UNIVERSITY OF COPENHAGEN
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Sole haemorrhages in Danish fattening dairy breed bulls

- A post-mortem study of sole haemorrhages and the relation to lesions in the rumen and liver

Master's thesis

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Title page

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Abstract

Lameness is a well known welfare issue in dairy production worldwide, and the most common causes hereof are diseases related to the claws and the distal skin. Sole haemorrhages have frequently been used as an indicator of the level of claw damage among cattle in dairy production. However, the occurrence of claw pathology amongst young dairy breed bulls in Denmark have not yet been investigated. In Denmark, the feeding regime of fattening dairy breed bulls is very intensive with a high amount of concentrates, which predisposes the animals to the development of ruminal acidosis and laminitis, and subsequently, to sole haemorrhages. The objective of this study was to investigate the prevalence of sole haemorrhages in young (≤550 days) Danish dairy breed bulls, and also of other claw-related lesions. Additionally, a possible correlation between sole haemorrhages and ruminal and liver lesions was investigated.

Hind feet from 125 young dairy breed bulls, and front feet from 21 of these animals, were collected at a local abattoir in Denmark. The rumen and liver from the animals in question were examined and observed lesions were documented. All claws were trimmed and thereafter examined for lesions; sole haemorrhages, white line lesions and other claw-related pathologies and their localization on the claws were registered. In order to evaluate the depth of the lesions and the position of the pedal bone, the claws were sectioned longitudinally. Some sole haemorrhages were not visible at the sole horn surface, but could only be observed in the depth of the horn once the claw was sectioned. The appearance of the sole haemorrhages observed were of a laminar type, with some being more diffusely laminar than others. The lesions observed in the rumen at the abattoir were characteristic for chronic ruminitis.

The percentage of affected animals with sole haemorrhages was 89.4%; white line disorders 31% (white line haemorrhage 28% and white line fissures 3.5%); ruminal lesions 57%; liver abscesses 8%; and subphrenic abscesses 7.2%. The sole haemorrhages were observed in the caudal part of the claw in 87% of the cases. Sole haemorrhages were significantly more severe on the lateral hind claw than on the medial hind claw ($p = 0.0073$ on left hind claws, and $p = 0.0046$ on right hind claws). No significant difference was observed between the severity of lesions on the front feet compared to hind feet. Neither was there any significant difference on the severity of lesions observed on the medial versus lateral claw on the front feet. A significant positive correlation was observed between daily weight gain and the severity of sole haemorrhages ($p = 0.031$).

This study provided information about a high prevalence of sole haemorrhages in fattening dairy breed bulls in Denmark. It can be suggested, that these lesions are a result of a laminitic insult, which could be a consequence to the intensive feeding strategy of these animals. Moreover, the high occurrence of overgrown claws might be a contributing factor to the development of sole haemorrhages due to altered weight-distribution. Future studies focusing on the impact of different

management strategies on the development of claw lesions, as well as studies that bring attention to welfare of these animals, are desired.

(**Key words:** Sole haemorrhage, fattening dairy bulls, laminitis, ruminitis, liver abscess, post-mortem study)

Resumé

Halthed er et velkendt og verdensomspændende velfærdsproblem hos malkekvæg, og den hyppigste årsag heraf, er sygdomme relateret til klove og den distale hud. Såleblødninger er jævnligt blevet brugt som en indikator for skadeniveauet på klovene hos kvæg i malkeproduktionen. Forekomsten af klovsygdomme blandt slagtekalve i Danmark er dog endnu ikke blevet undersøgt. I Danmark er fodringen af slagtekalve meget intensiv og med store mængder kraftfoder. Denne fodringsstrategi prædisponerer dyrene for udviklingen af ruminal acidose, forfangenhed og, til sidst, såleblødninger. Formålet med dette studie er, at undersøge forekomsten af læsioner relaterede til klovene hos unge slagtekalve (≤550 dage), med særligt fokus på såleblødninger. Desuden undersøges der, om der er en sammenhæng mellem såleblødninger og læsioner i vom og lever.

Distale bagben fra 125 slagtekalve, samt forbenene fra 21 af disse dyr, blev samlet ind ved et slagteri i Danmark. Vommen og leveren fra de pågældende dyr blev undersøgt, og alle læsioner blev registreret. I løbet af to dage efter indsamlingen på slagteriet, blev samtlige klove beskårne, og derefter undersøgt for, læsioner; såleblødninger, læsioner i den hvide linje og andre klovrelaterede læsioner og deres lokalisation på klovene blev registreret. For at vurdere dybden af læsionerne samt klovbenets position, blev klovene skåret igennem på langs. Nogle blødninger kunne ikke observeres på overfladen, men kun ses i dybden af sålehornet efter gennemskæring. De observerede blødninger var af en laminær type, og nogle var mere diffust laminære end andre. De ruminale fund der registreredes på slagteriet fandtes karakteristiske for kronisk rumenitis.

Prævalensen af dyr med såleblødninger var 89,4%, læsioner i den hvide linje 31% (blødning i den hvide linje 28%, fissur i den hvide linje 3,5%), ruminale læsioner 57%, leverabscesser 8%, og subfreniske abscesser 7,2%. I 87% af tilfældene, blev såleblødningerne observeret i den caudale del af kloven. Der var signifikant alvorligere såleblødninger på bagbenets laterale klov end på den mediale klov ($p = 0,0073$, og $p = 0,0046$ på hhv. venstre og højre bagben). Der blev ikke observeret signifikante forskelle mellem sværhedsgraden af såleblødninger på forbenene i forhold til bagbenene, og der blev heller ikke påvist nogen signifikant forskel på sværhedsgraden af såleblødninger observeret på den mediale og laterale klov på forbenene. En signifikant positiv sammenhæng blev observeret mellem kalvenes daglige tilvækst, og sværhedsgraden af såleblødninger ($p = 0,031$).

Dette studie viser at der er en høj forekomst af såleblødninger hos slagtekalve i Danmark. Disse læsioner kan muligvis skyldes tilfælde af laminitis, der kan have opstået som følge af den intensive fodring af disse dyr. Desuden kan forekomsten af forvoksede klove have en betydning for udviklingen af såleblødninger på grund af ændret vægtfordeling. Det anbefales at fremtidige studier fokuserer på, hvilken betydning forskellige fodringsstrategier og staldmiljøer har på udviklingen af såleblødninger, samt fokuserer på velfærden hos slagtekalve.

(**Nøgleord:** Såleblødning, slagtekalve, laminitis, rumenitis, leverabscess, post-mortem studie)

Preface

The present study was conducted from August 2015 to January 2016 at the Departement of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, as part of the Danish master degree in veterinary medicine. The thesis applies to fellow students, veterinarians, researchers and others with interest in claw pathology of fattening dairy breed bulls. The thesis is composed of a literature review of the anatomy, physiology and relevant pathology of the bovine claw, rumen and liver, followed by a section where the experimental work, statistics and results of the study are presented.

We would like to express our gratitude and appreciation to all the people that have helped and supported us throughout this study. We especially want to thank:

- SEGES for funding the project and giving us the opportunity to be an active part of it.
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1. Introduction

Lameness is a well known welfare issue amongst cattle in the dairy industry because of its high occurrence in herds worldwide and its devastating effects (Bicalho and Oikonomou, 2013; Capion, 2008a; Manske et al., 2002a; Vermunt, 2007). The issue has been investigated thoroughly by numerous authors (Capion et al., 2008a; Chesterton et al., 2008; Leach et al., 1998). Pathological conditions localized to the claws and the digital skin are the most common causes for lameness, and examples of frequently occurring conditions are; laminitis, white line lesions, digital dermatitis and solear ulceration (Bergsten, 1994; Jubb and Malmo, 1991; Manske et al., 2002a; Somers et al., 2003). However, to the authors' knowledge, the majority of earlier studies have focused their investigations on cows and heifers in dairy production, but little attention has been brought upon the occurrence of claw disorders amongst young dairy bulls.

1.1 Rearing of dairy breed bulls in Denmark

In Denmark, dairy breed bull calves are traditionally fattened in-doors with high amounts of concentrates until they are slaughtered at an age of about 11-12 months (Nielsen and Thamsborg, 2002). Due to the high starch load in the feed, problems with ruminal acidosis and related pathology (i.e. ruminitis, rumen parakeratosis, laminitis and liver abscesses) could be expected in these intensively fed animals, as suggested by Huuskonen (2009).

1.2 Purpose of this study

Sole haemorrhage is a commonly encountered condition associated with laminitis, and several authors have used sole haemorrhages as an indicator of the level of damage to the claw (Bergsten, 1994; Frankena et al., 1992; Greenough and Vermunt, 1991; Leach et al., 1997,1998; Manske et al., 2002a). The overall aim of this study was to calculate the prevalence of sole haemorrhages in young (≤550 days) Danish dairy breed bulls. We also wanted to investigate if there was an association between sole haemorrhages and ruminal and/or liver lesions in these animals, and furthermore, if there was a correlation between lesions in the rumen and liver. A possible relationship between the daily weight gain and the severity of sole haemorrhages was another subject of interest. Additionally, we wanted to compare the lesions on the right and left foot and the medial and lataral claw, respectively, to investigate if lesions were more severe on one particular foot or claw.

1.3 Hypothesis

On the basis of the aims of the present study, the following hypotheses were considered:

- There is a high prevalence of sole haemorrhages amongst Danish fattening dairy breed bulls.
- The severity of sole haemorrhages is greater on hind feet compared to front feet, and is greatest on the lateral hind claw.

- Due to the intensive feeding regime of dairy breed bull calves in Denmark, ruminits is commonly observed in the current study population.
- There is a correlation between ruminitis, liver abscesses and sole haemorrhages.
- There is a correlation between daily weight gain and the severity of sole haemorrhages.

1.4 Background

1.4.1 General anatomy and physiology of the bovine foot

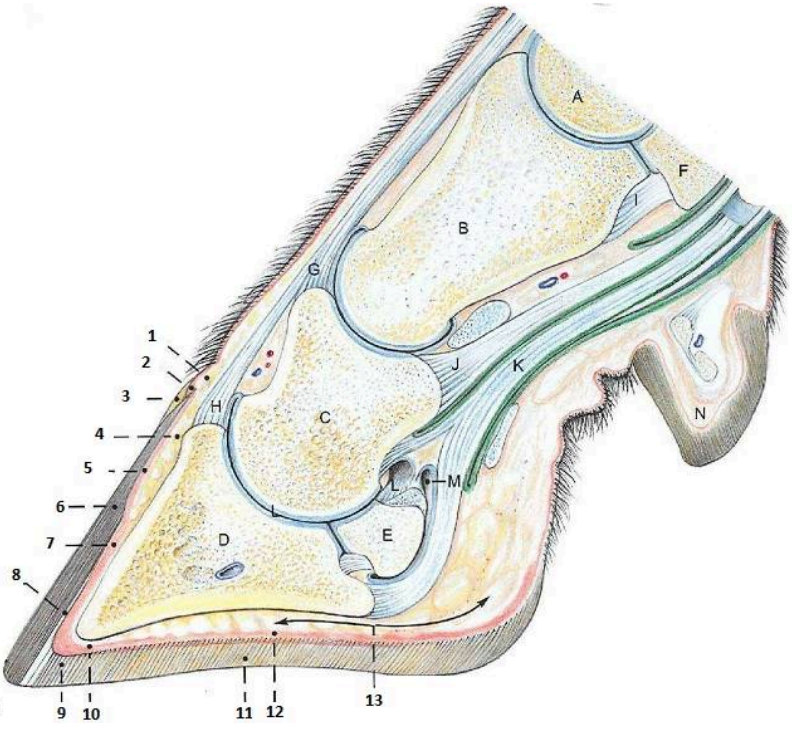


Figure 1. The anatomy of the bovine foot
(Edited illustration from Capion, 2011)

A: 3rd and 4th metacarpal/metatarsal bone
B: Proximal phalanx
C: Middle phalanx
D: Distal phalanx (pedal bone)
E: Distal sesamoid (navicular) bone
F: Proximal sesamoid bone
G+H: Common digital extensor tendon
I: Cruciate sesamoid ligament
J: Superficial digital flexor tendon
K: Deep digital flexor tendon
L: Distal interphalangeal joint
M: Navicular (podotrochlear) bursa
N: Accessory/dew claw

1: The perioplic segment / coronary band
2: The papillary corium of the perioplic segment
3: Perioplic horn
4: Coronary cushion
5: The papillary corium of the coronary segment
6: Horn wall
7: Laminar corium
8: Transition between coronary horn and lamellar horn / beginning of the white line area
9 + 11: Sole horn
10 + 12: Corium of the sole
13: Digital cushion

The bovine foot consists of two main claws – the lateral (4th digit) and medial (3rd digit) claw – and two accessory claws (rudimentary 2nd and 5th digit, also called dewclaws) (Reese et al., 2009; Leach, 1996). Figure 1 shows the inner structures of the bovine foot.

The pedal bone and related structures are surrounded by extensively modified skin, which, just like the skin elsewhere in the body, consists of the following layers;

- **Subcutis.** Subcutis is found in the heel region and in the perioplic and coronary segment of the claw, i.e. in areas where extra padding is required between the skin and the inner structures of the claw (Greenough, 2007; Warzecha, 1993). In the heel segment, the well-developed subcutis

forms the digital cushion, which consists of collagen and elastic fibres mixed with adipose tissue (Reese et al., 2009). Together with the soft, elastic heel horn and the retinaculum of the digit*, the digital cushion functions as a shock absorber during locomotion (Greenough, 2007). The subcutis in the perioplic and coronary region forms the coronary cushion, which is less well-developed than the digital cushion (Leach, 1996). The coronary cushion has the function to absorb the compression that occurs in the last stage of the stride when the pedal bone is forced dorsally in the claw capsule (Greenough, 2007).

- **Corium (dermis).** The corium is composed of loose connective tissue, which contains bundles of both collagen and elastic fibers (Leach, 1996). It is called the "living" part of the claw because it is richly supplied with nerves and blood vessels and provides nutrients and oxygen for the non-vascularized horn producing epidermis (Leach, 1996; Warzecha, 1993). Moreover, the corium acts as a support structure to suspend the pedal bone within the claw capsule (Blowey, 2008).

  The macroscopic structure of the corium divides it into two parts – the proximal papillary corium and the distal laminar corium. The papillary corium lies immediately distal to the coronary band and is characterized by its fingerlike projections called papillae, which penetrate the overlying horn like pegs (Blowey, 2008). Distal to the papillary corium lies the laminar corium, where the dermis forms folded sheets or leaflets (laminae), which provides a tight attatchment for the inner part of the claw to the horn capsule (Blowey, 2008). The dermal laminae are present only on the wall (and not on the sole), and run all the way from the bulb of the heel to the toe on the abaxial wall. On the axial wall, on the other hand, the dermal laminae run along the first third of the distal border, but run proximally up towards the interdigital space at the level where the wall is no longer weight-bearing (Blowey, 2008).

  Microscopically, the corium is composed of two cell layers, the profund stratum reticulare and the more superficial stratum papillare/lamellatum (Figure 2) (Lischer et al., 2002; Warzecha, 1993). The stratum lamellatum is the layer that interdigitates with the epithelial lamellae of the horn wall.

