Histopathology of Oligofructose-Induced Acute Laminitis in Heifers

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ABSTRACT

Histopathology of the dermo-epidermal junction in the lamellar region of front claws was examined in 6 dairy heifers given an alimentary oligofructose overload and compared with sections from a control group of 6 heifers. Four of the 6 heifers administered oligofructose developed clinical signs of acute laminitis before they were euthanized. Postmortem samples from front claws were processed for histology. Eleven histopathologic characteristics were selected from the existing literature and used in a blinded evaluation of sections. In total, 104 front claw samples, including 8 samples from 2 cows having spontaneously occurring acute laminitis, were evaluated histologically using hematoxylin and eosin as well as periodic acid-Schiff staining. The major morphological features associated with oligofructose-induced acute clinical laminitis were stretching of lamellae, dermal edema, hemorrhage, changes in basal cell morphology, presence of white blood cells in dermis, and signs of basement membrane detachment. Changes at the lamellar junction of claw tissue affected by oligofructose-induced clinical laminitis resembled tissue from the 2 cows suffering from spontaneous acute clinical laminitis, and generally were consistent with existing descriptions of laminitis histopathology. Important exceptions to existing descriptions in the literature were stretching of lamellae and basement membrane changes. Not previously described, we considered these early signs of acute laminitis. In conclusion, this study documents that oligofructose-induced clinical laminitis is associated with histopathological changes at the lamellar interface. A weakened dermo-epidermal junction is a possible intermediate stage in the pathophysiology of bovine sole ulceration at the typical site.

(**Key words:** histopathology, bovine, laminitis, oligofructose)

Abbreviation key: WBC = white blood cells.

INTRODUCTION

Laminitis is a common cause of lameness in cattle and causes significant loss in production. Descriptions of the disease include a number of different stages: acute, subacute, chronic, and subclinical. It has been difficult for researchers to establish the mechanism that links aseptic inflammation of the lamellae: *laminitis* or pododermatitis aseptica diffusa to the less acute forms of the disease. Current understanding of the etiology and pathophysiology is incomplete, and it is unknown whether aseptic inflammation plays an important role in the development of the disease or merely exists as a side effect with minor influence.

Severe cases of chronic laminitis are characterized by a deformation of the claw, the so-called slipperfoot (Ossent et al., 1997): the claws become flattened, broad, with a concave and furrowed dorsal wall, the white line becomes widened, soft, and discolored. In addition, sole and white line hemorrhages, double sole, and ulcers at the typical site (Rusterholz ulcers) are considered possible consequences of laminitis. Although some of these lesions, for example, sole ulcers, can undoubtedly be related to mechanical factors such as excessive abrasion caused by concrete floors (Bergsten and Frank, 1996), an association with chronic laminitis has been recognized in epidemiological studies (Bergsten, 1994; Lischer et al., 2000; Manske et al., 2002). One explanation for development of laminitis-associated sole ulcers was recently provided (Lischer et al., 2002). Postmortem findings in 19 cows with sole ulcers at the typical site demonstrated decreased height (depth) of solar soft tissue compared with normal cows without ulcers. The authors argued that solar compression, which induced corium hypoxia and eventually sole ulceration, developed because the third phalanx sank from its normal suspended position in the claw capsule (Mülling and Lischer, 2002). Further, laxity of the “suspensory apparatus” of the third phalanx was considered responsible for the downward dislocation of the bone. The suspensory apparatus was defined to include all structures between the surface of the third phalanx and the inner aspect of the cornified hoof capsule, thus consisting of a deeper dermal component and an epidermal component

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including the living cell layers of the epidermis. The authors considered the dermal component the weakest link (the locus minoris resistentiae) in which loosening and elongation of tissue occurred, because a separation between dermis and epidermis has never been demonstrated in cattle, unlike the situation in horses. The theory was further supported by *in vitro* evidence of weakened connective tissue near the time of first calving, in which sole ulcers are particularly frequent (Tarlton et al., 2002). It was assumed that periparturient hormonal changes (e.g., relaxin or estrogen) resulted in increased elasticity of connective tissues leading to increased susceptibility to sole ulcers.

