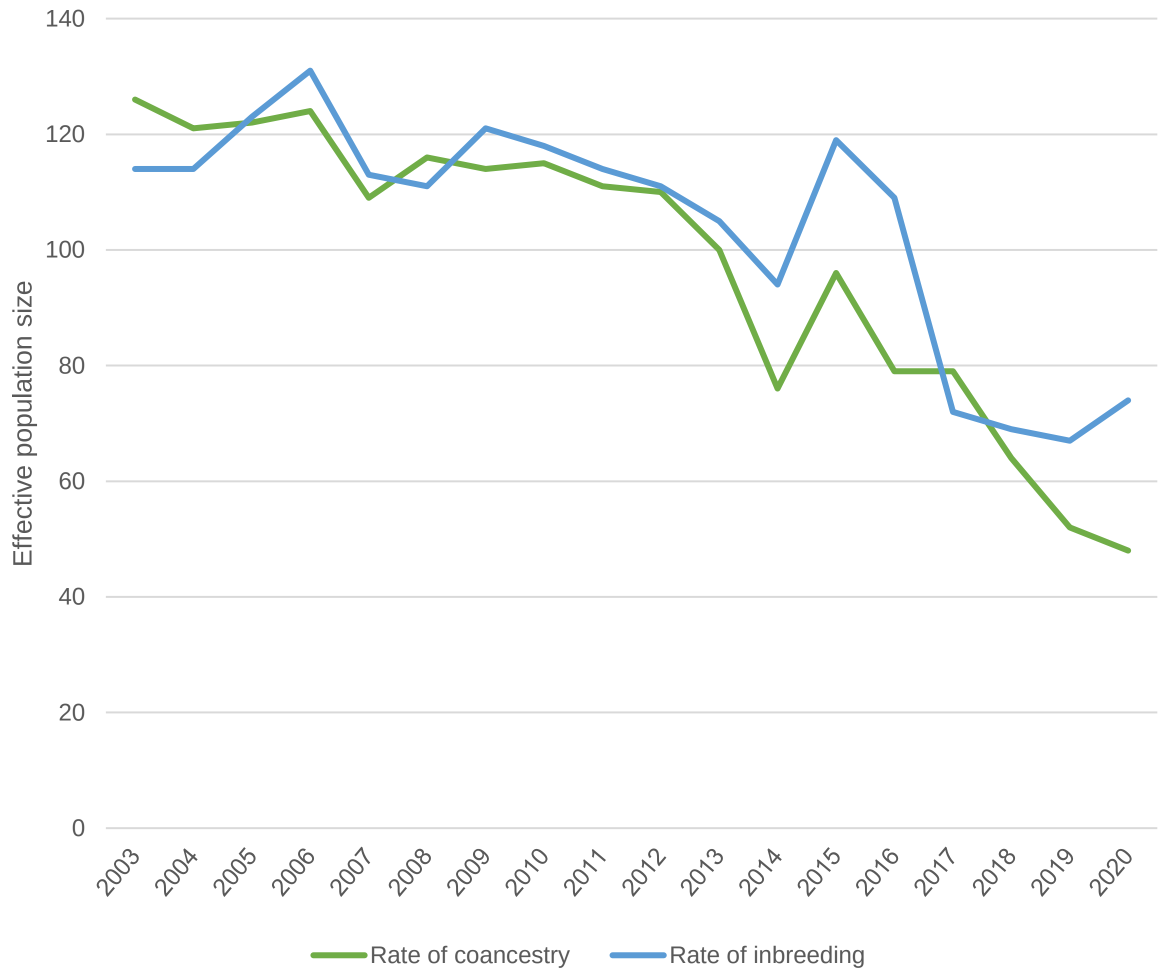

Figure 25. Evolution of the effective population size of the Belgian draft horse.
Evolution of the effective population size when calculated based on both the rate of inbreeding and rate of co-ancestry from pedigree records dating from 2003-2016 of the Belgian draft horse.
Adapted from "Conservation genomics of living heritage breeds" by L. François, 2018, (Inaugural dissertation), Faculty of Bioscience Engineering, Catholic University of Leuven, Belgium, and "Genetische variatie bij het Belgisch Trekpaard (deel 2)" by S. Janssens et al., 2021, Ledenblad V.F.B.T. 2, 14-15.

2.9. CONCLUSIONS AND PERSPECTIVES

Chronic progressive lymphedema in the Belgian draft horse is a disorder that, due to its high prevalence, has a significant impact on the survival of the current Belgian draft horse breed. Clinical signs are severe, compromise the horse's quality of life, affect animal welfare, and even cause a decreased life expectancy. Despite the severity of this condition, many uncertainties about its etiology, pathogenesis and subsequent possible treatment still remain to date. Established scientific research on CPL is rather limited, despite the urgent need for answers. Several aspects of this condition require further exploration and the possibilities for future research are numerous. The Belgian draft horse is national intangible cultural heritage, was once the national pride of Belgium, but is currently threatened with extinction.