- **Epidermis.** The epidermal layer of the claw is the horn producing layer, which covers the corium and forms the claw capsule (Blowey, 2008; Greenough, 2007). It is separated from the corium by the basement membrane (also referred to as the dermo-epidermal junction (Mülling, 2002)) and is composed of four cell layers; stratum basale, stratum spinosum, stratum granulosum and stratum corneum (Fig. 2) (Greenough, 2007). The basement membrane serves three important functions;
  - it provides mechanical support to the epidermis
  - it is part of the suspensory apparatus of the claw by the attachment of its collagen fibers to the network of collagen fibers in the corium
  - it enables and regulates the passage of substances, such as nutrients and growth regulating factors, to the epidermis from the vasculature of the corium (Greenough, 2007; Leach, 1996)

* The retinaculum of the digit is a fibroelastic band that binds the digital cusion and the deep digital flexor tendon to the pedal bone and the nacivular bone (Greenough, 2007; Reese et al., 2009).

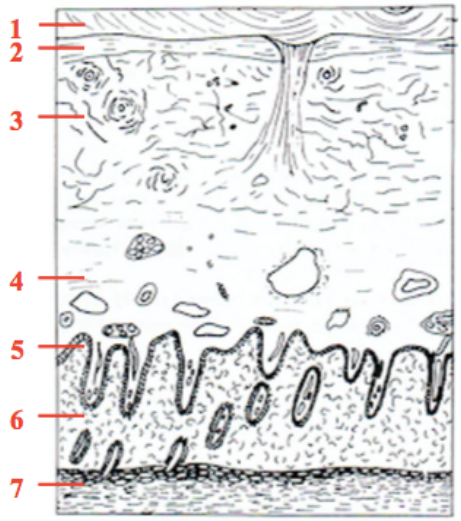


Figure 2. The microscopic structure of the bovine claw. (Edited illustration from Boosman, 1990)

1: The distal phalanx (pedal bone)
2: Periosteum
3: Corium – Stratum reticulare
4: Corium – Stratum papillare / lamellatum
5: Epidermis – Stratum basale
6: Epidermis – Stratum spinosum
7: Epidermis – Stratum corneum

The stratum basale is the innermost epidermal layer, and consists of a single layer of columnar cells. This is the layer in which the cells generate by mitotic division (Leach, 1996). The epidermal cells are filled with sulphur-rich proteins that accumulate in the cells and matures in the next cell layer, the stratum spinosum, to become aggregated into keratin filaments (Blowey, 2008; Leach, 1996). The keratin filaments form an internal cytoskeleton that establishes the mechanical strength of the epidermal cells (Greenough, 2007). As the cells advance to the outer epidermal layers, the maturation of keratin progresses (a process called cornification or keratinization), making the outermost layer – the stratum corneum – extremely hard and resistant (Blowey, 2008; Leach, 1996). The cells in this layer, called squames, are flattened and lack both nuclei and organells when they are completely keratinized (Leach, 1996). The third cell layer, the stratum granulosum, exists only in the areas of the claw where soft horn is produced, i.e. the periople and the bulb (Greenough, 2007).

Cells in the epidermis are tightly bound to each other at focal points by intercellular junctions called desmosomes (Leach, 1996), to which the intracellular cytoskeleton is attached (Greenough, 2007). Moreover, the cells constituting the basal layer are firmly attached to the basement membrane by structures called hemidesmosomes (Leach, 1996). To enable migration of cells through the epidermal layers and also, to allow the downward movement of the horn wall, the desmosomes and hemidesmosomes must be continuously released and reattached (Danscher, 2008; Leach, 1996). This is possible because of the strictly regulated balance between activators and inhibitors of the enzymes (matrix metalloproteinases (MMPs)) responsible for the break down of these structures (Danscher, 2008).

Due to special features of the modified skin at various locations on the claw, it can be subdivided into five segments; the perioplic segment, the coronary segment, the white line (also called the wall segment), the sole segment and the bulbar segment (the heel) (Blowey, 2008; Greenough, 2007).

The perioplic segment

The periople, also called the coronary band, is the hairless area of soft horn which forms the

proximal termination of the claw and acts as a junction between the flexible skin and the rigid keratinized claw wall (Blowey, 2008). The perioplic dermis carries narrow papillae that are directed distally, and the soft horn, which has a tubular structure, represents the outermost layer of the horn wall. In contrast to the horse, the perioplic horn does not reach the weight-bearing border of the claw, but is seen as a smooth, waxy band on the proximal third of the horn wall (Warzecha, 1993). At the palmar/plantar aspect of the claw, the periople merges with the bulb horn (Leach, 1996).

The perioplic horn is thought to be a part of the regulation of the water content of the proximal claw segments (Blowey, 2008).

The coronary segment

The claw wall (coronary epidermis) is produced by the epidermis covering the papillary corium. As it grows, the wall is pushed towards the tip of the toe and constitutes most of the visible part of the claw capsule (Greenough, 2007). The coronary horn is composed by both horn tubules, formed by cells at the tips of the dermal papillae, and intertubular horn, formed by epidermal cells at the sides and crypts of the dermal papillae, which glue the horn tubules together (Warzecha, 1993). A horn tubule has the shape of a pipe, where the central part – the medulla – is composed of degenerated cell components resulting in a soft and frail structure, and the horn cells constituting the outer part – the cortex – are more solid and stable (Greenough, 2007). In the cortex, the keratinized cells are arranged like a spiral, which gives the structure even stronger and flexible characteristics (Greenough, 2007). The horn tubules, which run longitudinally parallel to the surface of the claw (Greenough, 2007), markedly increase the horn strength (Blowey, 2008). The number of tubules varies considerably between various regions of the claw (Warzecha, 1993), and the regions with the most number of tubules are the regions with the hardest horn (Blowey, 2008).

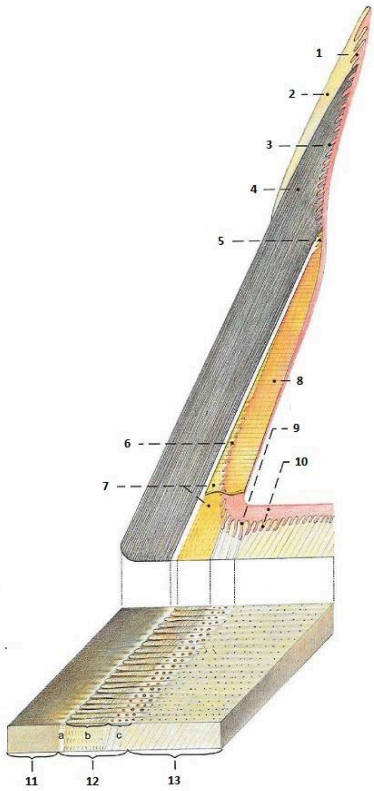


Figure 3. The composition of the dorsal part of the claw capsule (Edited figure from Capion, 2011)

1: Dermal papillae of the perioplic segment
2: Perioplic horn
3: Dermal papillae of the coronary segment
4: Horn wall / coronary horn
5: Transition between dermal papillae and dermal lamellae
6: The beginning of the transition between dermal lamellae and solear papillae
7: Epidermal (horn) lamellae
8: Dermo-epidermale lamellae
9: Transition between the lamellear papillae and the solear papillae
10: Solear papillae
11: Horn wall / coronary horn
12: The white line
13: Sole horn

a: Horn leaflets
b: Cap horn + horn leaflets
c: Terminal horn

The white line (the wall segment)

The segment producing the horn that emerges as the white line on the ground surface of the claw, is made up by the interdigitating dermal laminae and epidermal lamellae in the wall region (Greenough, 2007). The white line consists of three different types of horn cemented together;

- *horn leaflets*, produced by the epidermal stratum basale that covers the sides of the dermal laminae,
- *cap horn*, produced by the epidermal stratum basale that covers the top of the dermal laminae in the distal half of the wall segment,
- *terminal horn*, consists of tubular and intertubular horn and are produced by the epidermal stratum basale that covers the short, thick terminal papillae which ends the dermal laminae distally. (Warzecha, 1993; Budras et al., 1996)

The areas of cap horn and terminal horn are situated between the horn leaflets. Those two types of horn are much softer and 3-4 times wider than the horn leaflets. Thus, the white line is composed of predominantly cap horn and terminal horn (Warzecha, 1993). In addition to the knowledge that the horn leaflets of the white line comprise the least mature stages of horn (Blowey, 2008), horn turnover is more rapid in the white line than in the other parts of the claw capsule (Collis et al., 2004). This results in incomplete keratinization and therefore reduced horn quality and strength, which leaves the structure more susceptible to damage (Blowey, 2008; Collis et al., 2004). Thus, the white line makes a weak but flexible junction between the rigid wall horn and the sole horn (Blowey, 2008; Budras et al., 1996).

The sole segment

The solear corium has papillae, and hence the sole horn consists of horn tubules and intertubular horn just as the coronary horn. The distribution of the tubules is less dense than in the wall, which contributes to the reduced strength of the sole horn compared to the wall horn (Greenough, 2007; Blowey, 2008). The horn tubules of the sole horn slope forward in alignment to the slope of the dorsal wall (Greenough, 2007; Mortensen, 1993). The epithelial cells in the intertubular horn are arranged in strands which appear like leaflets. This gives the sole horn a striped appearance (Warzecha, 1993).

The bulbar segment (the heel)

Warzecha (1993) and Leach (1996) divide the bulbar segment into three regions; the apical part with hard horn (which in this study, just like in the studies by Greenough (2007) and Blowey (2008), is included as a part of the sole), the middle part with a convex surface (also called ‘the bulb of the heel’), and the proximal part which is situated on the palmar/plantar aspect of the claw. The bulbar segment is characterized by the previously mentioned well-developed subcutaneous layer – the digital cushion (Warzecha, 1993) – and is the main weigh-bearing part of the claw (Reese et al., 2009). The horn of the heel is tubular, and the horn tubules follow the convex shape of the heel towards the sole

(Blowey, 2008). The proximal part of the bulbar segment is a continuation of the perioplic segment, and the horn in this area of the claw has therefore the same soft consistency as seen in the periople (Blowey, 2008; Warzecha, 1993). The flexible characteristics of the heel horn allows the underlying digital cushion to be compressed during weight-bearing, thus reducing the pressure load on the skeleton. When the pressure is removed, the cushion returns to its original shape. Thus, the digital cushion serves as a very important shock absorber during locomotion and weight-bearing (Blowey, 2008). Moreover, the digital cushion has an important role in the maintenance of adequate blood flow within the foot (Blowey, 2008).

The suspensory apparatus of the digit

The suspensory apparatus involves collagen fibres that stretch between the pedal bone and the basement membrane between the dermal and epidermal lamellae (Figure 4). Its function is to transfer the load at weight-bearing from the pedal bone to the horn capsule (Greenough, 2007). The maximal weight-bearing function of the suspensory apparatus is on the dorsal surface of the claw (Greenough, 2007). In synergy with the digital and coronary cushion, the suspensory apparatus thus stabilizes the position of the pedal bone within the claw capsule during locomotion (Greenough, 2007).

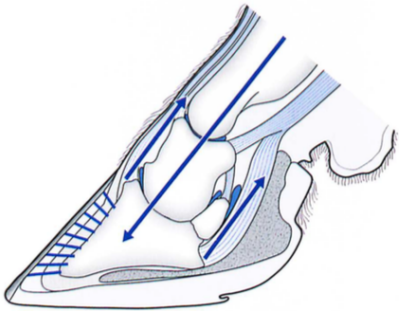


Figure 4. The support system of the pedal bone.
(Greenough, 2007)

The collagen fibres responsible for the suspension of the pedal bone anchor to periosteum-free ridges on the pedal bone and extend through the corium to attach to the basement membrane.

1.4.2 Diseases of the bovine foot

Diseases related to the skin

Digital dermatitis

Digital dermatitis, which is the same as papillomatous digital dermatitis (Read and Walker, 1998), is an infective contagious condition that usually affects the area of the distal dermal region of the foot (Manske et al., 2002c), usually near the skin horn border (Holzhauer et al., 2008). It is suggested that digital dermatitis can develop due to the presence of multiple microorganisms, but spirochaetes from the genus *Treponema spp.* is the one most frequently involved (Zinicola et al., 2015). Macroscopically, the lesions are typically circular and ulcerative with granulation tissue, often surrounded by long hairs (Refaain et al., 2013). As the disease progresses, the lesions increase in size and turn in to raised plaques with a filamentous surface. They are also characterized by an unpleasant

smell. Microscopically, hyperplasia and parakeratosis of the epithelium can be seen in the early stages, along with microabscesses and haemorrhages (El-Ghoul and Shaheed, 2001).

Interdigital dermatitis

Interdigital dermatitis is an infection of the interdigital skin (Frankena et al., 1993). The causative agent is typically *Dichelobacter nodosus* (Greenough, 2007). Moist and warm slurry conditions facilitate spreading of the bacterium between animals (Peterse, 1985). The conditions start with an infection of the the horn-producing epidermal layer in the interdigital space. Initially there is inflammation of the skin accompanied by bad smelling exudate, and in this stage the animal is not lame. As the condition becomes chronic, the infection spreads to the bulbar area, which results in grooves in the skin. These progress into fissures of varying depth, which stretches from the axial side of the claw to the abaxial side. At this point lameness might be evident (Kasari and Scanlan, 1987). As the disease evolves, the heel horn starts to erode, which is very painful and therefore forces the animal to shift weight from the heel area, causing the heel horn to thicken due to less wearing, leading to gait alterations (Greenough, 2007). Severe lesions can predispose to other foot related diseases such as digital dermatitis, ulceration and haemorrhage (Somers et al., 2005).

Interdigital necrobacillosis (foot rot)

Interdigital necrobacillosis (also called 'foot rot', 'interdigital phlegmon' and 'foul in the foot') is a necrotizing infection mainly caused by the opportunistic gram-negative bacterium *Fusobacterium necrophorum* (*F. necrophorum*). In addition to *F. necrophorum*, the bacteria *Porphyromonas levii* and *Prevotella intermedia* (previously considered a subspecies to *Bacteroides melaninogenicus*), are also commonly isolated from foot rot lesions (Morck et al., 1998; Nagaraja et al., 2005; Walter and Morck, 2002). *F. necrophorum* is an inhabitant of the normal flora in the alimentary tract of animals, and the primary source of infection in foot rot is considered to be fecally excreted bacteria (Greenough, 2007). However, the presence of *F. necrophorum* in cattle feces is rare, and, therefore, the need of a disturbance in the gut flora to induce the proliferation of *F. necrophorum* and thereby increase the amount excreted in faeces, has been discussed (Nagaraja et al., 2005).

The disease is characterized by an acute or subacute necrotizing infection in the skin and adjacent underlying soft tissues of the feet (Greenough, 2007; Nagaraja et al., 2005). For the bacterium to be able to penetrate the interdigital skin and invade into the deeper tissues, there has to be a damage or weakening in the protective skin barrier, e.g. by traumatic damage, hyper hydration of the skin or skin irritants (Greenough, 2007; Nagaraja et al., 2005). When the bacterium has invaded into the dermis in the interdigital skin, it produces a toxin that causes necrosis and tissue degeneration. Initially, a swelling is seen interdigitally and around the heel. In severe cases, when tracked up the tendon sheaths, the swelling can be seen around, and above, the fetlock (Blowey, 2008; Clark et al., 1985). Within a few days, the swelling fissures and malodorous pus with debris of degenerating and necrotizing tissue discharges. Other common clinical signs are fever and lameness (Blowey, 2008;

Clark et al., 1985; Nagaraja et al., 2005). If the infection penetrates into the distal interphalangeal joint, septic artritis with severe and prolonged lameness and rapid reduction in bodyweight can occur (Blowey, 2008; Clark et al., 1985).