Although these plausible theories explain how laminitis-associated sole ulcers develop and why lesions are related to calving, it is not clear why chronic laminitis cases develop lesions originating from the epidermal component such as a widened white line with soft and discolored keratin. In equine laminitis research, changes taking place at the dermo-epidermal junction in the early stage of the disease have been studied extensively using experimental models. A close relation between changes of the ultra structural anatomy, elongation of epidermal lamellae and eventually separation between the dermis and epidermis has been established by the use of immunohistochemistry and electron microscopy (French and Pollitt, 2004a,b). Until now, experimental induction of bovine laminitis has been difficult; and currently no histological description of the lamellae in the early stage of acute bovine laminitis exists. The present study takes advantage of a recently discovered experimental model for induction of laminitis in heifers (Thoenes et al., 2004), and postulates that stretching of the epidermal lamellae as well as other histological changes are indeed taking place at the dermo-epidermal junction during the early hours of acute laminitis in cattle. Therefore, the objective of the present study was to examine the histopathology of claw tissue from heifers in which acute laminitis was induced with an alimentary oligofructose overload.

MATERIALS AND METHODS

Animals

Twelve nonpregnant dairy heifers (1 Guernsey, 3 Jersey, 6 Holstein, and 2 Ayrshire) weighing between 325 and 505 kg were fed mixed grass-lucerne (alfalfa) hay to ensure good ruminal function. They were trained to accept close handling for 4 wk and kept in a paddock with a soft surface. After acclimation, the heifers were assigned to 2 groups of 6 heifers each. Treated heifers were administered a bolus of oligofructose (Raftilose P95; Orafit Group, Tienen, Belgium) via a stomach tube, whereas the other group served as the control

group and was sham-treated with tap water. Oligofructose was dissolved in tap water (solubility = 80 g/100 mL, Orafit product book version 03/98) and both groups received volumes equivalent to 6 L per 100 kg of BW. Treated heifers given oligofructose were allocated to 3 subgroups of 2 heifers each and dosed with 13, 17, or 21 g of oligofructose per kg of BW. Five percent of the bolus dose was given as a priming dose twice daily for 3 d before the experiment, to gradually adjust the forestomach microflora to the new source of carbohydrate. The 3 doses were used to investigate any dose-response trend in the clinical effect of oligofructose dosing.

The rationale for using oligofructose and the clinical response to this particular sugar was described elsewhere (Thoenes et al., 2004). Three monitors (all veterinarians), blinded to previous results, recorded the clinical responses of the heifers in a nonconsecutive rotation. Lameness was classified as none, mild, moderate, or severe; and response to hoof testers was positive or negative (Thoenes et al., 2004). In short, rumen acidosis, diarrhea, mild dehydration, metabolic acidosis, and transient fever were characteristics associated with oligofructose administration. Four of 6 heifers given oligofructose displayed lameness beginning 39 to 45 h after oligofructose dosing and showed positive pain reactions when the sensitivity of the claws was examined using hoof testers (a commonly used instrument to check for pain reaction during a routine examination of the claws). Acute laminitis (Ossent et al., 1997), a clinical disease with a rapid onset of foot pain and detectable lameness, is seen shortly after alimentary overload of readily fermentable carbohydrate. Signs of claw inflammation (e.g., warmth and increased pulsation) may be present, but no claw lesions can be identified visually. Following this clinical definition, 4 heifers could be classified as laminitis positive after the oral oligofructose overload. Two heifers given oligofructose (one dosed with 13 g/kg and the other with 21 g/kg) and all control heifers displayed no clinical signs of acute laminitis. No obvious trend between lameness and oligofructose dose was detected.

The protocol was approved by the Animal Ethics Committee, The University of Queensland, and heifers were inspected by the Animal Welfare Officer during the study (Animal Ethics Approval Certificate SVS/125/02/RVAUC). Ten heifers were euthanized, by captive bolt and exsanguination, 48 h after oligofructose administration, and 2 were euthanized at 72 h.