3. EFFECTS OF 0.5% MOXIDECTIN TOPICAL SOLUTION ON CHORIOPTES BOVIS INFESTATION AND LESIONS ASSOCIATED WITH CHRONIC PROGRESSIVE LYMPHEDEMA IN BELGIAN DRAFT HORSES

ABSTRACT

The purpose of this prospective, single-blinded, placebo-controlled clinical trial was to investigate the efficacy of topical moxidectin for the treatment of *Chorioptes bovis* infestation in Belgian draft horses, and to evaluate the effect of this treatment on the clinical signs associated with chronic progressive lymphedema (CPL). The efficacy of a topical moxidectin solution (Cydectin 0.5% Pour-On; Zoetis) at a dose of 1.5 mg/kg body weight, evenly distributed over the distal legs, was evaluated. Nineteen privately owned Belgian draft horses were therefore assigned to either a treatment group (topical moxidectin, n = 10) or a placebo group (PBS, n = 9). On Day 0, all 19 horses were positive for the presence of *C. bovis* in superficial skin scrapings. Treatment was applied twice with a 1 week interval on the distal limbs. Prior to treatment, all feathering of the horses had been clipped. Follow-up examinations consisted of determination of presence of living mites in superficial skin scrapings, scoring of pruritus, and scoring of mange associated and CPL associated lesions (skinfold score and skin lesion score) up to 7 weeks after treatment.

One week after treatment, no mites were detected in any of the treated horses, in contrast to the horses in the placebo group, where all horses, except for one, still displayed living mites in skin scrapings. In addition, a complete resolution of pruritus was seen in all horses in the treatment group. No reduction in mange lesions or skinfold score was obtained, although the skin lesion scores had improved in almost all treated horses.

At the end of the trial (7 weeks post treatment), still no mites ($p = 0.0010$) or signs of pruritus ($p < 0.0001$) were detected in the horses of the treatment group. Mange associated lesions significantly ($p < 0.0001$) improved in all horses treated with topical moxidectin. No reduction in the skinfold score could be obtained, however, the skin lesion score did improve significantly ($p < 0.0001$).

In conclusion, the results of this study demonstrate that topical moxidectin as used in this study is effective for the treatment of *Chorioptes bovis* in feathered draft horses and has a positive effect on skin lesions in CPL affected horses.

Keywords: *Chronic Progressive Lymphedema; Moxidectin; Chorioptes bovis; Belgian draft horse*

3.1. INTRODUCTION

Chorioptes bovis is a common cause of pastern dermatitis, with a special predilection for draft horses and other heavily feathered horses (e.g., Belgian draft horses) (Moriello et al., 1998; Rüfenacht et al., 2010). In draft horses, a remarkably high prevalence of *Chorioptes* infestation has been described in several studies, ranging from 50% up to 95% (Cremers, 1985; Geburek et al., 2005a; De Cock et al., 2003; Rüfenacht et al., 2010). The heavy feathering of the pasterns provides an ideal environment for mite multiplication (Paterson and Coumbe, 2009). The feathering traps skin scales and epidermal debris which provides a food supply for mites. As a result of the mite associated pruritus, the horse will be restless, frequently stamping its legs, and eventually exhibit auto-mutilation. Clinical signs are usually more severe during winter as mite activity and egg production are both increased at lower temperatures (Wall and Shearer, 1997). Currently, there is no registered pharmaceutical product available for the treatment of mange in horses in Europe (Vetcompendium, 2022). This results in the regular off-label use of veterinary drugs for other target animal species with varying results. The cascade system offers the attending veterinarian the possibility to deviate from the strict use of veterinary drugs registered in the respective country, to use a veterinary drug registered for

a similar condition in another animal species, or even for a different indication (Belgian Royal Decree of 14 December 2006, art. 230 and 231).
In addition, various draft horse breeds are known to suffer from an incurable, hereditary type of pastern dermatitis, named chronic progressive lymphedema (CPL). CPL is characterized by progressive swelling with associated skinfolds, hyperkeratosis, fibrosis, skin nodules and ulcerations on the distal limbs of the horse. Clinical signs of CPL can start to develop at an early age, progress over several years and may end in severe deformity of the distal limbs, severely affecting the horse's quality of life and welfare, often resulting in premature euthanasia (Ferraro, 2003; Affolter, 2013). Despite the serious nature of this condition, the etiology and pathogenesis of CPL remain unclear to date. Secondary infections (bacterial, fungal or parasitic) frequently occur in CPL affected draft horses and are known to potentially aggravate clinical signs. Moreover, chorioptic mange can influence CPL progression (De Cock et al., 2003; Walraff, 2003; Geburek et al., 2005a). Mites have already been associated with CPL in various studies but still, it remains unclear whether an infestation with mites represents a consequence of CPL or rather a causative factor in this pathology (Ferraro, 2001; Geburek et al., 2005a). Some authors suggest that CPL may develop from an inflammatory dermatitis (Geburek et al., 2005b; Mittmann et al., 2010; Mömke and Distl, 2007; François, 2018). The frequent presence of *C. bovis* in heavy draft horse breeds is consistent with breeds in which CPL has been described. Moreover, horses presenting with CPL are frequently infested (Barbet, 2014; Rüfenacht et al., 2010; Cremers, 1985; Geburek et al., 2005a). However, the impact of an effective treatment of *C. bovis* mites on the clinical signs associated with CPL has not been established to date. An effective treatment for *C. bovis* infestation would be of great value for both veterinary practice and future research on the effect of mites on CPL in draft horses. Therefore, the aims of this study were to evaluate the efficacy of topical moxidectin against *C. bovis* infestation, applied directly onto the distal limbs of draft horses and to investigate the effect of this treatment on the clinical signs associated with CPL.