Interdigital hyperplasia

An interdigital hyperplasia (corns, interdigital fibroma) is a firm, tumour like mass in the interdigital space which is a result from subcutaneous proliferation due to chronic irritation to the skin (Blowey, 2008; Kathiresan and Rani, 2008). There is evidence that the condition may have a hereditary component (van der Spek et al., 2013), especially in heavy breeds of dairy cows (Greenough, 2007) and in some breeds of beef bulls, e.g. Hereford (Blowey, 2008) and Simmental (Koenig et al., 2005). The hyperplasia is triggered by over-tension and slow fibrosis of the subcutaneous layer of the interdigital skin. Contributory factors for development of the condition are; poor foot conformation such as splayed digits, inflammation of the skin (e.g. chronic digital/interdigital dermatitis or low-grade foot rot), and other causes to splayed digits such as poor footing (Blowey, 2008; Divers and Peek, 2008; Greenough, 2007). Interdigital hyperplasia is not painful in itself, but lameness can be observed in cases where the growth has become large enough to be compressed and pinched by the claws during walking (Divers and Peek, 2008).

Diseases related to the horn

Laminitis (coriosis)

Laminitis (*Pododermatitis aseptica diffusa*) is, as the name implies, defined as a diffuse aseptic inflammation of the corium (Bergsten, 1994). Although acute laminits is a relatively rare disease in cattle (Bichalo and Oikonomou, 2013), the subclinical form of the disease is frequently considered a herd problem due to its environmental, managemental and nutritional risk factors (Bergsten, 1994). Moreover, even the subclinical form of laminitis is an important predisposing factor to the development of other claw lesions (Greenough and Vermunt, 1991; Westwood et al., 2003). Much research has been done in order to closer define the pathogenesis and pathophysiology behind the disease. However, this has not yet been completely clarified (Danscher, 2008; Greenough, 2007). Danscher (2008) divided the theories behind the pathophysiology of laminitis into four major categories; vascular, enzymatic, endocrine and traumatic.

The vascular theory

This theory is based on the suggestion that the blood flow, and hence both the nutrient supply and the oxygen supply, to the claw tissue is impaired (Danscher, 2008). Many researches have proposed that likely aetiological factors causing laminitis are systemically disseminated vasoactive substances that are produced and released to the systemic circulation in periods of disease, such as ruminal acidosis, metritis and mastitis (Blowey, 2008; Boosman et al., 1991; Ossent, 1999; Westwood et al., 2003). These substances, which include histamine, lactic acid, and endotoxins (Westwood et al., 2003), cause

a pathological response in the vasculature of the corium and are responsible for the signs of acute inflammation, i.e. pain, vasodilation, haemostasis (because of vessel wall paralysis), congestion and eventually erythema of the corium, in the acute stage of laminitis (Ossent, 1999). Because of the vascular effects, the arteriovenous shunts of the claw open, which allows the blood to bypass the lamellar corium (Ossent and Lischer, 1998). Damage to the capillary walls in the corium due to the inadequate oxygen supply results in leakage and thrombosis (Boosman, 1990; Nilsson, 1963; Ossent, 1999). Leakage of fluid into the interstitial space increases the pressure within the claw capsule, causing intense pain and a further reduction of the blood flow (Ossent, 1999). Moreover, impeded nutrient supply and metabolic exchange of the horn-producing epidermal cells, leads to deterioration of the cells, production of horn of inferior quality, and, eventually, loss of structural integrity at the dermal-epidermal junction (Ossent and Lischer, 1998). A weakening of this junction would cause a failure in its function to withstand the forces arising under weight-bearing, and consequentially, the whole foot can eventually sink within the horn capsule (Ossent and Lischer, 1998).

The enzyme theory

Another theory is the enzymes (MMPs), that normally have a part in the physiological remodelling in the healthy claw, become over-activated and degrade the components constituting the suspensory apparatus (Danscher, 2008). The resulting weakening of the suspensory apparatus allows the pedal bone to move within the claw capsule, and, because of the weight of the animal, it is forced downwards (Bichalo and Oikonomou, 2013; Danscher, 2008; Greenough, 2007). Consequently, the pedal bone crushes the soft tissue located between the pedal bone and the horn capsule and/or sole/heel horn (Danscher, 2008), resulting in haemorrhages, circulatory alterations, impaired horn production and lameness (Danscher, 2008; Lischer et al., 2002). Mungall et al. (2001) showed that certain *Streptococcus spp.* products are able to activate MMPs in the horse, and because the ruminal flora are dominated by these and other gram positive bacteria during ruminal acidosis, it is suggested that MMPs may be a part of the pathogenesis of laminitis caused by grain overload (Danscher, 2008).

The endocrine theory

This theory is based on the suggestion, that some hormones have a relaxing effect on the suspensory apparatus in the claw. During parturition, the circulating level of the hormones relaxin and oestrogen are elevated, which causes the ligaments in the birth canal to relax to facilitate parturition (Bagna et al., 1991; Danscher, 2008). It has also been shown that the collagen fibres constituting the suspensory apparatus in the claw are affected by the higher hormonal levels during parturition, which reduces the claw tissue strength (Danscher, 2008).

The trauma theory

The last theory is that weakening of the claw tissue comes as a result after over-exertion or trauma (Danscher, 2008). When the capacity of the tissue to repair and remodel are exceeded by repeated

traumatic events, the structures are permanently damaged (Danscher, 2008). Lesions related to laminitis have been documented in cattle after long-lasting transportation, long walking distances or prolonged standing time on hard surfaces (Danscher, 2008).

Independent of the underlying cause to the laminitic event, the resulting lesions follow the growth of the horn and are visible on the horn surface after 8-10 weeks (Blowey, 2008; Vermunt and Greenough, 1995). The horn changes are of variable severities and range from compromised horn production with softening of the horn, discolouration of the horn in the white line, sole or heel regions; to double soles, white line separation, and, eventually, to sinkage of the pedal bone and the formation of sole and heel ulcers (Ossent, 1999). The discolouration of the horn can be either yellow due to serous fluid transudation and incorporation in the horn, or red due to haemorrhage in the corium, which leads to deposition of blood pigments in the horn tubules (Greenough and Vermunt, 1991; Ossent and Lischer, 1998) and/or intertubular horn (Blowey, 2008). If the horn is blood-stained in multiple layers, it is an indication of repeated injury of the corium from repeated bouts of laminitis (Ossent, 1999). Greenough and Vermunt (1991) claimed that haemorrhages in several parts of the sole is indicative of subclinical laminitis. Chronic cases of laminitis often result in deformation of the whole claw capsule (Ossent and Lischer, 1998), and the toe wall can appear concave (Peterse, 1985).

Characteristic histopathology

- **Acute laminitis**

  Boosman (1990) observed hyperemia, oedema, haemorrhages and thrombi throughout the corium. These observations do, however, not match the ones that Danscher (2008) did in her study, and neither does the finding of infiltration of round cells, particularly lymphocytes, which is described by Boosman (1990). Epithelial cells in the stratum basale are enlarged and disorganized, and their nuclei are pale due to a low chromatin density (Boosman, 1990; Danscher, 2008). The basement membrane can appear thick, blurry and wavy (Danscher, 2008).

- **Chronic laminitis**

  According to Boosman (1990), vascular changes are the predominant pathologic processes in the corium. However, Nilsson (1963) observed less oedema, hyperemia and congestion in the chronic cases compared with the acute ones. Moreover, Nilsson (1963) describes consistent findings of cell infiltration, neo-capillary formation and intensive sclerosis throughout the corium. The epithelial cells in the stratum basale have resumed their original shape and arrangement and appear normal (Boosman, 1990).

White line disease

White line disease is one of the most common sequels to subclinical laminitis (Greenough, 2007). Because the soft horn constituting the white line is produced by the epidermal laminae, any

disturbance to this structure, as occurs in bouts of laminitis, will produce even weaker horn in this area of the claw. Once weakened, the horn is prone to wear and penetration by foreign material from the environment (Blowey, 2008; Greenough, 2007; Shearer and van Amstel, 2000). The most common site for a white line lesion to occur, is at the abaxially side of the claw and towards the heel (Blowey, 2008), and the lateral claw of the hind feet (usually both) is most frequently involved (Greenough, 2007). Following penetration of the white line, two scenarios are possible;

- The continued growth of the horn capsule (and white line) may force the impaction to the surface, where it eventually is shed, leaving healthy horn behind (Blowey, 2008).
- If the horn is further weakened, the foreign material may ascend the white line and penetrate all the way to the corium. The introduction of a contaminated foreign material this deep into the claw causes a site of infection and further damage to site of production of the white line horn (Collis et al., 2004). If the drainage is blocked, the infection and inflammatory response will result in an abscess (Blowey, 2008; Divers and Peek, 2008). The accumulation of pus increases the pressure inside the claw capsule and causes intense pain and lameness (Blowey, 2008).

Additionally, the turnover of the white line horn is more rapid than in the other horny structures of the claw. This often results in incomplete keratinization, and therefore softer horn of impaired quality, which makes it more susceptible to damage (Collis et al., 2004).

Barker et al. (2009) proposed that increasing herd size (as well as solid and slippery floor) might be a risk factor for the occurrence of white line lesions, in that a bigger herd can disrupt the social hierarchy and result in more avoidance behaviours between the animals.

Sole haemorrhage

Sole haemorrhage occurs commonly in dairy cattle, prevalences between 13.9-79% have been reported (Andersson and Lundström, 1981; Bergsten, 1994; Capion et al., 2008b; Frankena et al., 1992; Leach et al., 1998; Manske et al., 2002a; van der Spek et al., 2013). Sole haemorrhage appear as a red to purple discoloration of the sole and can occur for two reasons, either a traumatic injury to the corium due to uneven weight distribution of the sole, or as a sequel to subclinical laminitis (Greenough, 2007). In the case of subclinical laminitis, detachment of the lamella from the corium leads to sinkage of the third phalanges followed by increased pressure on the underlying corium. This will eventually lead to destruction of the capillaries and haemorrhage, which gradually is transferred outwards as the horn grows The erythrocytes constituting the haemorrhages are localized in the horn tubulues, and in the occurance of more than one episode of injury to the area there might be several layers of intratubular haemorrhage (Ossent and Lischer., 1998; Greenough, 2007). It is not possible to see the difference between a laminitic insult or a traumatic injury (Greenough, 2007), but laminits can be suspected if there are haemorrhage around the edge of the phalanges, the toe or the typical place for

sole ulceration (Watson, 2007). Moreover, subclinical laminitis is a likely cause when sole haemorrhages are present in several parts of the sole (Greenough and Vermunt, 1991). Cows housed indoors have more severe bleeding compared to cows living in dry lots (Vermunt et al., 1996). Age also has influence on the prevalence of haemorrhages, Frankena et al. (1992) showed that for every month the calves aged, they were 1.2 times more likely to get sole haemorrhage.

Sole ulcers (*Pododermatitis cicumscripta*)

Ulceration of the sole, also known as *Pododermatitis circumscripa*, is a common cause of lameness in dairy cattle. The condition can arise from several predisposing factors such as horn overgrowth, overtrimming and laminitis (Greenough, 2007). Ulceration of the sole due to laminitis occurs when pressure from the rotated pedal bone induce necrosis of the underlying tissues, which leads to inhibition of horn production. The area of the corium subjected to the highest amount of pressure will usually habit the site of ulceration. Most frequently, it is the flexor process of the pedal bone causing the compression leading to necrosis (Ossent and Lisher, 1998), which can be seen on the border between the sole and the heel (Weaver et al., 2005). Related structures, such as the navicular bursa and the deep digital flexor tendon, can also be affected, and if so, a very serious and potentially life threatening condition develops (Ossent and Lisher, 1998).

Influence of the hormone prolactin in relation to calving causes ligaments to relax, there among ligaments involved in the suspensory apparatus, which causes sinkage of the pedal bone and results in necrosis and ulceration (van Amstel and Shearer, 2006). Anatomical features such as sole thickness and fat composition in the digital cushion also play a role (van Amstel and Shearer, 2006), as well as environmental factors like concrete flooring (Greenough, 1986).

Double sole

Double sole occurs when horn production is temporarily being discontinued due to damage of the microvasculature in the dermis, followed by leakage of serum, which forces the dermis and the epidermis to separate (Greenough, 2007). Since the damage is temporary, the epithelial basal cells will eventually recover and start to produce horn again. As a result of the pause in horn growth there will be a pronounced separation between the old and the new sole (Ossent and Lisher, 1998).

Heel horn erosion

Greenough and Vermunt (1991) defines erosion of the heel as “an irregular loss of heel horn in the form of multiple irregular depressions or deep oblique grooves”. Heel horn erosion is a common finding in cattle kept in intensive production systems, and moist, unhygienic conditions, possibly combined with grooves in the heel horn after bouts of laminitis (Greenough and Vermunt, 1991), have been postulated as the aetiology of the disorder (Divers and Peek, 2008). Divers and Peek (2008) associate heel horn erosion with interdigital dermatitis caused by infection with *Dichelobacter nodusus*, and describe the condition as a spreading of the interdigital infection across the heels.

The disorder results in impaired function of the heel as the primary shock absorber, whereby the mechanical stress is transferred to other areas of the claw in severe cases, which may contribute to the development of sole ulcers and white line lesions (Greenough and Vermunt, 1991).

Fissures

Vertical fissures, also known as sand cracks, are fissures of the claw horn that stretch from the coronary band in a distal direction (Greenough and Weaver, 1997). Sand cracks occurring on the abaxial surface are rarely seen in dairy cattle, instead the axial surface of the claw is the most common location among this group (Divers and Peek, 2008). Axial fissures are rare, but do cause lameness (Greenough 2007). The cause of sand cracks is not completely understood but factors such as weight (Goonewardene and Hand, 1995), dehydration of the claw, age, laminitis and trauma are thought to predispose to the condition (Greenough and Weaver, 1997; Greenough, 2007).

A horizontal fissure is defined as horizontal penetration of the entire claw wall. If only an indentation in the horn is present, it is referred to as a groove. Horizontal ("hardship") grooves are usually painless, and occur when the animals are subjected to stressful events such as change in feed or transport. However, horizontal fissures are extremely painful. (Greenough and Weaver, 1997)

Other diseases

Overgrown claw

Overgrowth is not painful in itself, but is considered to be a predisposing factor of claw horn lesions (Divers and Peek, 2008). Although the horn grows at the same rate, some parts of the claw have softer horn than others, making the wear unevenly distributed. The horn wall at the toe of the claw comprises the hardest horn, making the wear in this part of the claw the least. Hence, although the horn growth rate is the same, the wear is generally greatest at the heel and least at the toe, making the toe the primarily area at which overgrowth is seen. (Blowey, 2008)

In severe cases of overgrowth, the dorsal wall surface becomes concave and the toe starts to deviate upwards. The concave shape of the dorsal wall is also a feature in cases of chronic laminitis, in which horizontal ("hardship") grooves/lines also may emerge around the circumference of the wall (Ossent and Lischer, 1998; Westwood et al., 2003). Because the horn wall only extends for one-third of the length of the axially part of the claw, a bigger overgrowth often occurs on the abaxial side of the claw. Hence, in extreme cases of overgrowth, the abaxial wall may be seen rolling under the sole (Blowey, 2008).