Claw Sections

The distal part of front limbs was separated from cadavers 5 to 10 min after euthanasia and kept on ice

during transportation to processing facilities (less than 1 h). A band saw was used to harvest a horizontally oriented tissue slice from the distal aspect of claws. The first cut was made through the long axis of the limb separating medial from lateral claws. A horizontal cut then removed the sole and the most distal 2 mm of the solar corium to expose the distal surface of the third phalanx. The third cut was placed 1.5 cm more proximally and parallel to the second, and generated a tissue slice of the distal claw with the sole removed. Finally, in preparation for later sampling of 2 tissue blocks (one dorsal and one abaxial), radial cuts into the slice were made 1 cm apart, just through the claw wall. Claw slices were fixed for a minimum of 24 h in 10% formalin phosphate-buffered saline; tissue blocks consisting of claw wall and adjacent soft tissue were then dissected free from the third phalanx. Faces of a tissue block were trimmed to avoid artifacts generated by the band saw. The inner 1 mm of the stratum medium and the lamellar layer were harvested for microtome sectioning. Formalin-fixed tissues were treated with alcohol, xylene, and paraffin, following the routine for histological samples, using an automatic tissue-processing machine. Sections (4 μ m thick) were cut and stained with Mayers hematoxylin and eosin and periodic acid-Schiff.

A list of characteristics, considered to represent possible pathologic changes of tissue affected by acute laminitis, was made from initial inspection of sections and from a literature review (Obel, 1948; Nilsson, 1963; MacLean, 1971; Andersson and Bergman, 1980; Boosman, 1991; Pollitt, 1996). To facilitate comparison and achieve consistency among samples, it was agreed to focus on the following 11 characteristics: tapering of epidermal lamellae, hyperemia, hemorrhage or edema of the dermis, presence of white blood cells (**WBC**) within blood vessels or outside vessels, changes in shape of lamellar basal cells, alterations in chromatin density of basal cells, necrosis of basal cells, number of suprabasal cell layers, and detachment of the lamellar basement membrane.

Tapering of epidermal lamellae was judged at the innermost tips of the dermo-epidermal transverse section. Epidermal lamellae were classified as stretched if the noncornified tips were stretched to a width equivalent to 1 to 2 basal cells. Hyperemia of the dermis was recorded if an increased number of red blood cells were present in the vessels. Hemorrhage was defined as red blood cells detected outside vessels. Edema was noted if normal tissue components were spread apart, giving the tissue a less distinct appearance, in a manner judged not to be artifact. Presence of WBC in vessels or outside vessels was classified positive. A change in shape of basal cells was recorded if a majority of basal cells were wider when compared with the normal, nar-

row, cylindrical cell morphology. Alteration of chromatin density was recorded if most basal cell nuclei displayed dispersed light-staining chromatin (euchromatin) judged against the normal condensed and intensely basophilic chromatin (heterochromatin) of epidermal basal cells. Pyknosis, karyorrhexis, or karyolysis of several basal cells were considered as signs of tissue necrosis. The number of suprabasal cell layers bordering the cornified part of the epidermal lamellae was estimated. One or 2 layers were classified as normal, whereas 3 or more were judged to be increased. Separation between basal cells and basement membrane was examined using periodic acid-Schiff—stained sections. Detachment of basement membrane was recorded if a discontinuity was observed in the tight association normally present between basal cells and basement membrane.

Heifer identity of sections was then blinded and the sections were evaluated in a systematic manner by one of the authors experienced in hoof and claw histopathology (Ove Wattle). Assessment of characteristics was recorded in only 2 classes as either yes/no or normal/abnormal according to the general impression of a characteristic in the section. Heifer identity code was then broken and findings from histopathology compared with the actual clinical diagnosis (laminitis: yes/no).

Claw sections from heifers given oligofructose were finally compared with front claw sections from 2 cows that displayed severe signs of acute laminitis for 1 wk. The cows were referred to the Large Animal Hospital, the Royal Veterinary and Agricultural University, Denmark. One cow was admitted because of persistent pneumonia unsuccessfully treated with antibiotics, the second because of dystocia, which was followed by peritonitis and retained placenta (sequelae of cesarean section).

RESULTS

Sections from 104 front claw samples (8 from each heifer), including 8 samples from the 2 cows referred to the veterinary teaching hospital, were evaluated for the 11 selected characteristics. Results from controls and heifers given oligofructose (96 samples) are summarized in Table 1 and illustrated in Figure 1.