3.2. MATERIALS AND METHODS

3.2.1. Experimental design

The present study was approved by the ethical committee of the Faculties of Veterinary Medicine and Bioscience Engineering of Ghent University (EC2021/102).
Horses were randomly divided into two experimental groups. On Days 0 and 7, all legs of all horses were topically treated with either moxidectin (treatment group, n = 10) or PBS (placebo group, n = 9). One day after each treatment, horses were examined for signs of adverse reactions. On Days 0, 7, 14, 28 and 56, all horses were scored for pruritus, mange associated lesions, and CPL associated lesions of which the latter consisted of both a skinfold score and a skin lesion score. The number of mites on all legs was determined on Days 0, 14 and 56. Owners were not informed of which group their horse was assigned to. For all horses, the study protocol started at the end of the winter of 2021-2022.

3.2.2. Horses

Privately owned Belgian draft horses were recruited in December 2021 after voluntary application for the study by the owners. Only Belgian draft horses with clinical signs of CPL were included in the study. Owners were informed of the study design and had to consent to clipping of the feathering on the legs of their horse. All horses were clinically healthy (with the exception of clinical signs of CPL) on physical examination. No previous treatment against endoparasites or ectoparasites or treatment with anti-inflammatory drugs (NSAID, glucocorticoids), less than 3 weeks prior to the study had been administered. Nineteen horses from 12 different stables fulfilled the inclusion criteria for the study (Table 3).

Table 3. Overview of animals included in the treatment group and placebo group.

Horse	Housing	Group	Age (years)	Sex	Contact horses
1		Treatment	10	mare	No
2		Treatment	14	mare	One warmblood horse
3	a	Treatment	18	mare	Three Belgian draft horses
4	a	Placebo	8	mare	Three Belgian draft horses
5	a	Placebo	5	mare	Three Belgian draft horses
6	a	Treatment	5	mare	Three Belgian draft horses
7		Treatment	7	gelding	No
8		Treatment	12	mare	No
9		Placebo	11	gelding	One Belgian draft horse
10	b	Treatment	10	mare	Two Belgian draft horses
11		Treatment	3	gelding	One Belgian draft horse and one warmblood horse
12	b	Placebo	14	mare	Two Belgian draft horses
13	b	Placebo	4	mare	Two Belgian draft horses
14		Placebo	6	gelding	No
15	c	Placebo	5	mare	Two Belgian draft horses and one Shetland pony
16	c	Placebo	11	mare	Two Belgian draft horses and one Shetland pony
17	c	Treatment	5	mare	Two Belgian draft horses and one Shetland pony
18		Treatment	7	mare	Two Belgian draft horses
19		Placebo	13	mare	Two Belgian draft horses

Horses that were housed in the same stable (housing) are marked with the same letters.

3.2.3. Treatment

All hairs on the distal limbs of all horses were clipped over an area ranging from the coronary band up to the level of the carpus/tarsus, both dorsally and palmar/plantar, to a final hair length of 0.5mm. Moxidectin (Cydectin 0.5% Pour-on; Zoetis) at a dose of 1.5 mg/kg body weight was then administered with a syringe directly onto the clipped skin and manually homogeneously distributed (treatment group). Horses in the placebo group received the same treatment but the moxidectin solution was replaced by PBS. Administration of moxidectin (treatment group) and PBS (placebo group) was repeated 7 days after the first treatment. One day after treatment, horses were examined for signs of adverse reactions.

3.2.4. Scoring CPL lesions

The CPL scoring was adapted from De Keyser et al. (2014a) (Table 4). A skinfold score was given based on swelling and deformation of the skin of the distal legs. The skin lesion score included changes in hair, superficial lesions and wounds. For each individual leg, a value ranging from 0 to 4 for both scorings was determined. The individual scores for each leg were added up, resulting in a total score for each parameter ranging from 0 to 16.

Table 4. Scoring system for CPL-associated lower-limb lesions in draft horses.

A. Skinfold score			B. Skin lesion score	
Severity	***Score***	**Swelling and deformation**	***Score***	**Skin surface and hairs**
Normal	***0***	Normal limb diameter	***0***	Hairs are supple
Mild: **Below fetlock**	***1***	Normal limb diameter Soft, compressible swelling 1-2 SF (P/PL)	***1***	Slight skin thickening Scaling Hyperkeratosis Hairs supple (Hock: hairs upright)
Moderate: **Until fetlock**	***2***	Normal limb diameter Moderate hard, diffuse swelling >2 SF and NOD (D/P/PL)	***2***	Moderate skin thickening Hyperkeratosis, scaling Small wounds, ulcera Hairs less supple (Hock: hairs upright + exudate)
Severe: **Above fetlock**	***3***	Increased limb diameter Hard, diffuse swelling SF and NOD (D/P/PL) Mechanical impairment	***3***	Severe skin thickening Hyperkeratosis, scaling Several wounds, ulcera, exudate Greasy skin + bad odour Hairs rough + broken Hock: hairs upright + exudate + wounds
Extreme: **Above fetlock**	***4***	Increased limb diameter Hard, diffuse swelling SF and NOD surrounding limb Severe mechanical impairment General loss of condition 'Final stage'	***4***	Very severe skin thickening Hyperkeratosis, scaling Numerous wounds, ulcera, blood, exudate Greasy skin + bad odour Hairs rough + broken Hock: hairs upright + excessive exudate + large open wounds

CPL-associated lesions are scored based on **A.** the severity of skinfolds and nodules and the dimensions of the affected region (skinfold score), and **B.** the condition of the skin surface and hairs on the distal limbs (skin lesion score).
SF = skin folds, NOD = nodules, D = dorsal, P = palmar, PL = plantar.
Adapted from "Chronic progressive lymphoedema in draught horses" by K. De Keyser et al., 2014, Equine Veterinary Journal 47(3), 260–266.