In addition to the external changes in the shape of the claw, there are also markedly internal changes occurring because of the mechanical forces arising during weight-bearing and the abnormal weight distribution on the overgrown claw. With an overgrown claw, the heel drops in relation to the toe, and as a result to this, the pedal bone rotates backwards inside the claw capsule. This causes abnormal pressure of the pedal bone on the corium in the heel region, and hence, even a simple overgrowth can predispose for the development of sole ulcers. (Blowey, 2008)

Toe ulcer

Toe ulcers can occur from different pathogeneses, where the most relevant ones are due to a thin sole (Shearer and van Amstel, 2009), and as a consequence of laminitis (Ossent and Lischer, 1998). A thin sole can occur when the claw is subjected to excessive wearing and abrasion due to, for example walking on harsh and uneven flooring (Shearer and van Amstel., 2009), moist conditions (van Amstel et al., 2004) and over trimming (Nuss and Paulus, 2006). The lesion starts out as a detachment of the white line that stretches across the depth of the epidermal layers (Shearer and van Amstel., 2009). This facilitates bacterial infiltration leading to subsolar abscess formation, and if the pedal bone is involved, it can lead to severe osteolysis (Kofler,1999). In the case of laminitis, rotation of the tip of the pedal bone causes compression of the corium, which leads to necrosis and hindered horn production, eventually leading to formation of an ulcer (Ossent and Lischer, 1998).

1.4.3 Anatomy, physiology and diseases of the forestomachs

A basic knowledge of the anatomy and physiology of the bovine forestomachs is necessary in order to fully understand the disease processes occurring. The following sections will therefore describe the basics regarding these fields.

Macroscopic anatomy

The bovine forestomachs are composed of rumen, reticulum and omasum, which are lined by non-glandular mucosa. The rumen is the largest forestomach and has the capacity to hold up to 100 liters. It is attached to the sublumbar musculature and the diaphragm by fibrous tissue (Hofmann, 1993). The rumen has five sacs; the largest sac is located dorsally and is called *saccus dorsalis*, the second largest is located ventrally and is called *saccus ventralis*. Located cranially are *saccus cranialis,* and caudally *saccus caecus caudodorsalis* and *saccus caecus caudoventralis* (Figure 5) (König et al., 2009). Externally, the sacs are divided by grooves in which blood and lymph vessels are located (Hofmann, 1993). Internally, the grooves correspond to the *pilae ruminis* which are internal projections consisting mainly of smooth musculature (Hofmann, 1993). The mucosal lining of the rumen is covered with

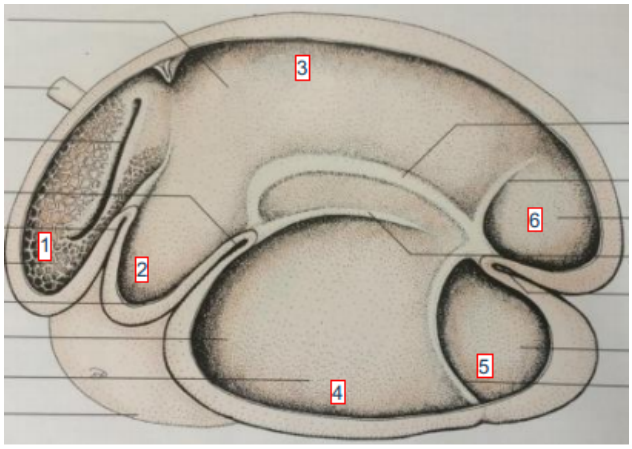


Figure 5. The internal macroscopic anatomy of the rumen and reticulum. (König et al., 2009)
1. Reticulum, 2. Saccus cranialis, 3. Saccus dorsalis, 4. Saccus ventralis, 5. Saccus caecus caudoventralis, 6. Saccus caecus caudodorsalis

Figure 6. Ruminal papillae (Webpage 1)

Figure 7. Honeycomb pattern of the reticular mucosa (Webpage 2)

papillae, which are small tongue shaped protrusions with the purpose to increase the absorptional surface of the rumen (Figure 6) (König et al., 2009). There are usually no papillae on the dorsal ruminal wall or the pilae (Hofmann, 1993).

The reticulum is located craniomedially to the rumen and is the smallest of the forestomachs. It is separated ventrally from the rumen by a deep fold, and dorsally they blend together (Berg, 2003). Due to the close anatomical and physiological relationship between the rumen and the reticulum they are commonly referred to as the *ruminoreticulm.* The mucosa of the reticulum is composed of primary, secondary and tertiary crests, which compose *cellulae reticuli* that together form a characteristic honeycomb pattern (Figure 7). The cells are largest ventrally and turn gradually into papillae dorsally (Hofmann, 1993).

The last of the forestomachs, the omasum, is an almost spherical structure located on the right side of the ruminoreticulum, to which it connects through the *ostium reticulo-omasicum*. It contains up to 100 leaf like laminae covered by rounded horny papillae (Budras and Habel, 2003; Frappier, 2006).

Microscopic anatomy

The ruminal surface is composed of a keratinized stratified squamous epithelium (Graham and Simmons, 2005). The stratum basale, which is resting on the basement membrane, consists of columnar cells. They are characterized by accommodating numerous mitochondria, ribosomes, large vesicles and Golgi complexes (Frappier, 2006; Lyford, 1993). The metabolism of molecules absorbed through the epithelium takes place in this cell layer (Lyford, 1993). As the basal cells differentiate, they move towards the lumen and turn in to the cells of the stratum spinosum, which contains mucus granules (Lyford, 1993). The next layer is the stratum granulosum, in which the cells are flat and contain keratohyaline granules. As they advance towards the outermost layer, the stratum corneum, the cells' nuclei become pyknotic (Frappier, 2006) and the cells fuse together with neighboring cells (Lyford, 1993).

The stratum corneum can be two to twenty cell layers thick, and its keratinized cells serves as a protective barrier against harsh ingested materials (Frappier, 2006). Keratin, which usually is a tough, insoluble, non-reactive material, is modified in the rumen, making it softer and therefore more

permeable to water and water-soluble substances (Lyford, 1993). Between the cells throughout the whole epithelium is a varying degree of distention due to the absorption of nutrients through the epithelium (Frappier, 2006). Beneath the epithelium lies the lamina propria-submucosa (an absent lamina muscluaris makes lamina propria and lamina muscularis blend together), which extend up into the papillae (Frappier, 2006). A dense network of venules in the propria-submucosa transports molecules absorbed through the epithelium to the ruminal vein, portal vein and eventually the liver (Hofmann, 1993). Underneath the propria-submucosa are the circular and longitudinal muscle layers, between which the plexus myentericus* is located (Frappier, 2006).

The epithelium has the same structure and composition throughout the forestomachs, however, there is some difference in the arrangement of the muscular layer. In the reticulum, the lamina muscularis is located in the upper part of the primary reticular crests, and they interconnect between the crests creating a system of muscle covering the reticular surface (Frappier, 2006).

Physiology

The cows' nutritional requirements are completely dependent on a vast and diverse microbial population inhabiting the ruminoreticulum. The microbial population consists of several species of bacteria, protozoa and fungi. They are central in process of fermentation in the rumen since they can hydrolyze complex carbohydrates such as cellulose, hemicellulose and pectin, which make up the building blocks of the bovine diet. (Hoover and Miller, 1991). The fermentation process eventually produces the volatile fatty acids (VFA), primarily acetate, propionate and butyrate, which can be utilized as energy by the cow. The VFAs are passively absorbed through the epithelium on the papillae, but the rate of absorption is dependent on several factors such as pH, osmolality, redox potential and the amount of forage provided. Figure 8 illustrates the fermentation process.

The ruminal papillae vary in size and shape depending the type of diet. The growth of papillae is primarily stimulated by butyrate and propionate, which are products of a concentrate rich diet. These VFA's stimulate blood flow to the rumen, which in turn promotes mitosis in the mucosa, which subsequently leads to proliferation of the papillae. A diet consisting mainly of fibrous forage produce primarily acetate, which leads to reversion of the papillae (Hofmann, 1993).

The muscular walls of the reticulorumen contract in a coordinated pattern and are govern by a complex intrinsic nervous system. This causes the ingesta to be mixed around, which further utilizes fermentation. Moreover, the muscular contractions move the ingesta to the reticulum where it goes either back up the esophagus for rumination, or continues to the omasum and abomasum for further digestions (Herdt, 2007).

* Plexus myentericus consists of nerve fibers from the autonomic nervous system, and regulates the motility of the gastrointestinal system (Frappier, 2006; König et al., 2009; Blood et al., 2007)

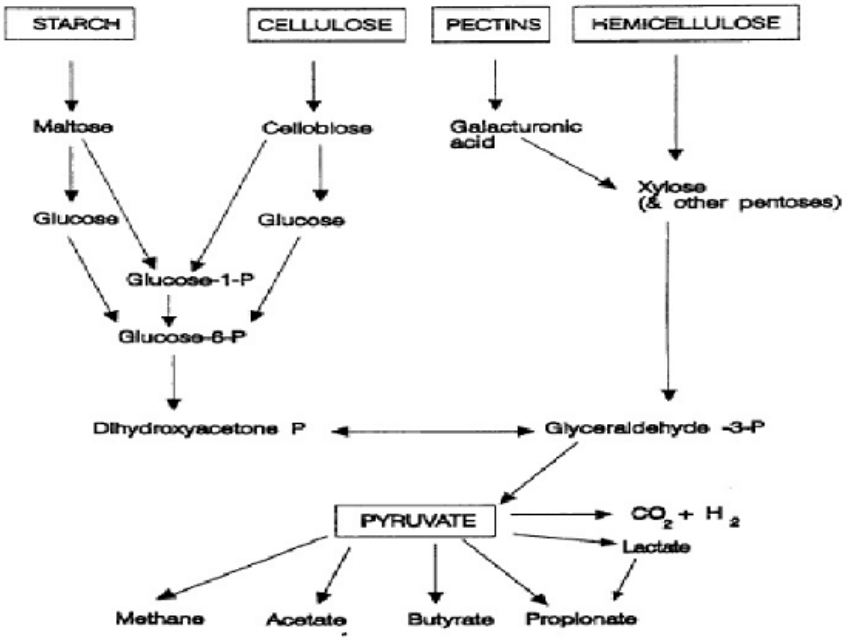


Figure 8. Hydrolysis of complex carbohydrates under the formation of volatile fatty acids. (Webpage 3)

Development of rumen in the calf

During the first couple of weeks in the calf's life, milk bypasses the rumen through the esophageal groove, which is a direct shortcut between the esophagus and the pylorus (Figure 9) (Budras and Habel, 2003). The rumen is therefore not functional in this period (Jami et al., 2013). It also takes up substantially less space than in an adult individual; in fact, in the neonatal animal, the abomasum account for half the volume of the forestomachs, and the rumen and its functional structures such as the papillae are underdeveloped (Hyttel et al., 2010). Because of the underdevelopment of the papillae, the texture of the ruminal mucosa resembles sandpaper in the neonate (Lyford, 1993). Upon introduction of solid, digestible concentrates to the diet, there will be a production of VFA´s. As previously mentioned, VFA´s have a stimulatory effect on epithelial development and proliferation. The development of the ruminal capacity and increase in ruminal wall musculature is also dependent on intake of solid feeds. However, studies have shown that calves given plastic sponges and wood shavings also develop a larger rumen and increased musculature in the ruminal wall. This indicates that the texture of the ingested material is the primary stimulant to the development of the musculature, and not the nutritional properties, in contrast to the development of the epithelium (Lyford, 1993).

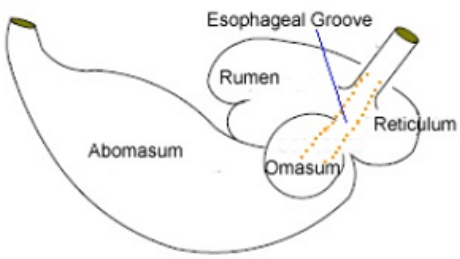


Figure 9. Illustration of the esophageal groove and the proportions of the forestomchs of the neonate (Webpage 4)

Diseases of the forestomachs

Ruminal acidosis

Ruminal acidosis can be either acute, subacute or chronic (Nocek, 1997), and the most economically important one is the subacute ruminal acidosis (SARA) (Gonzáles et al., 2012). SARA is defined by a lowered pH in the rumen, and the pH threshold for SARA is considered to be below 5.5-5.8 for an extended period of time (Nordlund et al., 1995; Plaizier et al., 2008). Rapid diet changes such as going from a low concentrate diet in the dry period, to a high concentrate diet in the lactation period is known to induce SARA (Nagaraja and Titgemeyer, 2007). In the dry period the ruminal papillae and epithelium are not adapted to a sudden increase in concentrate (Martens et al., 2012), whereby the produced VFA's accumulate, thus lowering the intraruminal pH (Plaizier et al., 2008).

The ruminal epithelium is not protected by mucus, and is therefore more sensitive than other gastrointestinal organs (Krause and Oetzel, 2006). The acidic conditions in the rumen can lead to epithelial damage (Dias et al., 2015), which eventually turn into chronic ruminitis characterized by papillar clumping, depapillation and formation of granulation tissue (Jensen et al., 1954). Histologically, epithelial hyperplasia, hyperkeratosis, rete peg formation, abundant granulation tissue in the submucosa, as well as infiltration of inflammatory cells can be seen (Jensen et al., 1954). The acidic state also increases the osmotic pressure in the rumen due to a larger amount of acids getting ionized and more glucose being available. If the osmotic pressure in the rumen becomes considerably higher than the osmotic pressure in blood, fluids from the blood will be pulled from the blood in to the ruminal wall, which causes the papillae to swell and the epithelium to get torn of. As these lesions heal the mucosa becomes hyper- or parakeratotic which further impairs absorption (Owens et al., 1998).

As the rumen becomes acidic, the microbial population shifts from mainly gram negative bacteria and protozoa, to gram positive bacteria (Plaizier et al., 2014). Ruminal acidosis is often associated with increased growth of *Streptococcus bovis* and other gram positive bacteria (Nocek, 1997). *S. bovis* is an acid tolerant bacterium that produce lactic acid when glucose is readily available (Nocek, 1997; Owens et al., 1998). As the result of bacteriolysis, substances capable of systemics effects*, are absorbed into the blood stream, facilitated by the lesions in the mucosa (Vermunt and Greenough, 1994). *Fusobacterium necrophorum*, which is part of the normal bacterial flora in the rumen, can infect the mucosal lesions and cause abscesses, from which emboli can be released into the bloodstream (Scanlan and Hathcock, 1983), and spread to other organs such as the liver, heart or kidneys (Figure 12) (Plaizier et al., 2008).

In order to keep the ruminal pH above the levels of subclinical ruminal acidosis, the maintenance of a buffering system for the rumen is essential. A way of doing this is to include sufficient rough forages to the diet, and to increase forage length. These ingredients stimulate chewing, which in turn causes saliva production to increase and subsequently buffering of the rumen (Plaizier et al., 2014). However, if the straw length is to long, cows have a tendency to sort them out (Leonardi and Armentano, 2003). Feeding smaller portions more often, adding higher quality forage and/or

* for example histamine and endotoxins, both known to, amongst other things, be involved in the pathogenesis of laminitis (Owens et al., 1998; Vermunt and Greenough, 1994)

moisten the feed might help this problem (Krause and Oetzel, 2006). Feeding more often can also contribute to a less fluctating pH in the rumen. Moreover, non-fibre carbohydrates should not be abundant in the feed (Plaizier et al., 2014).