In controls, the dermal and epidermal lamellae interlocked tightly. The tips of epidermal lamellae were rounded (Figure 1A) and, in periodic acid-Schiff—stained sections, always in a close association with the distinct basement membrane (Figure 1B). The basement membrane was wider and more blurred away from the lamellar tip (closer to the hoof wall). The noncornified part of the epidermal lamellae (just before the tip) always consisted of several cell layers. Most epidermal basal cells were cylindrical and oriented perpendicular

Table 1. Frequency of abnormal findings in claw sections from 6 heifers (1 to 6) given oligofructose compared with sections from 6 control heifers (7 to 12).¹

Characteristic	Class	Treatment ²															
		Oligofructose								Controls							
		1*	2	3*	4*	5	6*	Sum	Abnormal	7	8	9	10	11	12	Sum	Abnormal
Pointed or stretched lamellae	No	5	8	5	2	6	3	29		6	8	7	8	7	8	44	
	Yes	3	0	3	6	2	5	19	40%	2	0	1	0	1	0	4	8%
Hyperemia	No	7	4	4	6	6	8	35		4	8	7	7	8	8	42	
	Yes	1	4	4	2	2	0	13	27%	4	0	1	1	0	0	6	13%
Hemorrhage	No	7	5	3	2	6	8	31		6	7	6	8	8	8	43	
	Yes	1	3	5	6	2	0	17	35%	2	1	2	0	0	0	5	10%
Basal cells wider	No	2	3	1	0	3	1	10		8	8	7	8	6	8	45	
	Yes	6	5	7	8	5	7	38	79%	0	0	1	0	2	0	3	6%
Chromatin density low	No	1	0	0	0	2	1	4		8	8	8	8	8	8	48	
	Yes	7	8	8	8	6	7	44	92%	0	0	0	0	0	0	0	0%
White blood cells in vessels	No	2	7	5	1	4	6	25		7	7	5	2	2	4	27	
	Yes	6	1	3	7	4	2	23	48%	1	1	3	6	6	4	21	44%
White blood cells in tissue	No	6	8	8	5	8	8	43		8	8	7	8	8	8	47	
	Yes	2	0	0	3	0	0	5	10%	0	0	1	0	0	0	1	2%
Edema	No	3	6	5	5	7	6	32		8	8	8	8	8	8	48	
	Yes	5	2	3	3	1	2	16	33%	0	0	0	0	0	0	0	0%
Basement membrane detached	No	6	8	7	7	8	8	44		8	8	8	8	8	8	48	
	Yes	2	0	1	1	0	0	4	8%	0	0	0	0	0	0	0	0%
Necrosis	No	8	8	8	8	8	8	48		8	8	8	8	8	8	48	
	Yes	0	0	0	0	0	0	0	0%	0	0	0	0	0	0	0	0%
Increased suprabasal cell layers	No	5	8	3	7	8	4	35		6	7	8	8	8	8	45	
	Yes	3	0	5	1	0	4	13	27%	2	1	0	0	0	0	3	6%

¹Two sections were harvested from each of the 4 front claws in all heifers: 1 section from the dorsal aspect and 1 from the abaxial aspect. Thus, 8 sections from each of 12 heifers were evaluated by light microscopy for abnormality in 11 histopathologic characteristics. Four heifers given oligofructose had a clinical diagnosis of acute laminitis, marked with an asterisk (*).

²Heifers 4, 5, 6, 7, 8, and 12 were Holstein-Friesians; heifers 1, 3, 9, and 11 were Channel Islands; and heifers 2 and 10 were Ayrshires. Heifers 1 and 2 were given 12 g of oligofructose per kg of BW; heifers 3 and 4 were given 17 g/kg, and heifers 5 and 6 were given 21 g/kg.

(or slightly angled) to the basement membrane. In hematoxylin and eosin sections, the basophilic nuclei (i.e., heterochromatin) of basal cells were elongated or oval and located away from the basement membrane with an orientation along the cell itself (Figure 1C). Normally 1 or 2, or occasionally 3, suprabasal cell layers were present between the basal cells and the cornified part of the epidermal lamellae. These suprabasal cells were elongated and flat and orientated parallel to the direction of the cornified epidermal lamellae. A nonstaining halo surrounding the nuclei of basal or suprabasal cells, most likely a result of cytoplasmic shrinkage due to the tissue processing, was sometimes seen. Only occasionally were isolated pyknotic cells seen. The weakly stained cornified part of the epidermal lamellae extended variably from about 75% to nearly the entire length of a lamella. The cornified cells were normally orientated along the axis of a lamella in a continuous cornified rod. Frequently, segments of cornification were separated by a few noncornified cells (Figure 1A). Infrequently, areas with a circular cell orientation forming an acidophilic horn-like body were seen. The amount of cap horn varied depending on the exact location of the section. White blood cells were found in vessels, but only in 1 section of 48 samples in the tissue.