3.2.5. Scoring mange lesions

Each leg was scored individually with a score ranging from 0 to 3 according to the presence of lesions (0 = No lesions, 1 = Mild skin scaling and/or crusting of the skin, 2 = Moderate skin scaling, crusting of the skin, potentially with focal areas of alopecia, 3 = Severe skin scaling, crusting of the skin, potentially with extensive areas of alopecia). All four individual scores were then added up to obtain one total score per horse, ranging from 0 to 12.

3.2.6. Scoring pruritus

Owners were asked to document signs of pruritus in their horse, such as rubbing, biting or stamping its legs. This information was complemented with the observations of the investigator during the visits to obtain a pruritus score ranging from 0 to 3 (0 = never, 1 = sometimes, 2 = regularly, 3 = continuously).

3.2.7. Mite count

Superficial skin scrapings were obtained after clipping the distal limbs by use of a sharp scalpel blade. Samples were taken from 3 different locations for each leg (palmar/plantar in the pastern region, palmar/plantar of the fetlock, and palmar/plantar on the cannon bone) over an area of 3x3cm each. The individual samples were pooled per leg. Within 48 hours after sampling, the number of live *C. bovis* mites was determined for each sample using a stereomicroscope at 25x magnification (Barth and Visser, 1985). The total mite count per leg was defined as the total number of nymphs, larvae and adults. The live mites present from each scraping were counted up to a maximum of 2000. Counts above 2000 were recorded as '>2000'.

3.2.8. Statistical analysis

The age distribution in the two groups was described in terms of means and significant differences evaluated with a non-parametric Mann-Whitney U-test. Differences in distribution of sex were evaluated using a Fisher's exact test. Mite counts, skinfold scores, skin lesion scores, pruritus and mange lesions scores were compared between both treatment groups using a non-parametric Mann-Whitney U-test for each time period after non-normally distribution of the data was established using a D'Agostino & Pearson test. The statistical analyses were performed with a threshold value for statistical significance of 0.05, and multiple hypotheses correction was used for multiple comparisons, using GraphPad Prism software (version 8.4.3).

3.3. RESULTS

3.3.1. Horses

The signalment and contact animals are summarized in Table 3. The mean ages of the horses in the treatment and placebo group were 9.1 years and 8.6 years respectively (range 3–18 years). Numbers of horses were unevenly distributed between the groups (10 horses in the treatment group and 9 horses in the placebo group) but the distributions of age and sex did not differ between the two groups ($p = 0.9201$ and $p > 0.9999$ respectively).

3.3.2. CPL-score

In all horses, skinfolds were observed and corresponding skinfold scores varied from 4 to 16 at the beginning of the study. The grades of skinfolds had not changed significantly on Days 14 and 56 in either group. Skin lesion scores were significantly reduced in all horses in the treatment group on Day 14 ($p = 0.0037$) and Day 56 ($p < 0.0001$) (Fig. 26 and 27). No significant difference in skin lesion scores was observed in the placebo group on Days 14 and 56. Skinfold scores and skin lesion scores are summarized in Table 5.