1.4.4 Anatomy, physiology and diseases of the liver

Macroscopic anatomy

The liver is the largest gland in the body and is located in the cranial part of the abdomen. In the ruminant, the liver is positioned in the right side due to the very space occupying rumen, and it is located roughly between the 6th intercostal space and the dorsal end of the 13th rib (Budras and Habel, 2003). In cattle, the liver grossly consists of a left and right lobe, as well as a quadrate and a caudate lobe. The exterior is covered in peritoneum, which is fused together with the underlying liver tissue. The hepatic porta, which is the entry port for the portal vein and the hepatic artery, is located on the visceral surface of the liver, where also the gallbladder is situated. The common hepatic bile duct exits the liver in the portal area. The liver is supported by the left and right triangular ligaments that stretch from the dorsal surface of the liver to the diaphragm, and the coronary ligament, which envelop the caudal vena cava on its direction to the diaphragm. The portal vein transports functional blood from the stomach, spleen, pancreas and intestine, and the hepatic artery brings oxygenated blood to the liver. After entering the liver, the artery and vein branch into intralobular arterioles and venules and eventually drain together into the sinusoids. (König et al., 2009)

Microscopic anatomy

Liver lobule, hepatocytes and sinusoids

The liver parenchyma is made up by hepatocytes, which are organized in rows in the *liver* lobules (Figure 10). Liver lobules are hexagonal divisions with a central vein in the middle and portal triads in the corners. Portal triads consist of an intralobular hepatic artery, an intralobular portal vein and a bile duct. Surrounding the hepatocytes are a network of sinusoids travelling from the intralobular hepatic artery and intralobular portal vein located peripheral in the lobule, towards the central vein. Sinusoids are a type of capillaries that supply the hepatocytes with blood so that exchange of molecules between the blood and the cell can take place. The blood is then transported via the central vein to larger veins

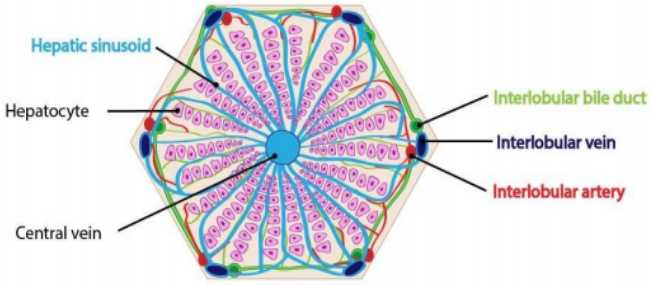


Figure 10. Schematic illustration of a liver lobule (Webpage 5)

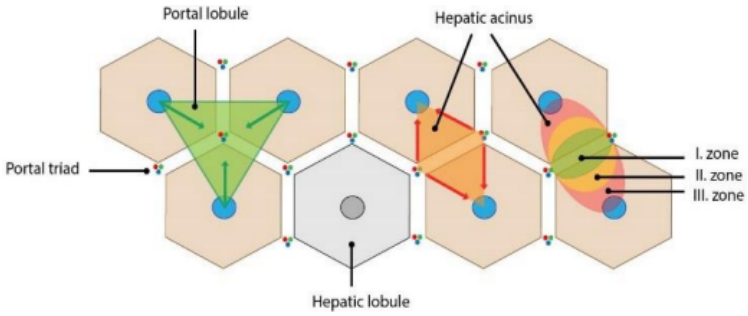


Figure 11. Illustration of the liver lobules, portal lobule and liver acinus with zones. (Webpage 5)

and eventually the caudal vena cava. The hepatocytes are characterized by their centrally located nucleus with at least one visible nucleolus, and the cytoplasm, which contains numerous lysosomes and mitochondria, Golgi complex, rough endoplasmatic reticulum (rER), smooth endoplasmatic reticulum (sER) and glycogen deposits. Each hepatocyte has three contact surfaces; one is in contact with another hepatocyte through tight junctions and desmosomes, one is in contact with the sinusoids, and one is in contact with bile canaliculi, in which bile is secreted. The bile travels through a system of increasingly larger ducts to eventually reach the extrahepatic biliary passages made up by the hepatic ducts, which drain the individual lobes, the cystic ducts, which drain the gallbladder, the two ducts, then unite and empties into the duodenum. (Frappier, 2006)

The portal lobule and liver acinus

The *portal lobule* is a triangular shaped area of liver parenchyma. The corners of the triangle are central veins from three neighboring liver lobules and in the center is a portal triad. This area shows the part of the parenchyma that excrete bile into the bile duct. Another area of the liver lobules is the *liver acinus*, which represents the vascular supply to the parenchyma. The acinus has an elliptical shape with two central veins at each pole, and two portal triads in the middle on the border between two lover lobules. The artery and vein of the portal triad branch out between the lobules forming the vascular axis of the acinus. Each half of the acinus is divided into three zones, zone 1 being closest to the axis and therefore the first to receive blood, zone 2 is in the middle towards the central vein, and zone 3 adjacent to the central vein. (Frappier, 2006). A schematic illustration of liver lobule, portal lobule and liver acinus is exemplified in Figure 11.

Physiology

As mentioned earlier, the portal vein brings blood to the liver from all the unpaired organs in the abdomen. The liver filters the molecules such as nutrients and xenobiotics arriving with the portal blood before reaching the peripheral tissues (Mukai et al., 2012). The liver is directly involved in many functions, for example glucose regulation (Mukai et al., 2012), which in the ruminant largely takes place through gluconeogenesis (Danfær, 1993). In ruminants, only an insignificant amount of glucose is absorbed through the gastrointestinal (GI) tract. Therefore, gluconeogenesis is one of the most important functions in the ruminant liver, since it ensures that there is sufficient levels of glucose available (Danfær, 1993). Propionate is the most important precursor to glucose in the

gluconeogenesis in ruminants.

Another major liver function is to eliminate xenobiotics, which are often very lipophilic. Therefore, in the liver, they undergo a two-stage process in order to become water-soluble, which make them more excretable. The enzyme cytochrome-P450 is central in this process (Mukai et al., 2012).

Diseases of the liver

Liver abscesses

Several studies have shown that rumenitis caused by acidosis significantly predisposes to, and is the most common cause for, the development of liver abscesses (Kanoe et al., 1979; O'Sullivan, 1995; Rezac, 2013; Scanlan and Hathcock, 1983; Zachary and McGavin, 2012). Ruminal acidosis causes damage to the mucosal barrier, which allows the otherwise commensal bacteria constituting the ruminal microflora, primarily *Fusobacterium necrophorum* and *Trueperella pyogenes*, to colonize the ruminal wall (Rezac, 2013; Zachary and McGavin, 2012). After colonization of the ruminal wall, bacterial emboli can enter the portal circulation, be carried to the liver, and subsequently colonize and cause abscessation within the liver parenchyma (Figure 12) (Rezac, 2013; Scanlan and Hathcock, 1983).

Liver abscesses can also develop as a sequel to traumatic reticulitis, omphalophlebitis (in the neonatal animal) and as a consequence to haematogenous spread of bacteria from other sites of infections, e.g. spread of *Fusobacterium necrophorum* from interdigital necrobacillosis (O'Sullivan, 1995).

All ages and breeds of cattle can develop liver abscesses, but feedlot cattle, especially Holstein steers (Bide, 1974), have been shown to have the highest incidence and economic impact. This is probably due to the intensive feeding with a high concentrate ration to these animals (Rezac, 2013). Usually, liver abscesses are incidental lesions, but they can cause weight loss due to reduced feed efficiency (Jensen et al., 1954; Scanlan and Hathcock 1983; Zachary and McGavin, 2012). Many hepatic abscesses resolve and, depending on their extent, leave a fibrous scar behind (Scanlan

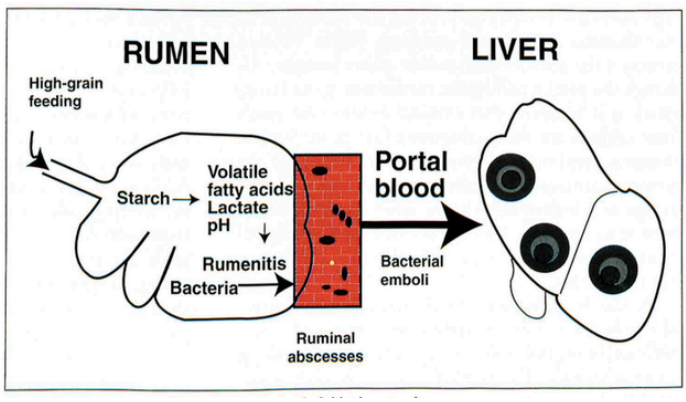


Figure 12. (Nagaraja et al., 1996) Illustration of the spreading of bacteria from the rumen, via the portal blood, to the liver, where abscesses develop.

and Hathcock, 1983) or are followed by regeneration of liver parenchyma (Jensen et al., 2010). However, liver abscesses do sporadically rupture into a hepatic vein or the caudal vena cava, which can lead to phlebitis and thrombosis. As a consequence to the blocked venous drainage, passive congestion of the liver and hypertension of the portal circulation can occur (Zachary and McGavin, 2012). Moreover, detachment of a part of a thrombus may cause a fatal septic embolization of the lungs (Zachary and McGavin, 2012).

Subphrenic abscesses

These abscesses are located in the subphrenic space, i.e. between the diaphragm and the liver. A subphrenic abscess can develop as a consequence to a suppurative infection within the abdominal cavity (Ochsner and Graves, 1933), where microorganisms are transported with the lymph through fenestrations in the diaphragm to lymph nodes in the thorax (Jensen et al., 2010). Another cause to the development of these abscesses is rupture of a liver abscess into the subphrenic space. Ochsner and Graves (1933) mentioned the following risk factors for subphrenic abscesses; biliary tract disease, liver infection, pancreatitis, gastrointestinal neoplasia and perforation of any abdominal organ.

2. Materials and methods

2.1 Data collection

The study of this Master's thesis was designed as a cross-sectional study and was carried out from August 2015 to January 2016. The sample collection took place at a cattle abattoir in Aarhus (Jutland, Denmark) on four occasions (once a week for four weeks) during August and September 2015. During each visit at the abattoir, the feet from about 30 selected young dairy cattle were collected (Table 1). In total, hind feet from 125 young dairy cattle (124 bulls and 1 heifer) were collected in the period, and out of these, all four feet were sampled from 21 animals. The distribution of the number of sampled feet during each visit at the slaughterhouse is presented in Table 1. The selected animals were between 256 and 776 days old, came from 14 different farms and weighted 301-593 kg. The majority of animals belonged to the Holstein breed, but Jersey, Danish red and crossbreeds were also represented. The specific information about the animals was received from the Danish Cattle Database after the last sampling tour, and was therefore not known at the time of collection. Following each tour to the abattoir, the material underwent the same procedure, described below.

Table 1. The distribution of the number of feet collected at each visit at the slaughterhouse.

	1st visit	**2nd visit**	**3rd visit**	**4th visit**
Front feet	5	5	6	5
Hind feet	27	24	28	25
Total	32	29	34	30

To facilitate the sample collection, calves were selected in groups of five. Shortly following slaughter, feet cut off at the carpus/tarsus were marked according to their location on the animal, i.e. a left hind foot was marked with “LH” (left hind) and a right front foot with “RF” (right front). Hereafter, all feet belonging to the same animal were put in a plastic bag, each which was marked with an ordinal number and the animal’s CHR-number. The ordinal number and CHR-number were also written on a pre-designed registration sheet (Appendix I). The material was then stored in plastic containers.

The liver and rumen of the animals in question were also examined. Every lesion observed on these organs was diagnosed, measured, noted on the registration sheet, and photographed. Representative tissue samples from selected lesions were collected for histological evaluation.

The material was transported to the Large Animal Teaching Hospital at the University of Copenhagen, where it was stored in a cold room at about 10°C during the night. The following day, the claws were trimmed using a small angle grinder, in order to reveal possible pathological findings on the sole- and bulbar horn. The first two times, only the weight-bearing surface of the claws was trimmed. However, the third and fourth time, we decided that the trimming should include the bulb horn as well, in order to better visualise the extent of the lesions. No check-up was done in order to trim the claws to the same level, whereby more horn was trimmed away on some claws compared to others. The length and width of the lesions were roughly estimated, and their localization were documented. Pathological findings such as sole haemorrhages, heel horn erosion and interdigital lesions, as well as other findings such as overgrown and/or assymetrical claw were photo documented. Immediately after finishing the trimming of the claws, they were transported to the pathology unit of the Department of Veterinary Disease Biology, where they again were put in a cold room at about 10°C for storage until the following day.

On the second day after collecting the material, all claws were examined for lesions, and all observed sole haemorrhages, white line lesions, horn fissures and skin related pathology were noted.

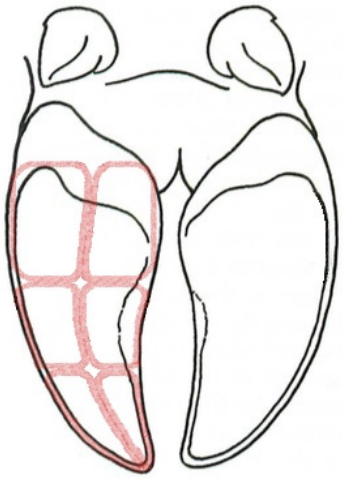


Figure 13. Illustration showing the division of the claw into six parts in order to thoroughly be able to register the localization of observed lesions.

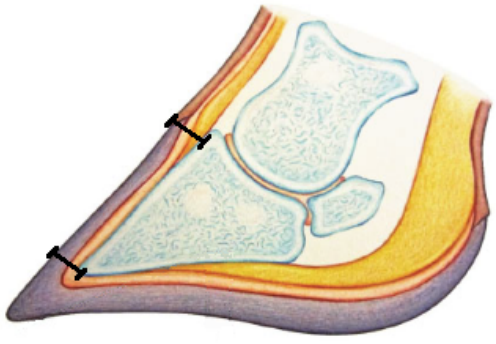


Figure 14. Illustration showing the points of measurement for evaluation of pedal bone rotation/sinkage.

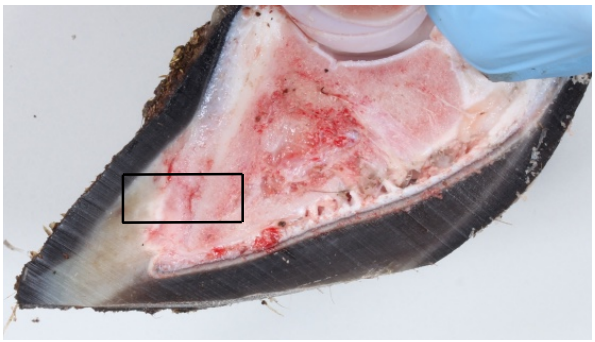


Figure 15. Sagittal section of a laminitic claw in a young dairy breed bull. The square shows the sampling area for histologic laminitis evaluation.

The lesions' spread and localization on the claw (Figure 13) were recorded on a pre-designed form (Appendix II), and the length and width of the lesions were measured and noted. Hereafter, the claws were sectioned longitudinally through all lesions to enable measurement of the depth of the lesions, which was measured from the sole horn surface. If a solear bleeding was extended in dorso-caudal direction in the depth of the horn, the additional length of the lesion was measured and recorded.