Moreover, hyperemia was present in some sections, and hemorrhage was seen only occasionally.

In heifers administered oligofructose, the inner part of epidermal lamellae often appeared stretched and reduced to a width of only 1 or 2 cells (Figures 1D and 1E). An increased distance between the tip and the cornified part of the lamella indicated that lamellar stretching occurred primarily in the noncornified tip of the epidermal lamellae (Figure 1D). In periodic acid-Schiff sections, the basement membrane had a more folded and blurred appearance along the perimeter of the stretched part of lamellae. In some sections, folds of basement membrane were seen at the very tip of the lamellae (Figure 1F). Occasionally, the basement membrane was separated from the basal cells, forming small, nonstained vacuoles between these 2 structures at epidermal tips (Figure 1E). Nearly all epidermal basal cells displayed subtle changes in their morphology. Nuclei frequently showed a coarser meshed chromatin network, stained less intensely, and appeared less cylindrical. When these changes were observed, the rounded enlarged nuclei containing more euchromatin often had a more central position in the cell. Mitotic figures were rarely seen. The basal cells themselves were often enlarged and wider. Many of them had a

Figure 1. Cross sections of the lamellar layer from control heifers (A, B, and C), and heifers given oligofructose (D, E, F, G, and H). The left side of the pictures is directed toward the inner parts of the claw. B = basal cells, SB = suprabasal cells, BM = basement membrane, DL = dermal lamellae, EL = epidermal lamellae. (A) Hematoxylin and eosin stain. Normal appearance of the inner part of the lamellar layer. (B) Periodic acid-Schiff stain. Rounded epidermal tip in the normal close association with its basement membrane. (C) Hematoxylin and eosin stain of the middle part of an epidermal lamella. Cylindrical basal cells with elongated and basophilic nuclei oriented along the long axis of the cells. (D) Hematoxylin and eosin stained section with severely stretched lamellae. The innermost part of the epidermal lamellae is only 1 to 2 cell layers wide. Often stretching was less obvious than this. (E) Details from epidermal tips, periodic acid-Schiff stain. Stretched lamellae with a few small, nonstained vacuoles formed by basement membrane separation at the epidermal tips. Inserted element in the bottom left hand corner of the figure is from a different heifer. (F) Periodic acid-Schiff stain. Details from tip of epidermal lamella. The basement membrane has a folded and blurred appearance. (G) Hematoxylin and eosin stain of the middle part of epidermal lamella. Cuboidal basal cells with rounded and rather centrally positioned nuclei. Nuclei have a coarse chromatin network. (H) Hematoxylin and eosin stain of the middle part of an epidermal lamella. Increased number of suprabasal cells, minor hemorrhage, and a white blood cell (WBC) in a small blood vessel.

more cuboidal appearance (Figure 1G). Often, the number of suprabasal cell layers along the cornified part of the lamellae increased to more than 3 layers (Figure 1H). Only occasionally were pyknotic cells noticed, but areas with obvious signs of tissue necrosis were not seen in any of the samples. Hyperemia and WBC in vessels were not general features, but were present in a number of sections (Figure 1H). Hemorrhage, edema, and WBC (mainly granulocytes) were sometimes seen outside vessels. Thrombotic emboli in blood vessels were not seen in the examined tissues.

Sections from the cows that developed severe clinical signs of acute laminitis had many characteristics in common with sections from heifers given oligofructose (data not shown in Table 1). Stretched and pointed lamellar tips with folded basement membrane were seen together with scattered areas of wider epidermal basal cells. Occasionally, areas with detached basement membrane were seen at the tips of the epidermal lamellae (Figure 2A and 2C). Detachment was especially prominent in a section from the cow with peritonitis (Figure 2D). However, nuclear chromatin had, in most sections, increased heterochromatin resembling control tissue. Numbers of suprabasal cell layers along the cornified epidermal lamellae were few. In the cow with pneumonia, the tips of some stretched lamellae had a bulb (resembling the end of a drum stick) containing basal cells that were outlined by a smooth basement membrane (Figure 2A). This was never seen in control heifers or those given oligofructose.