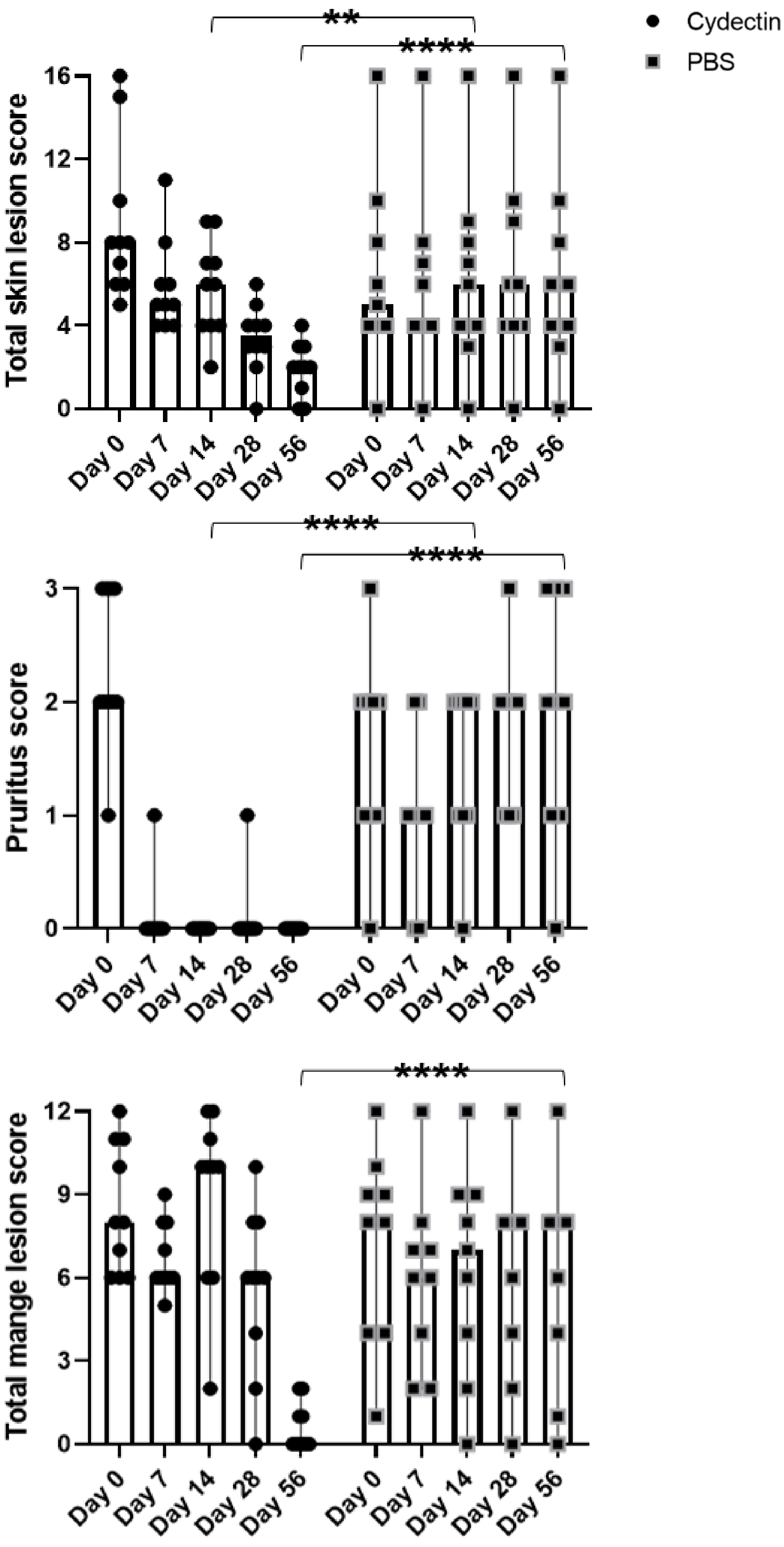

Figure 26. Evolution of total skin lesion scores, pruritus score and total mange lesion scores. Medians and ranges of the scores for each parameter at different time points are displayed for both the treatment group and placebo group. Statistical significance between the two groups was assumed at **p<0.01, ***p<0.001, and ****p<0.0001.

Figure 27. Improvement of skin lesions in a Belgian draft horse (horse 18) after treatment with topical moxidectin.
A. Skin lesions on the medial side of the distal leg are present on Day 0 (skin lesion score = 3). **B.** On Day 28, wounds were healed and the overall condition of the skin had improved (skin lesion score = 1). **C.** Healing of a persistent wound on the palmar side of the carpus on Days 7, 28 and 56.

Table 5. Total skinfold score and skin lesion score per horse.

		Skinfold score					Skin lesion score				
Horse	**Group**	***Day 0***	***Day 7***	***Day 14***	***Day 28***	***Day 56***	***Day 0***	***Day 7***	***Day 14***	***Day 28***	***Day 56***
1	1	12	12	12	12	12	8	5	7	5	2
2	1	8	8	8	8	8	6	5	4	4	0
3	1	16	16	16	16	16	16	8	6	3	3
6	1	10	10	10	10	10	8	4	4	2	1
7	1	10	10	10	10	10	8	4	7	4	3
8	1	6	6	6	6	6	5	5	4	3	2
10	1	12	12	12	12	12	10	6	6	3	2
11	1	4	4	4	4	4	6	4	2	0	0
17	1	6	6	6	6	6	7	6	9	4	2
18	1	16	16	16	16	16	15	11	9	6	4
4	2	12	12	12	12	12	8	7	8	9	8
5	2	6	6	6	6	6	6	6	6	6	6
9	2	16	16	16	16	16	16	16	16	16	16
12	2	6	6	6	6	6	4	4	7	6	6
13	2	6	6	6	6	6	4	4	3	4	3
14	2	4	4	4	4	4	5	4	4	4	4
15	2	4	4	4	4	4	0	0	0	0	0
16	2	4	4	4	4	4	4	4	4	4	4
19	2	8	8	8	8	8	10	8	9	10	10

An individual score for each leg was established, after which these scores were added up to obtain a total score per horse for each parameter.

3.3.3. Mange lesions

On Day 0, all horses showed mange associated lesions in various degrees (Fig. 26 and Table 6). On Day 14, initial mange lesion scores seemed to increase as excessive scaling of the skin had occurred after treatment with moxidectin (Fig. 29 and 30). However, this excessive skin scaling had resolved by the end of the trial, and in the treatment group, mange lesions were significantly decreased on Day 56, compared with the placebo group ($p < 0.0001$) (Fig. 26 and 28). The horses in the placebo group showed no improvement in mange associated lesions throughout the trial.

Figure 28. Resolution of mange associated lesions on the distal leg of a Belgian draft horse (horse 11) after treatment with topical moxidectin.
A. On Day 0, extensive areas of alopecia were present on the lateral aspect of the cannon bone. **B.** After clipping, severe skin scaling and crusting was seen on the lesions (mange lesion score = 3). **C.** On Day 28, skin scaling and crusting had resolved and hair had grown back (mange lesion score = 0).

Figure 29. Scales and crusts from the skin of a Belgian draft horse after treatment with topical moxidectin.
Excessive skin scaling occurred on Day 14 and resolved gradually over the following weeks. The skin scales consisted of thick layers of hyperkeratosis.

Figure 30. Excessive skin scaling one week after treatment with topical moxidectin in Belgian draft horses (horses 18 and 1).

3.3.4. Pruritus score

Prior to treatment, all horses displayed signs of pruritus, except for one horse in the placebo group (Table 6). On Day 14, all signs of pruritus in the horses in the treatment group had resolved ($p < 0.0001$) (Fig. 26). Pruritus remained absent until the end of the trial (Day 56) ($p < 0.0001$). In the placebo group, pruritus was not eliminated.