A longitudinal section was also made in the middle of the claw to enable evaluation of the pedal bone and a potential rotation/sinkage of it. In order to evaluate a rotation or sinkage of the pedal bone more accurate, the horizontal distance from the border of the horn wall to the tip of the pedal bone and the extensor process of the pedal bone, respectively, was measured (Figure 14). In representative cases (four out of nineteen) where the distance between the apex of the pedal bone to the border of the horn wall was ≥0,5 cm longer than the distance from the extensor process to the border of the horn wall, a sample for histological evaluation was collected to assess the possible occurrence of laminitis. Photo documentation was also obtained of representative findings.

Horn samples were taken from areas in the sole where haemorrhage was macroscopically observed. Samples collected from claws susptected for laminitis, were taken from the distal dorsal horn wall, involving both horn and bone tissue from the pedal bone (Figure 15).

All samples from ruminal and liver lesions, as well as sole horn haemorrhages and suspected laminitic lesions, were initially stored in a formalin solution. The ruminal, liver and horn samples were cut into an appropriate size and placed in a processing cassette, which was marked with the animal's ordinal number and a number representing the lesion in question. The samples for laminitis evaluation were put in formic acid for about five weeks to soften the bone in order to facilitate cutting.

Hereafter, the samples were imbedded in paraffin, processed and sliced to 1-2 µm, after which they were stained with hemoatoxylin eosin (HE) for regular histological evaluation. To detect erythrocytes and confirm presence of eosinophilic granulocytes, a LUNA stain was conducted. In order to the detect the haemoglobin residue iron in the horn samples, a Perl stain was done. Furthermore, a Masson trichrome stain was done to evaluate the extent of connective tissue in the ruminal samples.

The histological samples were evaluated using a light microscope (Olympus BX51), and pictures of representative histological findings were taken with a camera microscope (Olympus BX60).

2.2 Data management

All obtained data were entered into a data set in Microsoft Excel 2015. The heifer, animals older than 550 days, and five animals with missing data, were excluded from the study. The areas of the sole haemorrhages were calculated by using the recorded measurements of the length and width of these lesions. Based on the areas, a grading scale of 0-5 was then established to allow a grading of the sole haemorrhages (Table 2). When all sole haemorrhages were scored, each animal received an overall grade, determined by the highest score of the sole haemorrhages on its claws. The overall score of each animal was thereafter used to calculate the sole haemorrhage prevalence on hind feet with a 95% confidence interval (CI) by using the website VassarStats. Moreover, the prevalence and 95% CI of sole haemorrhages only visible on the sole horn surface was calculated, as well as the prevalence and 95% CI for white line lesions.

Likewise, the recordings of the ruminal and liver lesions were also put into a data set in Microsoft Office Excel (2015). The scoring system of the ruminal lesions was determined on the basis of the measured length and width of the lesions. An approximate average of the size of the ruminal

Table 2. The grading scale of sole haemorrhages.

0	No visible haemorrhage on the horn surface
1	Singular haemorrhage with an area of ≤1.5 cm^2
2	Multiple haemorrhages of <1.5 cm^2 or a singular haemorrhage with an area of 1.5-4 cm^2
3	Singular haemorrhage with an area of 4-9 cm^2 or multiple haemorrhages of 1.5-4 cm^2
4	Multiple haemorrhages of 4-9 cm^2 or a singular haemorrhage of 9-16 cm^2
5	A haemorrhage with an area of >16 cm^2 or a haemorrhage of 9-16 cm^2 with additional smaller haemorhages

Table 3. The grading scale of ruminal lesions.

0	No lesion
1	Partial or complete depapillation in an area of ≤225 cm^2 or papillary clumping
2	Partial or complete depapillation in an area of >225 cm^2 or a lesion with necrosis

Table 4. The grading scale of liver lesions.

0	No lesion
1	Cicatrices and other intrahepatic fibrous lesions
2	Abscesses or perihepatitis

lesions, i.e. 15x15 cm, was used when calculating the threshold area (225 cm^2) for the grading scale (Table 3). A scoring system was established for the liver lesions as well (Table 4). The prevalences with 95% CI's of the ruminal and liver lesions were calculated by using the previously mentioned website.

2.3 Statistical methods

To implement the relevant statistic analyses for the collected data, we used the two programs Microsoft Office Excel (2015) and GraphPad PRISM 6.0h Trial (2015). The section of Biostatistics at the University of Copenhagen provided a statistical advisory service, which we consulted at two occasions with particular focus on relevant statistical methods and correct interpretation of our results. The threshold value was set to 5%, indicating a significant result when $p \leq 0.05$.

Goodman-Kruskal gamma test

The statistical test used to investigate if there is an association between sole haemorrhages and lesions in the rumen and liver, respectively, must take the ordinal variables of the grading scales into account. This also applies to the analysis of a correlation between ruminal and liver lesions. Therefore, the Goodman-Kruskal gamma test, which is a paired nonparametric test that measures rank correlations, was used for these analyses.

The Wilcoxon test

The analysis for comparison of the lesions on the right and left foot and the medial and lateral claw, respectively, is a paired nonparametric t-test, and we decided to use the Wilcoxon test to implement these analyses.

Spearman's rank correlation

To investigate if there is an association between the daily weight gain and the total score of sole haemorrhages, a Spearman's rank correlation analysis was made. The daily weight gain was calculated based on the information about slaughter weight and age that was received from the Danish Cattle Database. In the calculation, the different birth weight of different breeds (42 kg for Holsteins and cross breeds and 35 kg for Jerseys) was also taken into consideration.

3. Results

3.1 Macroscopic evaluation of observed lesions

3.1.1 Claw

The majority of animals had abnormal claw shapes, and the claws were also extremely overgrown in many cases; rolling under the sole was observed of the abaxial wall horn, and the heel horn was very long and in some cases even expanding to cover the caudal part of the sole. Additionally, many animals had a concave distortion of the dorsal horn wall of the claws, and in some cases, horn of very

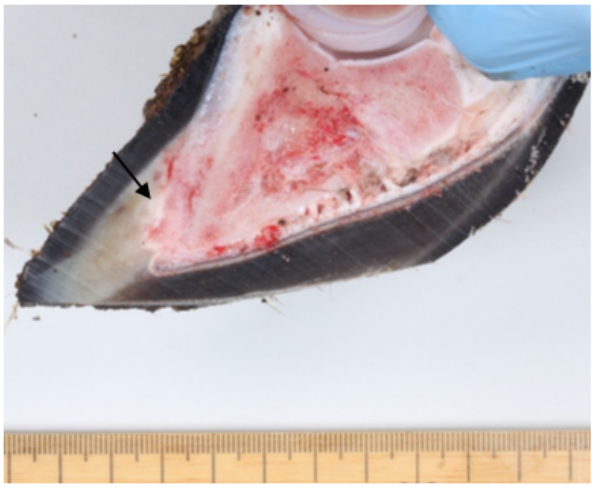


Figure 16. Longitudinal section of a laminitic hind claw from a young dairy breed bull. Downward roation of the pedal bone, a widened area of pale horn between the dorsal surface of the pedal bone and the dorsal horn wall, and an indistinct and irregular dermal border (arrow) are present.

bad quality was observed. These findings are consistent with the description of claws affected with chronic laminitis in a study by MacLean (1966).

The sectioned claws showed in some cases a widened area of pale horn between the dorsal surface of the pedal bone and the dorsal horn wall (Figure 16). This also corresponds to the findings observed in the study by MacLean (1966).

Sole haemorrhages

Virtually all claws, with only a few exceptions, presented with some degree of haemorrhage. The intensity of the haemorrhages varied between being barely visible, to showing up as profound dark red-violet discolorations. The edges of the haemorrhages on the solear and bulbar horn surface varied between having a blurry and sometimes nearly undefinable appearance, to being sharp and distinctive. In the longitudinal sections, the depth of the haemorrhages could be visualised in most cases, and ranged between 0.1-1.5 cm. In some cases, the haemorrhages could, however, only be observed on the

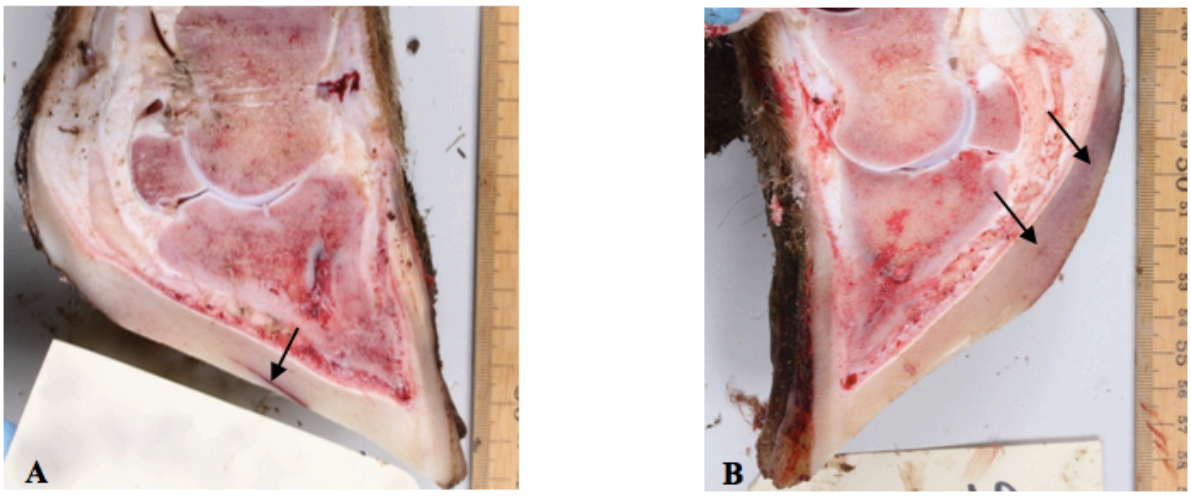
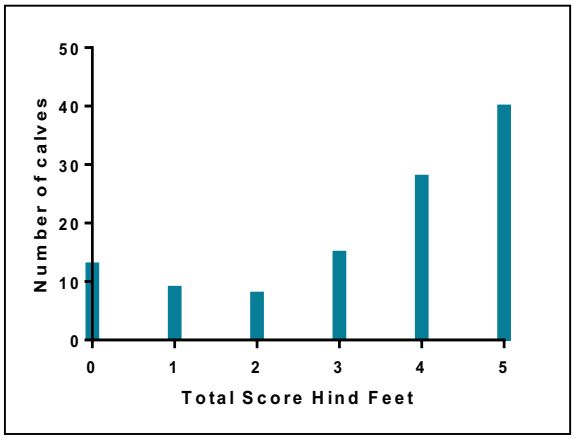
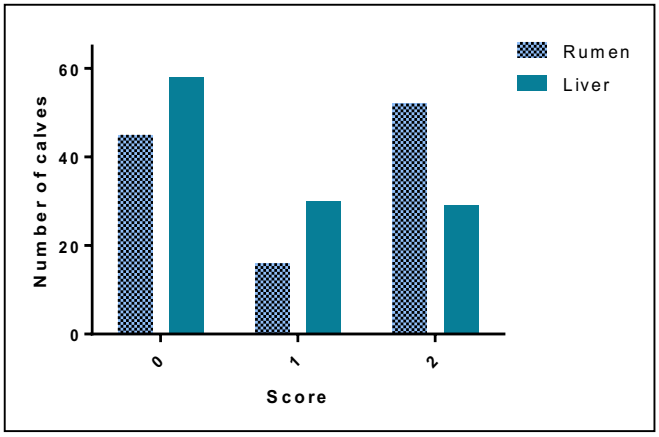


Figure 17. Longitudinal section of two hind claws from young dairy breed bulls. A distinct laminar sole haemorrhage is present in figure A (arrow). In figure B, a diffuse laminar sole haemorrhage is present in the bulbar area (approximately pointed out by the arrows).





horn surface. Additionally, haemorrhages not visible on the horn surface could be observed in the depth of the horn in some animals.

In the longitudinal sections, the haemorrhages were characterized as being of a laminar type, in that they appeared as horizontal lines in the horn, parallel to the corium. In some cases, the haemorrhagic lines were very distinct and clearly noticeable, while in other cases, they had a more diffuse and indistinct appearance (Figure 17). Multiple layers of laminar haemorrhages were observed in some animals.

Haemorrhages were recorded in all areas of the sole and bulb, however there was an evident overrepresentation in the bulbar area (see Table 5). Some claws were pigmented and therefore difficult or impossible to evaluate. As mentioned in the methods' section, the haemorrhagic lesions were given a score of zero to five. Examples of lesions given the different scores are presented in Figure 20. Score 4 and 5 were overrepresented, as illustrated in Figure 18.

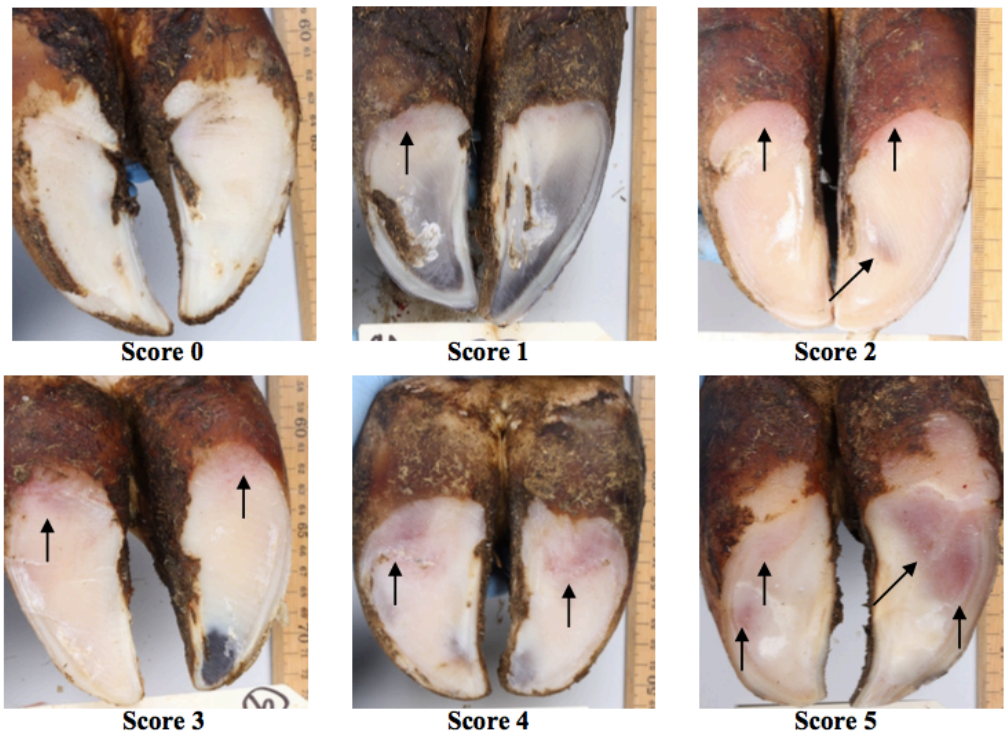


Figure 20. Hind claws from young dairy breed bulls, ventral view. Representative sole haemorrhages for each score (arrows).