DISCUSSION

Stretching of lamellae, dermal edema, hemorrhage, changes in basal cell morphology, presence of WBC in dermis, and signs of basement membrane detachment were associated with oligofructose-induced acute clinical laminitis in this study. Some variation was observed in a preliminary examination of sections, and a new evaluation system was developed to reduce observer discrepancies. All sections were therefore evaluated by one person, using a set of defined criteria in assessment of characteristics that were considered attributable to actual tissue differences. Nevertheless, tissue sections from the heifers administered oligofructose did not display identical histopathology, and within the controls, some variation was also apparent.

The 4 heifers, however, that developed clinical laminitis had a noticeable and recognizable histopathology (Table 1). For example, 17 of 32 (53%) sections in laminitis-positive heifers showed stretching of lamellae compared with 2 of 16 (13%) in the 2 laminitis negative heifers given oligofructose. In controls, stretching of lamellae was recorded in 4 of 48 (8%) sections. The

latter showed only slight stretching of lamellae, but an overlap between control tissue and tissue affected by oligofructose-induced laminitis was present for other characteristics as well. Variation in tissue characteristics was also noted among sections belonging to the same individual. Except for changes in chromatin density, none of the heifers that developed laminitis had identical findings in all 8 sample locations belonging to that heifer. Variation among sections from the same individual also was reported (Obel, 1948; Linford, 1987) when evaluating sections from horses with acute laminitis. A possible explanation for this may be an irregular distribution of lesions. This is further supported by a patchy distribution of dermal hyperemia found in a clinical case referred to the university hospital (Figure 2B). Whether this is a consequence of some areas being less susceptible than others or just a random incident, caused by delays in the development of lesions at different sites, is uncertain. When lesion distribution was examined across claws (medial vs. lateral), feet (left vs. right front), or position in the claws where samples were harvested (abaxial vs. dorsal), no trend favoring one site was obvious in the present study (data not shown). The present study examined only front feet due to practical implications. A comparison of lesion distribution between hind and front claws is therefore not available. From the literature, however, it is well known that laminitis-associated chronic claw lesions are most prevalent in the hind limbs and this aspect should be included in future investigations.

The histopathology of spontaneous, acute bovine laminitis has been described previously. With some variation among heifers, Nilsson (1963) reported edema, hyperemia, hemorrhage, and thromboses to be the histopathological characteristics in a group of 14 heifers classified as suffering from acute clinical laminitis (signs < 9 d). These were also the findings of MacLean (1971), and, with the exception that no thromboses were seen in the heifers given oligofructose, agree with the present study. Changes in epidermal basal cells were found regularly by all investigators. Basal cells were enlarged and more rounded or oval, and the color of the nuclei was lighter than normal (Nilsson, 1963; MacLean, 1971). Stretching of lamellae, number of suprabasal cell layers, and basement membrane changes were not described by these investigators. However, both MacLean (1971) and Nilsson (1963) reported that the keratin pillar appeared to have contracted away from the base of the lamellae (these authors named the innermost part of the lamellae as the base), which corresponds to the finding of the present study, in which an increased distance between the tip and the cornified part of the lamellae was recorded. Furthermore, these researchers (Nilsson, 1963; MacLean, 1971) also de-

Figure 2. Samples from the 2 cows with spontaneously occurring acute clinical laminitis. The heifers displayed severe lameness, positive pain reactions with hoof testers, and increased claw temperature for a week before euthanasia. DL = dermal lamellae, EL = epidermal lamellae, BM = basement membrane. (A) Cross-sections of the lamellar layer, periodic acid-Schiff stain (cow with laminitis as sequela to pneumonia). The innermost part of the top lamella can be seen at greater magnification in the top right-hand corner. Small loops of detached basement membrane are seen (arrows) in the stretched tip of this lamella. The bottom 2 lamellae are also stretched, but their bulb formed tips are outlined by a smooth basement membrane. (B) Lateral view of the corium of left front lateral claw (cow with laminitis as sequela to pneumonia). The claw capsule has been removed after heating to 55°C for 30 min. There is a patchy distribution of dermal hyperemia. (C and D) Cross-sections of the innermost part of the lamellar layer showing moderate (C) and severe (D) detachment of basement membrane from the basal cells at the tips of stretched EL, periodic acid-Schiff stain (cow with laminitis as sequel to peritonitis).