3.3.5. Mite count

On Day 0, *Chorioptes bovis* mites were found in variable numbers in the skin scrapings of all horses (Fig. 31). Total mite counts varied from 2 up to >8000 (Table 6). One week after treatment (Day 14), no mites were detected in any of the horses in the treatment group ($p = 0.0133$). In the placebo group, all horses but one, tested positive for living mites one week after treatment. At the end of the trial (Day 56), still no mites could be detected in the horses of the treatment group ($p = 0.0010$). In contrast, on Day 56, variable numbers of *C. bovis* were still present in the skin scrapings of all horses in the placebo group.

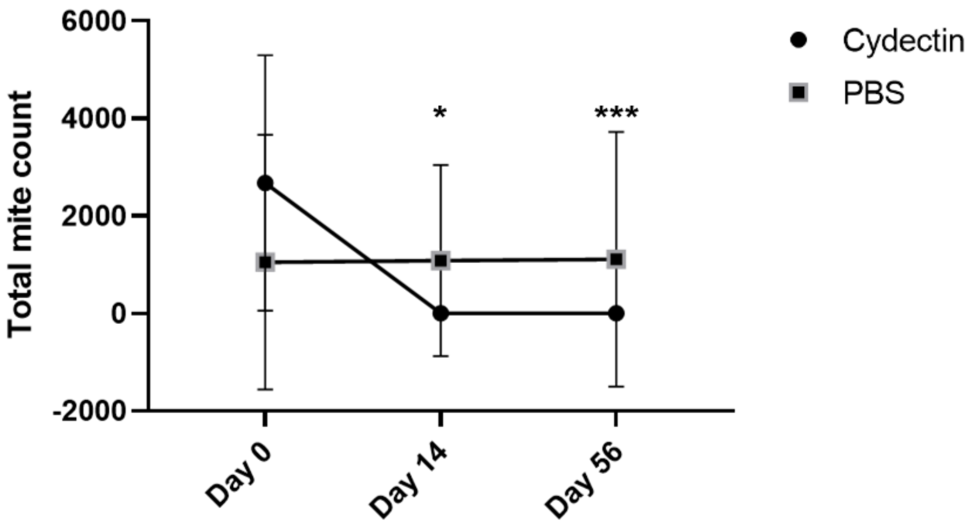

Figure 31. Evolution of total mite counts.
Mean total mite counts with standard deviation are displayed for each group at different time points. In the treatment group (Cydectin) no living mites were found on Day 14 in any of the horses. On Day 56, mites were still absent in skin scrapings of the treatment group. Statistical significance between the two groups was assumed at *p <0.025, **p<0.01, ***p<0.001, and ****p<0.0001.

Table 6. Total mange lesion score, pruritus score and total mite count per horse.

		Mange lesions					Pruritus score					Total mite count		
Horse	**Group**	*Day 0*	*Day 7*	*Day 14*	*Day 28*	*Day 56*	*Day 0*	*Day 7*	*Day 14*	*Day 28*	*Day 56*	*Day 0*	*Day 14*	*Day 56*
1	1	7	7	12	6	0	1	0	0	0	0	727	0	0
2	1	6	5	6	10	0	3	0	0	0	0	4826	0	0
3	1	11	6	10	6	2	2	0	0	0	0	4627	0	0
6	1	10	9	11	8	0	2	0	0	0	0	59	0	0
7	1	8	6	10	6	1	3	0	0	0	0	1020	0	0
8	1	6	6	10	6	0	3	1	0	0	0	2	0	0
10	1	8	6	10	2	2	2	0	0	0	0	825	0	0
11	1	12	8	2	0	0	2	0	0	0	0	3185	0	0
17	1	6	6	6	4	0	3	0	0	0	0	3509	0	0
18	1	11	8	12	8	1	2	0	0	0	0	79	0	0
4	2	9	8	9	8	8	2	1	2	2	2	105	45	59
5	2	4	4	4	4	4	2	1	2	2	2	3	18	33
9	2	12	12	12	12	12	1	2	1	2	3	553	129	70
12	2	8	6	7	8	8	2	1	2	2	3	12	0	5
13	2	4	2	2	2	1	1	0	1	1	1	184	9	8
14	2	9	7	9	8	8	3	1	2	3	3	344	4376	1112
15	2	1	2	0	0	0	0	0	0	1	0	74	2	17
16	2	10	6	6	6	6	2	2	2	2	2	>8000	4677	>8000
19	2	8	7	8	8	8	1	1	1	1	1	180	489	681

An individual score for each leg was established for the mange lesion score, after which these scores were added up to obtain a total score per horse. Mite counts were also determined for each leg individually, and added up, resulting in a total mite count per horse.

3.4. DISCUSSION

Moxidectin is a macrocyclic lactone, belonging to the milbemycin family. This compound acts by binding to ligand-gated chloride channels, more specifically gamma-aminobutyric (GABA-A) mediated and glutamate-gated subtypes (Shoop et al., 1995). This bond results in an increased permeability of the cell, leading to an influx of chloride ions and flaccid paralysis of the parasite leading to death. For this study, moxidectin was chosen because of its significantly longer plasma half-life (17 days) in horses, in comparison with ivermectin (2.3 days) or doramectin (3 days) (Gokbulut et al., 2001). Pour-on formulations decrease the risk of injury for both user and animal, and are particularly convenient for animal owners who can apply the product. Moreover, Rüfenacht et al. (2010) demonstrated that oral moxidectin (0.4 mg/kg body weight, administered twice with 21 days interval) in combination with environmental acaricide treatment is ineffective in the treatment of *C. bovis* in feathered horses. Rendle et al. (2007) were able to eliminate pruritus and obtain a statistically significant reduction in mite counts with subcutaneous administration of doramectin (0.3 mg/kg body weight, administered twice with 14 days interval). However, neither a total eradication of the mites, nor a reduction of skin lesions (scales, crusts and lichenification) could be achieved.

In this study, 1.5mg/kg body weight of a pour-on formulation of moxidectin was administered, while the oral recommended dose of moxidectin in horses amounts 0.4mg/kg body weight. The recommended dose for bovine is 0.5 mg/kg body weight, poured over the back of the cow from the neck up to the basis of the tail. Since administration of pour-on moxidectin in horses is off-label use and no recommendations for pour-on dosage are available, we determined the dosage for this study considering available research in horses and several possible complicating factors for bioavailability, such as the presence of lesions including hyperkeratosis on the site of application in draft horses with CPL. Moreover, in horses, poor plasma availability has been observed after pour-on administration of macrocyclic lactones at the same dose used for oral administration, which could result in subtherapeutic plasma concentrations, thereby promoting the development of drug resistance in (gastrointestinal) parasites (Gokbulut et al., 2010). Therefore, a higher dose (1.5mg/kg topical, compared with 0.4mg/kg oral) for the use on the distal legs in CPL affected draft horses was justified. Since to the author's knowledge there is no research available on the pharmacodynamics and/or pharmacokinetics and persistence of effect of pour-on moxidectin in horses, it was decided to administer treatment twice with one week interval, considering the life cycle of *C. bovis*. Treatment resulted in a total eradication of mites one week after treatment ($p = 0.0133$). At the end of the trial (Day 56) still no mites were detected in the horses in the treatment group ($p = 0.0010$) although several horses in this group were continuously challenged by direct contact with infested horses and no environmental therapy had been implemented. It has been demonstrated that *C. bovis* mites could survive up to 69 days in the environment (Liebisch et al., 1985). Ideally, persistence of treatment effect should exceed 69 days, however, due to time constraints of the study, this could not be demonstrated. Further research should be aimed at unravelling pharmacodynamics and pharmacokinetics of topically administered moxidectin in horses. In addition, dose determination and dose confirmation studies should establish the most appropriate dose for sufficient efficacy against both endo- and ectoparasites in order to limit the risks of drug resistance development. Moreover, ecotoxicity studies to evaluate the effect on the environment of the use of pour-on moxidectin in horses are recommended.

Clipping of the feathering could also possibly affect the mite population and the general condition of the skin. Paterson and Coumbe (2009) did achieve a total eradication of mites and a significant reduction of pruritus scores with lime sulphur when applied as a 5% solution in draft horses. However, the authors noted that possibly some of the improvement in their study may have been due to clipping of the feathering, which would have physically removed some mites from the hair coat (Paterson and Coumbe, 2009). Therefore, in our study, a placebo group was included in which feathering had been clipped. Overall, no significant improvement in lesions or reduction in pruritus was observed. One week after clipping (Day 7), some horses in the placebo group also displayed a reduction in pruritus score, what could be explained by physical partial removal of mites, but this effect disappeared in the following weeks. These findings indicate that clipping of the feathering has no significant effect on lesion scores, mite counts and pruritus in CPL affected horses, however, clipping is recommended for appropriately applying topical treatment.

Based on clinical examinations, pour-on moxidectin at a dose of 1.5mg/kg body weight was not associated with any adverse effects in this study. It should be noted that because moxidectin is very lipophilic (Hayes, 1994), high serum concentrations may be obtained when moxidectin is administered to horses with little body fat, such as foals or debilitated horses (Rendle et al., 2007). When the concentration of moxidectin in the serum is high, moxidectin is able to cross the blood-brain barrier and cause neurotoxicity. The less developed blood-brain barrier of neonates can make them more susceptible to overdoses of moxidectin (Müller et al. 2005). However, since CPL is a progressive disease and clinical signs in draft horses are often not present until the age of 3 years (De Keyser et al., 2014b), and in addition, pour-on administration results in lower plasma availability, the toxicity potential for the use of moxidectin in draft horses affected by CPL will be minimized.

Because pruritus can range from severe to non-existent in chorioptic mange, it is presumed that, as with other mite infestations, hypersensitivity plays a role in the pathogenesis of lesions (Barbet, 2014). The pruritus accompanying a mite infestation might result from a combination of mechanical irritation and hypersensitivity to the mite and by-products of the mite (i.e., faeces) (Martineua, 1987). However, there have been no studies to determine the role of the immune system in the pathogenesis or resistance to *C. bovis* in horses. Variations in clinical severity and response to treatment among individual horses within the same environment may be interpreted as indirect evidence for differences in host susceptibility and resistance (Wilson, 2014). In this study, all horses displayed signs of pruritus, except for one horse in the placebo group. One week after treatment with moxidectin, all signs of pruritus had resolved ($p < 0.0001$) and remained absent until the end of the trial ($p < 0.0001$), which seems to be consistent with the eradication of mites one week after treatment until the end of the trial.

The mange lesion score was mainly based on the presence of skin scaling, since hyperkeratosis represents a main feature of the skin in case of mite infestation (Sinclair, 1990). It should be noted, however, that in draft horses with CPL, scaling of the skin and hyperkeratosis also represent main features of the affected skin (De Keyser, 2014; Geburek et al., 2005a; De Cock et al., 2003). Geburek (2002) hypothesized that a reduced exfoliation of keratinised cells from the surface of the stratum corneum may lead to hyperkeratosis in affected draft horses. It is suspected that a non-regular transformation of intercellular components in the stratum compactum is responsible for this (Geburek, 2002). The triggering stimulus may be chronic irritation due to the nutritive behaviour of mites (Geburek, 2002). Alternatively, the hyperkeratotic reaction may be part of an immune response to an antigenic stimulus emanating from mites (e.g. faecal pellets) (Geburek, 2002). Highly remarkable, after treatment with pour-on moxidectin, all horses displayed excessive skin scaling for a variable period of time, increasing the mite lesion scores. Hyperkeratosis as seen in CPL affected horses and/or mite infestation in this study appeared to clinically improve after treatment with pour-on moxidectin. This seemed to be consistent with the shedding of crusts and resolution of hyperkeratosis after administration of macrocyclic lactones (subcutaneous injection) for the treatment of mange infestation in other animal species (Singla et al., 1996; Jadhav et al., 2020; Kaya et al., 2010). After microscopic evaluation of the skin scales of the horses in this study, it became apparent that these scales contained thick layers of hyperkeratosis that had detached from the skin. Since this observation is consistent with the previously described effects of mite eradication in other animal species, it can be concluded that this excessive skin scaling is the result of the presence and consequent treatment of mites. However, a direct effect of moxidectin cannot be excluded.

Since both CPL and mite infestation can cause similar skin lesions at the same region, the parameters used for scoring of both lesions overlap. For example, initial mange lesions include erythema, papules, and crusts, progressing to more extensive crusting, ulceration, lichenification, and secondary infection (Barbet, 2014). Similar, in CPL, the clinical onset is characterized by crusting, scaling and lichenification of the skin (Affolter, 2013; De Keyser, 2014; Akucewich and Anthony, 2007; Beck, 2015). In our study, mange and skin lesion scores both significantly improved after treatment with moxidectin, indicating that at least part of the skin lesions associated with CPL were induced by the presence of mites. These lesions can be attributed to auto-mutilation, as pruritus caused by mites often leads to stamping, rubbing the legs or even biting. In addition, in literature, edematous skin thickenings and skinfolds are described as characterizing mange lesions in draft hoses and CPL is described as the result of a chronic, untreated infestation of *C. bovis* (Beck, 2015; Gehlen and Niedermaier, 2009), and this appears to be entirely consistent with the clinical signs of CPL. In any case, the treatment used in the present study offers new possibilities to investigate non-complicated CPL lesions.

Despite numerous attempts to unravel the etiology and genetic origin of CPL, the causative factor and subsequent pathogenesis of this disease remain unknown. One hypothesis has been formulated that considers CPL as an overregulated inflammatory (auto)immune response which is initiated by an inflammatory dermatitis, with edema and lesions developing from scaling into hyperkeratotic and hyperplastic lesions (De Cock et al., 2003, 2006a; De Cock et al., 2009; Geburek et al., 2005a; Mittmann, 2009; Mittmann et al., 2010; Mömke and Distl, 2007; Wallraf, 2003; François, 2018). The characteristic CPL lesions are considered to be a consequence of bacterial infection, mange infestation, vasculitis or contact with chemical irritants (Ferraro, 2001; Geburek et al., 2005a; Mittmann, 2009; Mömke and Distl, 2007; François, 2018). Our study indicates that effective treatment of mite infestation significantly improves clinical signs of CPL ($p < 0.0001$). However, a reduction in skinfolds could not be obtained. The presence of skinfolds could represent the result of chronic inflammation, since the onset of this clinical sign of CPL only starts in most draft horses at the age of 3 years old, progressively worsening with age. Chronic inflammation plays a crucial role in the pathogenesis of fibrotic diseases (Meyer et al., 2011). Fibrosis is a main feature of the affected skin in the distal limbs in CPL, which contributes to skinfold formation and thickening of the skin (De Cock et al., 2003). In CPL affected draft horses, an increased number of CD 3-positive T-lymphocytes in combination with an increased number of MHC II-positive cells in the upper dermis of the distal limbs suggest a non-acute confrontation of the skin with antigenic material, resulting in chronic inflammation (Geburek, 2002). Further research is needed to determine the exact role and possibility of *C. bovis* as a causative factor in the pathogenesis of CPL in draft horses under the hypotheses of CPL as an inflammatory dermatitis.

In conclusion, *C. bovis* mite infestation could be eradicated with a dual application of pour-on moxidectin in CPL affected Belgian draft horses, resulting in significant improvement of mange associated lesions, elimination of pruritus and significant improvement in skin lesions associated with CPL.

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4. GENERAL CONCLUSION

To this day, many uncertainties and unanswered questions still remain concerning chronic progressive lymphedema in draft horses. Further research will need to be directed towards the possible etiology and pathogenesis in order to establish possible strategies for managing this disease. *Chorioptes bovis* mites seem to represent an important factor in the development of clinical symptoms associated with CPL. Whether these mites could constitute a causative factor and whether the pathogenesis of CPL is based on an inflammatory dermatitis, both represent interesting candidate topics for future research. In breeds such as the Belgian draft horse, CPL poses a major threat to animal welfare and the preservation of the breed. Despite the limited established scientific research on this disease, the necessity of further research is currently more urgent than ever.

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