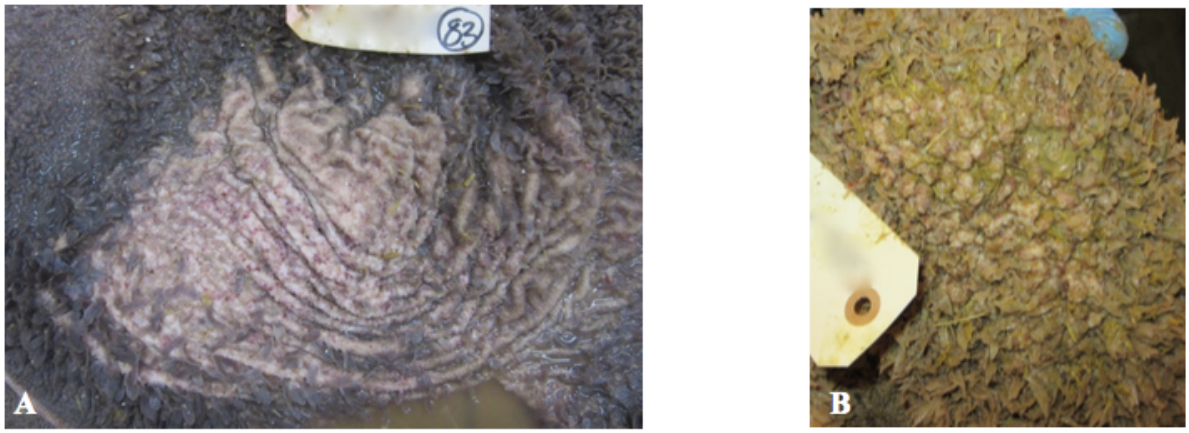


Figure 21. Young dairy breed bull, the ventral sac of the rumen. A) Complete depapillation with multifocal necrosis is present. B) Focal clumping of ruminal papillae can be observed.

Other lesions

White line lesions such as white line haemorrhages and separation of the white line were relatively commonly observed. These lesions were quite evenly localized along the white line, but a somewhat more common occurrence was observed on the lateral side of the lateral claw.

Heel horn erosion was seen in a large number of animals. Double sole was noticed in two animals, and skin lesions, such as mild digital dermatitis, were seen in a few animals. One animal had severe interdigital hyperplasia.

3.1.2 Rumen

Examination of the inside of the rumen revealed that a considerable part of the animals had some degree of rumen pathology. Findings observed in the selected animals' rumen include partial to complete depapillation with or without multifocal necrosis (Figure 21A), and focal clumping of the papillae (Figure 21B). All lesions were located in either the ventral sac or ruminal atrium, and their size varied from 30 cm^2 to 1280 cm^2. The distribution of ruminal scores is illustrated in Figure 19. Multiple warts, 0.5 cm in diameter, were observed on the ruminal pilae in one animal.

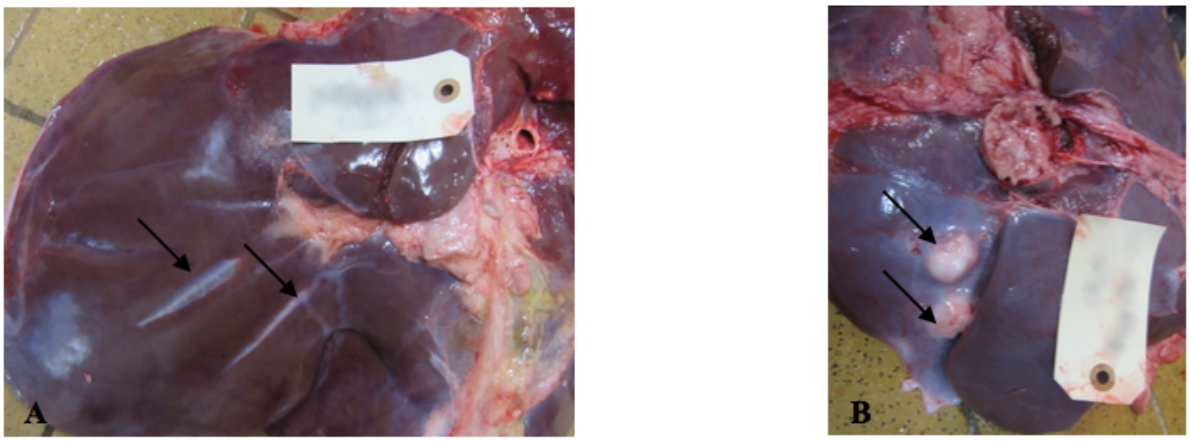


Figure 22. Livers, visceral surface, young dairy breed bulls. A) Multifocal elongated fibrous lesions on the left hepatic lobe (arrows). B) Multifocal liver abscesses on the right hepatic lobe (arrows)

3.1.3 Liver

The most common findings in the liver were focal and multifocal liver abscesses of varying size and number (Figure 22B). Moreover, subphrenic abscesses with or without fibrous adhesion were seen in a number of animals. Another common finding was cicatrices (scars), most of which were located on the diaphragmatic surface of the liver. These lesions had various shapes; round, rod- and star shaped cicatrices were observed. Furthermore, multifocal elongated fibrous lesions (Fig. 22A), between 2 and 10 cm in length, were observed on the visceral surface of the liver of some animals. Finally, chronic fibrous adhesive perihepatitis were observed in some animals. The distribution of liver scores is illustrated in Figure 19.

3.2 Histologic evaluation of observed leisons

3.2.1 Claw

In the HE samples, no erythrocytes were observed to confirm the presence of haemorrhage in the sole/bulbar horn. However, an unidentified yellow coloured content was observed in the horn tubules in these samples (Figure 23B). Attempts with several staining techniques (described in the methods' section) were performed in order to try to identify the presence of erythrocytes and/or erythrocyte residues in the horn, as well as to characterize the yellow tubular content. However, all attempts were unsuccessful.

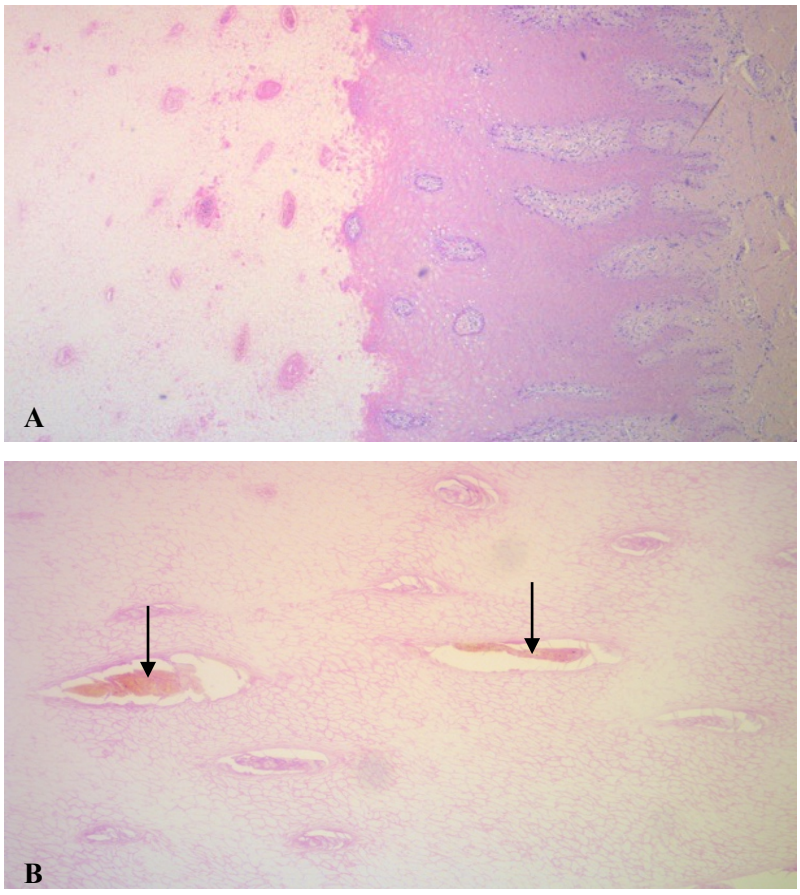


Figure 23. Histologic images of the sole horn, young dairy breed bull. HE staining. A) Overview of the sole horn structure. B) Unidentified yellow coloured content in two horn tubules (arrows).

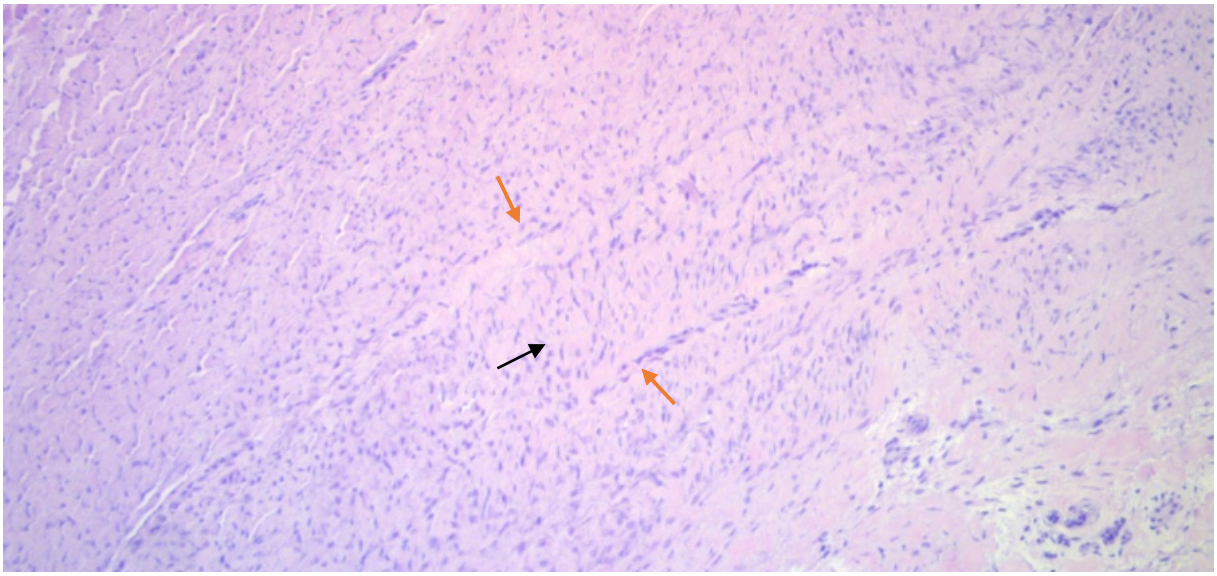


Figure 24. Histologic image of the corium in a laminitic claw, young dairy breed bull. HE staining. Fibroblasts are arranged perpendicular (black arrow) to the blood vessels (orange arrows), which is indicative of granulation tissue.

Some of the histologic samples for laminitis evaluation were of such bad quality that a thorough evaluation was impossible. The samples of acceptable quality revealed granulation tissue and neovascularization in the stratum reticulare of the dermis, which is characteristic of a chronic laminitic state (Figure 24). Moreover, a few congested blood vessels were observed.

3.2.2 Rumen

At low magnification, widespread papillar atrophy, rete peg formation and hyperkeratosis were present (Figure 25A). Progressing to a higher magnification, varying degree of epithelial hyperplasia was evident. In one sample, there was marked intercellular distension in the innermost layers of the epithelium, with visible demosomes, which is compatible with spongiosis.

Furthermore, a prominent finding in the propria-submucosa was dilated lymphatic vessels (Figure 25B). A varying degree of infiltration of neutrophilic granulocytes, eosinophilic granulocytes, lymphocytes and giant cells were also found in the propria-submucosa, as well as neovascularization. The presence of giant cells implies a granulomatous inflammation. Microabcsess formation in the

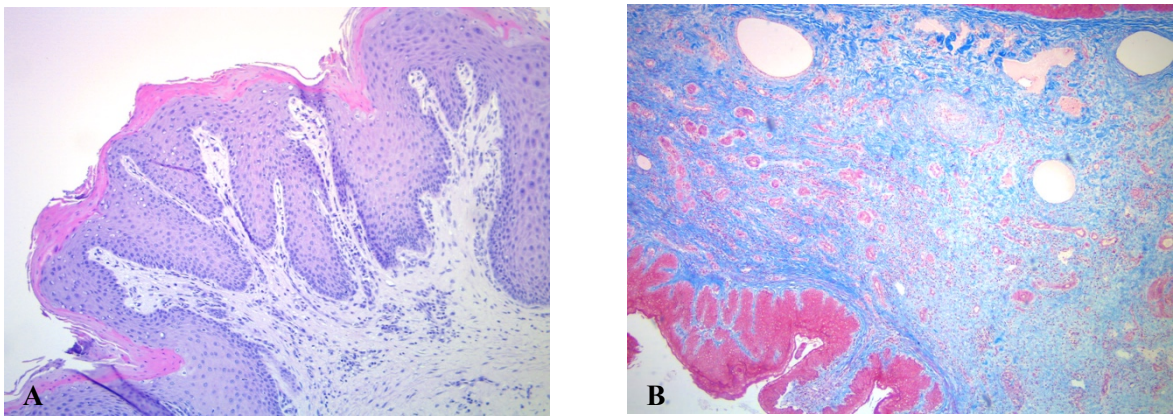


Figure 25. Histologic images of ruminal lesions, young dairy breed bulls. A) Rete peg formation, hyperkeratosis and hyperplasia of the epithelium can be observed. HE stain. B) Dilated lymph vessels and excessive connective tissue are seen. Masson trichrome stain.

propria-submucosa and epithelium were observed in some samples, as well as intraepithelial exudation (exocytose). Moreover, a single intraepithelial keratin cyst was observed in the epithelium of one animal, and epithelial necrosis was encountered in a few samples.

Presence of an excessive amount of connective tissue was confirmed in the Masson trichrome samples (Figure 25B).

3.2.3 Liver

The histologic examination of the liver samples revealed hyperplasia of the bile ducts in one sample from an elongated fibrous lesion. Furthermore, a thickening of the fibrous capsule surrounding the liver (equivalent to granulation tissue) was present in the areas of macroscopically observable cicatrices.

3.2 Statistics

3.2.1 Prevalences

The prevalence of sole haemorrhages was on hind feet 89.4%, with a 95% confidence interval of 82-94%. When sole haemorrhages only observed in the depth of the horn were excluded, a prevalence of 88.5% (95% CI = [0.81;0.93]) was obtained. In order to evaluate the distribution of different locations of the bleedings on the claws, the prevalence of lesions occurring on the caudal, central and cranial third of the claw, respectively, was calculated. These prevalences are seen in Table 5, as well as the prevalences of white line lesions and lesions in the rumen and liver. The distribution of sole

Table 5. Prevalences in percent (%) of observed lesions

Lesion	Prevalence	95% confidence interval
Sole haemorrhage		
- All sole haemorrhages	89.4	82 – 94
- Only superficial haemorrhages	88.5	81 – 93
- Localization:		
• Caudal third	87	79 – 92
• Central third	38	29 – 48
• Cranial third	11	5.9 – 18
White line lesion	31	23 – 41
- White line haemorrhages	28	20 – 38
- White line fissures	3.5	1.1 – 9.4
Ruminal lesion	57	47.7 – 65.9
Liver lesion	48	39 – 57
- Liver abscess	8	4,1 – 14.6
- Subphrenic abscess	7.2	3.6 – 13.6

haemorrhage scores is shown in Figure 18, and the scores of lesions in the rumen and liver are presented in Figure 19.

The information about abnormal claw shapes was recorded for a number of animals, but due to a too small sample size, no objective statistics were made.