scribed presence of acidophilic horn-like bodies in the majority of cases except the most acute ones. Presence of these bodies was very unusual in the present study and were seen both in tissue from controls as well as oligofructose-treated heifers. Presence of WBC in dermis was noted in all 3 studies in contrast to tissue necrosis, indicating more severe or progressed tissue damage, which was only recorded by MacLean (1971) and Nilsson (1963). In summary, the findings of the present study are generally in accordance with previous descriptions. The lack of other studies reporting lamellar stretching may be explained by methodological difficulties. Until now it has been impossible to study the early phases of laminitis because no reliable model for induction of acute clinical laminitis existed. The advan-

tage, however, of the new model used to induce acute clinical laminitis herein (Thoefner et al., 2004), is that the time lapse between exposure to oligofructose, occurrence of clinical signs, and tissue sampling can be accurately determined and controlled. The 4 heifers that developed clinical laminitis had signs of foot pain between 9 and 33 h before euthanasia (Thoefner et al., 2004). The histopathology described in the present study, therefore, documents what occurred early in the disease, and the discrepancies among studies might represent differences in progressive stages of the disease.

The investigators of the present study acknowledge that laxity of the suspensory apparatus of the claw is a plausible explanation and central tenet for the down-

ward displacement of the third phalanx and hence, development of laminitis-associated sole ulcers in cattle. Complementary to another theory (Lischer et al., 2002; Tarlton et al., 2002), which suggested that the weakest point of the suspensory apparatus resides in a loosening of the collagen fiber bundles in the dermal component, the present study documents changes of the living epidermal cell layers including stretching and occasional basement membrane detachment. This was a feature of oligofructose-induced acute laminitis as well as in spontaneously occurring laminitis. Consequently, a failure of epidermal attachment resulting in increased length of the epidermal component, which then becomes the weakest structure in the suspensory apparatus of a laminitis-affected claw, needs to be considered in clarification of bovine laminitis pathophysiology.

The fact that all types of lesions, demonstrated in the present study, were also characteristics of equine acute laminitis (Obel, 1948; Pollitt, 1996; Wattle, 2000) indicates the existence of analogies in laminitis pathophysiology between cattle and horses. Thus, bovine laminitis might be initiated by a factor that disturbs the homeostasis of epidermal basal cells or activates proteases that influence important molecular structures responsible for the normal attachment between basal cells and basement membrane. Recent reports (French and Pollitt, 2004a,b) showed that loss of hemidesmosomes, cytoskeleton failure, and cleavage of laminin-5 were associated with basement membrane dysadhesion in horses in which laminitis was experimentally induced. In cattle, a weakened dermo-epidermal junction might allow the downward displacing forces, operating through the weight of the animal, to stretch the lamellae and eventually, in the most severe affected cases, to detach lamellae that might or might not be accompanied with hemorrhage. It is likely that lesions documented in the present study, with repeated bouts of laminitis would lead to the production of horn with inferior quality and a widening of the white line with or without white line hemorrhage and discoloration. These changes are chronic manifestations of laminitis that can be difficult to explain if lesions are assumed only to affect the dermal component of the suspensory apparatus. It is possible, however, that stretching of tissue could take place in more than one area or structure, and further, that the location is related to different predisposing factors (e.g., rumen acidosis or calving) as well as related to the severity of the clinical manifestation.

Lack of other publications reporting lamellar stretching (in terms of a visually detectable tapering of lamellae) suggest that stretching of lamellae is a transient phenomenon and therefore seen only early in the acute phase. Disappearance of the stretched form might be

explained by a rapid healing of lamellae taking place when a new equilibrium between the downward displacing forces and the epidermal laxity is reached. The bulbs at the epidermal tips (Figure 2A) are suggested to represent such epidermal healing indicating proliferation of basal cells that are filling out shrunken areas (folded basement membrane) or even empty pockets (basement membrane vacuoles). Eventually the basement membrane is pushed back to its normal position and tips reappear rounded. In horses, a marked proliferation of epidermal basal cells left in pockets of residual basement membrane was found in a study that followed healing after surgical hoof wall resection (Pollitt and Daradka, 2004). In the light of acute and chronic types of bovine laminitis, the mechanisms of epidermal lamellae stretching and healing require further work to fully understand the findings of the current study.

CONCLUSIONS

The present report describes the histopathology of acute clinical laminitis using a novel induction model. Stretching of lamellae, dermal edema, hemorrhage, changes in basal cell morphology, presence of WBC in dermis, and signs of basement membrane detachment were associated with oligofructose-induced acute laminitis. These findings document that changes are taking place at the dermo-epidermal junction during the early hours of acute laminitis.

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REFERENCES

- Andersson, L., and A. Bergman. 1980. Pathology of bovine laminitis especially as regards vascular lesions. *Acta Vet. Scand.* 21:559–566.
- Bergsten, C. 1994. Hemorrhages of the sole horn of dairy cows as a retrospective indicator of laminitis—An epidemiologic study. *Acta Vet. Scand.* 35:55–66.
- Bergsten, C., and B. Frank. 1996. Sole hemorrhages in tied primiparous cows as an indicator of periparturient laminitis: Effects of diet, flooring and season. *Acta Vet. Scand.* 37:383–394.
- Boosman, R. 1991. Bovine Laminitis. Histopathologic and arteriographic aspects, and its relation to endotoxaemia. Ph.D. Dissertation, University of Utrecht, The Netherlands.
- French, K. R., and C. C. Pollitt. 2004a. Equine laminitis: Cleavage of laminin-5 associated with basement membrane dysadhesion. *Equine Vet. J.* 36:242–247.
- French, K. R., and C. C. Pollitt. 2004b. Equine laminitis: Loss of hemidesmosomes in hoof secondary epidermal lamellae correlates to dose in an oligofructose induction model—An ultrastructural study. *Equine Vet. J.* 36:299–303.

- Linford, R. L. 1987. A radiographic, morphometric, histological, and ultrastructural investigation of lamellar function, abnormality and the associated radiographic findings for sound and footsore thoroughbreds, and horses with experimentally induced traumatic and alimentary laminitis. Ph.D. Dissertation, Univ. Calif., Davis.
- Lischer, C. J., A. Etrich-Hunkeler, H. Geyer, J. Schulze, and P. Ossent. 2000. Clinical and biochemical findings in dairy cows with uncomplicated sole ulcers with regard to laminitis as a predisposing factor. *Schweiz. Arch. Tierheilkd.* 142:496–506.
- Lischer, C. J., P. Ossent, M. Raber, and H. Geyer. 2002. Suspensory structures and supporting tissues of the third phalanx of cows and their relevance to the development of typical sole ulcers (Rust-erholz ulcers). *Vet. Rec.* 151:694–698.
- MacLean, C. W. 1971. The histopathology of laminitis in dairy cows. *J. Comp. Pathol.* 81:563–570.
- Manske, T., J. Hultgren, and C. Bergsten. 2002. Prevalence and interrelationships of hoof lesions and lameness in Swedish dairy cows. *Prev. Vet. Med.* 54:247–263.
- Milling, C., and C. Lischer. 2002. New aspects on etiology and pathogenesis of laminitis in cattle. Pages 236–247 in *Recent Developments and Perspectives in Bovine Medicine*. Proc. 22th World Buiatrics Congress, Hannover, Germany. Klinik für Rinderkrankheiten, Tierärztliche Hochschule Hannover, Germany.
- Nilsson, S. A. 1963. Clinical, morphological and experimental studies of laminitis in cattle. *Acta Vet. Scand.* 4(Suppl. 1):188–222.
- Obel, N. 1948. Studies on the histopathology of acute laminitis. Ph.D. Dissertation, Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Ossent, P., P. R. Greenough, and J. J. Vermunt. 1997. Laminitis. Pages 277, 279, and 287–288 in *Lameness in Cattle*. 3rd ed. P. R. Greenough and A. D. Weaver, ed. Saunders, Philadelphia, PA.
- Pollitt, C. C. 1996. Basement membrane pathology: A feature of acute equine laminitis. *Equine Vet. J.* 28:38–49.
- Pollitt, C. C., and M. Daradka. 2004. Hoof wall wound repair. *Equine Vet. J.* 36:210–215.
- Tarleton, J. F., D. E. Holah, K. M. Evans, S. Jones, G. R. Pearson, and A. J. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. *Vet. J.* 163:196–204.
- Thoenner, M. B., C. C. Pollitt, A. W. van Eps, G. J. Milinovich, D. J. Trott, O. Wattle, and P. H. Andersen. 2004. Acute bovine laminitis: A new induction model using alimentary oligofructose overload. *J. Dairy Sci.* 87:2932–2940.
- Wattle, O. 2000. Studies on the cytokeratins of the equine hoof wall, chestnut and skin with special reference to laminitis. Ph.D. Dissertation, Swedish University of Agricultural Sciences, Uppsala, Sweden.