3.2.1 Goodman-Kruskal gamma test

As mentioned in the methods' section, this test was used to investigate a possible correlation between sole haemorrhages and lesions in the rumen and/or liver, and also between the ruminal and liver lesions. When comparing sole haemorrhages and ruminal lesions, a p-value of 0.098 was found, whereas the comparison between sole haemorrhages and liver lesions resulted in a p-value of 0.25. Moreover, the setup of ruminal lesions versus liver lesions resulted in a p-value of 0.23. Thus, we did not obtain any clearly significant results, although a possible correlation between sole haemorrhages and ruminal lesions still might exist and needs to be discussed.

3.2.2 The Wilcoxon test

The results of the Wilcoxon tests performed to investigate if there is a significant difference between the severity of sole haemorrhages on different claws/feet, are presented in Table 6.

3.2.3 Spearman's rank correlation

This analysis resulted in a positive significant association between daily weight gain and total score of sole haemorrhages, with a Spearman r-value of 0.2027 (95% CI = [0.013;0.38]) and p-value of 0.031. Figure 16 illustrates the result.

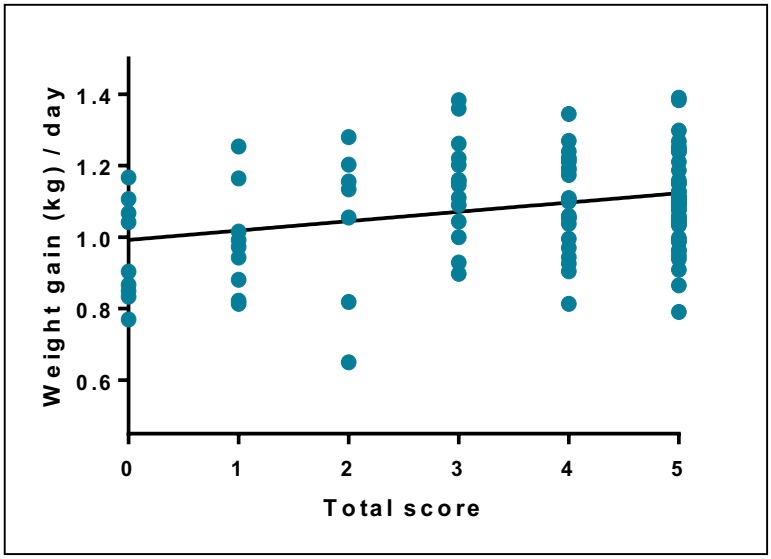


Figure 25. Graph showing the distribution of daily weight gain between the different severities of sole haemorrhages.

Table 6. The results of the Wilcoxon test.

Tested lesions	Lesions on medial vs. lateral claw on all *left* hind claws	Lesions on medial vs. lateral claw on all *right* hind claws	Lesions on left hind claws vs. lesions on right hind claws	Lesions on left *front* claws vs. lesions on left *hind* claws	Lesions on right *front* vs. lesions on right *hind* claws	Lesions on medial vs. lateral claw on all *left* front claws	Lesions on medial vs. lateral claw on all *right* front claws
P-value	0.0073	0.0046	0.86	0.20	0.36	0.18	0.51
Mean score of lesions	Medial claw: 2.42 Lateral claw: 2.76	Medial claw: 2.25 Lateral claw: 2.53	Left hind claws: 3.03 Right hind claws: 3.00	Left front claws: 3.05 Left hind claws: 3.38	Right front claws: 3.24 Right hind claws: 2.90	Medial claw: 2.57 Lateral claw: 2.90	Medial claw: 2.76 Lateral claw: 2.67
Interpretation	There is a significant difference on the severity of lesions on medial and lateral claws, respectively. The mean of the score on lateral claws are higher than the average on medial claws, indicating a possibility that lesions are more severe on lateral claws.		There is not a significant difference on the severity of lesions on left hind claws compared to right hind claws. The mean of the score of the legs are quite similar.	There is not a significant difference between the severity of lesions on the front claws compared to the hind claws.		There is not a significant difference on the severity of lesions on medial and lateral claws, respectively.	

4. Discussion

4.1 Results

4.1.1 Gross pathology

The sole haemorrhages observed in the present study were most likely the result of some sort of traumatic event. Depending on the extent and localization of the initial trauma, the haemorrhages varied in size, intensity and localization. In cases where multiple haemorrhages were present on the same claw, subclinical laminitis could be considered a cause, as proposed by Greenough and Vermunt (1991). Because of the high prevalence of ruminal lesions, which probably have developed as a sequel to ruminal acidosis due to an intensive feeding regime, it is likely that subclinical laminitis is a problem in Danish dairy bulls. However, due to the fact that the majority of sole haemorrhages were observed in the caudal part of the claws, the presence of overgrown claws might also have been a contributing factor for the development of sole haemorrhages. Overgrown claws, in the extent of the ones observed in the present study, can be assumed to cause an adverse weight distribution on the claws by moving the weight-bearing surface caudally. One can expect that this subsequently leads to an increased pressure on the dermis in this area, which will cause damage to the dermal blood vessels, and consequently result in a haemorrhage – seen as a discolouration on the sole/bulbar horn surface several weeks later. It has been suggested, that claw trimming can reduce the trauma of the corium that contributes to laminitis-associated claw lesions by restoring and maintaining a correct shape of the claw (Manske et al., 2002b). Although we can not prove this statistically in this study, one can nevertheless argue the need of regular claw trimming as a preventive measure to improve the claw health of fattening dairy bulls.

The presence of multiple haemorrhagic lesions in the sagittal sections of the horn could be explained by several traumas to the dermal area, giving it the characteristic streaky appearance as described by Greenough (2007).

The diffuse, barely distinguishable lesions observed in some cases, may be attributed to diffusion of blood down the horn tubules, following trauma to the dermis (Greenough, 2007). Whether or not the aetiology behind the more diffuse appearance and the more clearly linear one is the same or different, remains unclear. The age, and therefore the time for the initial insult, is difficult to determine due to great variance in the rate of horn growth. It has been proposed that factors such as excessive wearing, high pressure on the dermis and intensive feeding promotes horn growth, and that the haemorrhagic lesion will reach the solar surface roughly 6-10 weeks post initial trauma (Greenough, 2007).

Earlier studies investigating sole haemorrhages in cattle (Bergsten, 1994; Capion et al., 2008a; Capion et al. 2008b; Greenough and Vermunt, 1991; Leach et al., 1998; Manske et al. 2002a; van der Spek et al., 2013) focused on living animals and, consequently, did not have the opportunity to

evaluate the depth of the lesions. Hence, the grading scales in these studies are built-up by the areas of the sole haemorrhages, without taking the depth of the lesions into consideration. In order to compare the results of the present study with the results of others, the basis for our grading scale are the areas of the lesions as well.

It can not be ruled out, that post-mortem events in the claws might have affected the size and intensiveness of the lesions, which then might have influenced our results. This suspicion comes from observations done during the trimming session on the first day after slaughtering, where preliminary estimations of the size of lesions were made and registered. When comparing these measurements to the ones obtained on the second day post-mortem, differences in size and intensity of lesions were recognized. However, differences in lighting and in the ability to properly measure the lesions varied between the two occasions, making it questionable as to how significant these differences are. Therefore, we have not pursued any further interest to this in this study.

4.1.2 Histology

No erythrocytes or residues from erythrocytes were visible in the histological samples. This might be explained by a shorter half-life of erythrocytes outside the blood vessels compared to inside the blood vessels. Another reason might be the fact that the lesions we macroscopically identified as haemorrhages were of a relatively old age, which may have led to disintegration of the substances that normally would get stained by HE, LUNA or Perl dye. However, it can not be ruled out that the yellow coloured tubular content observed in the HE stain was some form of residue from erythrocytes.

4.2 Statistics

4.2.1 Claw

The prevalence of sole haemorrhages in this study (89.4%) is higher than the prevalences presented in other studies on both young and old animals, which reach between 13.9-79% (Andersson and Lundström, 1981; Bergsten, 1994; Capion et al., 2008b; Frankena et al., 1992; Leach et al., 1998; Manske et al., 2002a; van der Spek et al., 2013). However, previous researchers have investigated the prevalence amongst dairy cows and/or heifers, and due to the differences in study populations between this and other studies, a direct comparison might be inaccurate. Moreover, in the present study, the claws were examined post-mortem, which enabled sagittal sectioning and visualization of deep haemorrhages as well as superficial haemorrhages. It was hypothesized, that this would result in a higher prevalence than when only superficial haemorrhages were registered, which was confirmed in the study (Table 5).

The prevalence of white line haemorrhages (28.3%) was noticeably higher than in other studies, where prevalences of 12% (Manske et al., 2002a), 9.6% (Koenig et al., 2005) and 6% (Capion et al., 2008b) have been observed. However, the results might not be comparable due to the different study populations. Moreover, the high prevalence presented in the present study supports the theory of

subclinical laminitis being a problem in the intensive production system of fattening dairy bulls.

The statistics made to enable a comparison between haemorrhages on different claws, showed a significant difference between the severity of lesions on the lateral and medial hind claws, and suggested that haemorrhages on the lateral hind claws are more severe than on the medial claws. This confirms the findings by MacLean (1971), Greenough and Vermunt (1991), Bergsten (1994) and Leach et al. (1998). On the contrary to other studies (Bergsten, 1994; Andersson and Lundström, 1981; Manske et al., 2002; Leach et al. 1998), no significant difference was found in the severity of lesions on front claws compared to hind claws. As the result of earlier studies implies, it was expected that lesions on the hind feet were more severe than lesions on the front feet. The absence of a significant result regarding this in the present study might be attributed to the small number of animals from which all four feet were collected and which had a complete set of data. The present study did neither find a significant difference on the severity of lesions on the medial and lateral claws on the front feet, where the medial claws generally are more severely affected, according to earlier studies (Vermunt and Greenough, 1996; Bergsten, 1994).

The positive significant correlation between the daily weight gain and the severity of sole haemorrhages could possibly be explained by heavier animals, larger forces affecting the claws, and therefore a higher risk of developing severe sole haemorrhages. A high daily weight gain might be associated with a high concentrate intake, which predisposes the animal to develop laminitis and, subsequently, more extensive sole haemorrhages.

4.2.2 Rumen

No statistical relationship between ruminal lesions and liver lesions was observed in this study. Neither was there a significant correlation between ruminal lesions and the severity of sole haemorrhages. However, the lack of significance might be due to a too small sample size. The absence of significant correlations might also be explained by having completely healed, and therefore not visible, lesions in some rumens and/or livers.

A prevalence of 57% of ruminal lesions was found in the present study. The lesions observed were consistent with chronic ruminitis, most likely as a result of an acidic state in the rumen. Previous studies investigating ruminitis in cattle are scarce, but some observed prevalences are 24.1% (Rezac et al., 2013), and 37.6% (Jensen, 1954), 50.9% (Thompson et al., 2008).

The gross pathologic findings as well as the histologic findings observed in the present study corresponds to the findings done by Jensen et al. (1954). However, Jensen et al. (1954) registered lesions with a size of maximum 20 cm^2, which stands in contrast to the present study, where notably larger lesions were registered.

4.2.3 Liver

There was no significant correlation between liver lesions and the severity of sole haemorrhages. This could be explained by the same factors as the ones mentioned in the rumen section.

At the abattoir, some of the livers were discarded before we had the opportunity to examine them for lesions. We recieved information about the cause of discard in all cases, but do not know if the livers in question also had other lesions.

The prevalence of subphrenic abscesses have, to our knowledge, not been previously investigated in a population of fattening dairy bulls. Therefore, it is difficult to evaluate if the prevalence of 7.2% in the current study population corresponds to the actual prevalence.

5. Conclusion

The purpose of this study was to investigate the prevalence of sole haemorrhages in Danish fattening dairy bulls, and also to find out if these lesions had a correlation to ruminal and liver lesions. To our knowledge, this is the first study that has examined postmortal material for sole haemorrhages in this population. The prevalence of sole haemorrhages on hind feet was high (89.4%), and the lateral hind claws were the most severely affected of all claws. A large proportion of the animals also had ruminal lesions (57%), which was consistent with chronic ruminitis, probably as a result of an acidic state in the rumen. We propose, that there is a correlation between ruminal lesions and sole haemorrhages, and that it is possible, that the sole haemorrhages develop as a sequel to subclinical laminitis. Another contributing factor for the high prevalence of sole haemorrhages, might be the high occurrence of overgrown claws.

6. Perspectives

For further investigation, studies focusing on the following subjects are of interest;

- How the housing (bedding material, flooring) and management (feeding regimes) of fattening dairy bulls affect the prevalence of sole haemorrhages.
- Examination of living animals to investigate the occurrence of lameness.
- Welfare aspects regarding the high prevalence of claw pathology, especially subclinical laminitis and sole haemorrhages, in the fattening dairy bull population.
- Examination of animals in the acute stage of an experimentally induced disease in order to clarify the pathogenesis behind the development of sole haemorrhages.
- Investigate if there is an association between sole haemorrhages and/or white line lesions with abnormal claw shape (overgrown horn).
- Prevalence of subphrenic abscesses and their impact on welfare and productivity in dairy cattle (including fattening dairy bulls).

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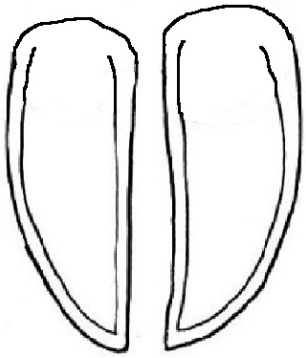
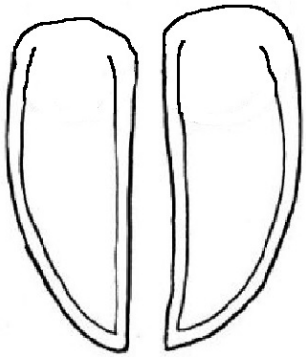
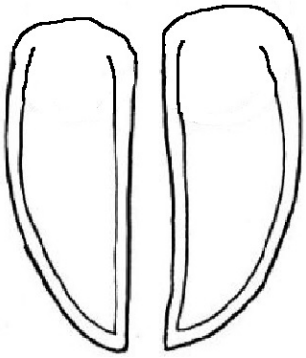
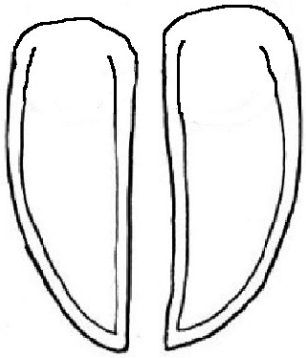
Appendix I: Registration sheet at the abattoir

Slagteriprotokol

Kalvenummer	CHR-nummer	Slagtenummer	Lever		Vom	
			Histologi		**Histologi**	
			Billede		**Billede**	
			Histologi		**Histologi**	
			Billede		**Billede**	
			Histologi		**Histologi**	
			Billede		**Billede**	
			Histologi		**Histologi**	
			Billede		**Billede**	
			Histologi		**Histologi**	
			Billede		**Billede**	

Appendix II: Registration sheet for observed lesions

Calf no.	
CHR-no.	

	Lesion, localization, size and description	**Measurments**
Left front		**Coronary band:** L: M: **Toe:** L: M:
Right front		**Coronary band:** L: M: **Toe:** L: M:
Left hind		**Coronary band:** L: M: **Toe:** L: M:
Right hind		**Coronary band:** L: M: **Toe:** L: M: