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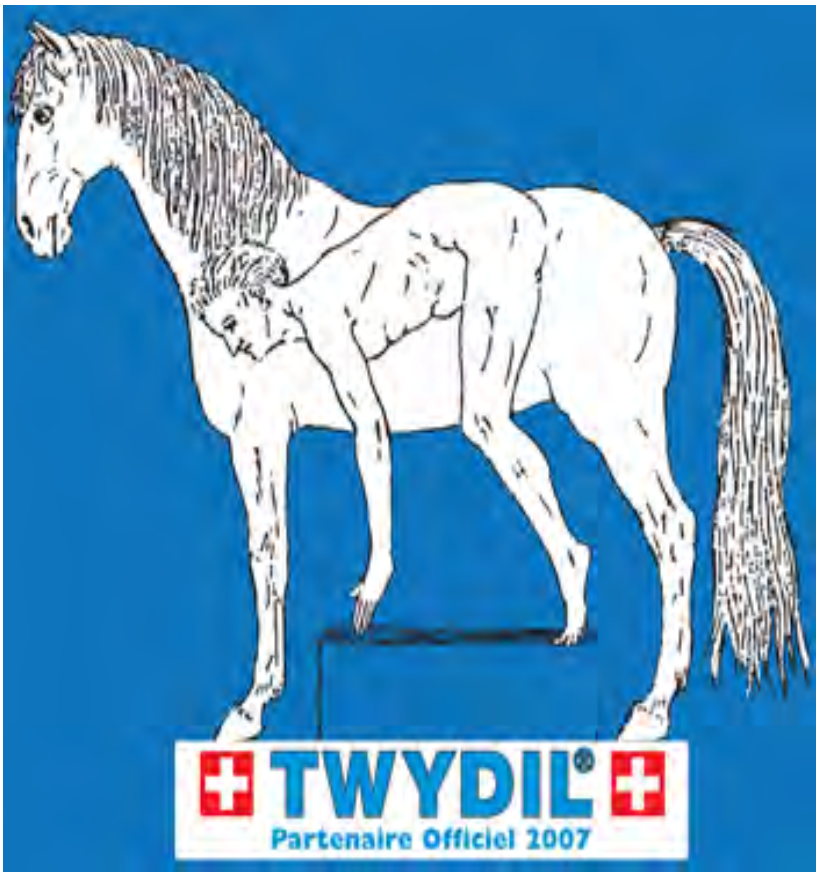
La pathologie du pied et son imagerie médicale

Hufpathologie und Deren Bildgebende Verfahren

Hoof Pathology and Medical Imaging



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LAMINITIS : WHAT TREATMENT AT WHAT STAGE ?

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The process initiating the perturbation of the lamellar attachment apparatus begins to operate during the developmental phase before the first clinical sign of laminitis, foot pain, is apparent. During the developmental phase the specific problems of the horse, often have to be attended to urgently (e.g. acute abdomen, grain overload acidosis, electrolyte imbalance, rhabdomyolysis, retained placenta) and unfortunately the feet are often left out of the therapeutic equation until the first signs of foot pain (shifting weight from one foot to the other, lameness when trotted out, especially when turned) appear. By the time foot pain is apparent lamellar pathology is underway. In other words foot pain is the clinical sign that lamellar compromise is occurring. To wait and see if foot pain is the sequel to a metabolic crisis is to miss the opportunity to prevent or at least ameliorate lamellar pathology. There is a good correlation between the severity of laminitis histopathology, as seen with the microscope, and the degree of lameness (Obel 1948) shown by the horse (Pollitt 1996). When a horse first starts to show laminitic pain, the anatomy of the hoof wall lamellae is being destroyed. The higher the lameness grade, the more severe the microscopic damage. Any activity that places stress on an already weakened lamellar attachment apparatus (such as forced exercise) causes further damage and is contraindicated. The use of nerve blocks to eliminate pain will also encourage locomotion and precipitate more damage.

LAMINITIS THERAPY

From the outset it must be stated that a therapeutic regime, using biological or chemotherapeutic agents, able to arrest or block the triggering of laminitis, does not exist. On the other hand, there is a plethora of remedies, used empirically, that symptomatically help the horse after it has acquired laminitis. It is more the extent and severity of the lamellar pathology that influences the outcome for the horse, not the treatment regimen itself. An effective laminitis preventive may emerge when the mechanism behind the disintegration of the anatomy of the hoof wall lamellae is fully understood. Our discovery that a class of enzymes appears to be involved in the lamellar failure of laminitis has led us to commence trials of proteinase inhibitor therapy, specifically targeted at hoof wall matrix metalloproteinases.

Since laminitis usually develops as a sequel to disease processes in body compartments other than the foot, it is of paramount importance that the primary disease is treated urgently and effectively. If the duration and severity of the primary disease can be reduced by intensive therapy, there is a strong chance that the severity of lamellar pathology may also be reduced, thus improving the prognosis for the horse. Nevertheless, severe laminitis is sometimes the outcome despite the best of current therapy.

When the laminitis process is triggered, there is virtually nothing, by way of drug therapy, that will stop its relentless progress. The administration of a nonsteroidal anti-inflammatory drug (NSAID) like phenylbutazone, during the developmental/acute stages, will ameliorate foot pain and create a more comfortable-looking horse, but the disease continues unabated. This creates an ethical dilemma; balancing the need to alleviate pain and suffering against the realisation that most of what is administered is only palliative. When NSAIDs are in use, the patient should be confined to a stall with deep bedding. Exercise, while under the influence of painkillers, such as phenylbutazone, is contraindicated.

CRYOTHERAPY

The results of experiments at the AELRU, continuously evaluating foot temperature (and by implication foot circulation), as horses developed laminitis, showed that vasoconstriction during the developmental stage of laminitis may have had a protective effect (Pollitt and Davies 1998). The induction of digital vasoconstriction may be a useful preventive strategy in the developmental phase of laminitis. Limited anecdotal evidence from practicing veterinarians suggests that cryotherapy may halt the development of the disease. The profound hypometabolic effect of cryotherapy is considered to be the most important mechanism by which cold limits the

severity of an injury. Tissue metabolic rate and oxygen consumption are inversely related to temperature. A reduced requirement of cooled tissue for oxygen, glucose and other metabolites enhances the survival of cells during periods of ischemia. This mechanism is thought to protect tissue and is the basis for the use of cryotherapy in organ transplant surgery. A reduction in metabolic enzymatic activity of approximately 50% has been observed with a reduction in tissue temperature of 10°C. The activity of collagenases and pro-inflammatory cytokines is significantly reduced at lower temperatures.
Cryotherapy causes potent local vasoconstriction. This is largely mediated by sympathetic nervous control; however, a direct constrictive effect on blood vessel walls may occur, particularly at lower temperatures. To date clinical recommendations for the duration and temperature of cryotherapy in horses has been extrapolated from human medicine. Our recent studies have challenged these recommendations.

CRYOTHERAPY: POTENTIAL MECHANISMS FOR PREVENTING LAMELLAR DAMAGE

The precise, molecular pathogenesis of acute laminitis is unknown. The diverse effects of cryotherapy, however, have the potential to interrupt many of the pathophysiological mechanisms that have been hypothesized to occur during the developmental and acute phases of the disease. A summary is presented in Table 1. Enzymatic degradation of lamellar attachments by matrix metalloproteinases (MMPs) forms the basis of our pathophysiological theory for developmental laminitis. It is hypothesized that the inappropriate release of excess, activated lamellar MMPs is mediated by "laminitis trigger factors" delivered to the foot via the digital circulation during developmental laminitis. The delivery of these triggers, which may include cytokines, protein fragments or bacterial products of hindgut origin, appear to be limited by cold-induced digital vasoconstriction during the developmental phase of laminitis. This was the basis for evaluating the use of cryotherapy for the prevention of laminitis. The potent local hypometabolic effect of cryotherapy could augment the vasoconstrictive effect on the digital vasculature. A cold-induced reduction in the local production and activity of MMPs would limit degradation of the lamellar attachments. A digital hypometabolic state would also limit the local production and activity of pro-inflammatory cytokines, such as interleukin and tumor necrosis factor, during the developmental stage of laminitis. Cryotherapy could also limit secondary inflammatory damage caused by white blood cell infiltration. Similar mechanisms are believed to be the basis for the efficacy of scalp cryotherapy in preventing alopecia in cancer patients undergoing chemotherapy. Vasoconstriction apparently reduces delivery of the chemotherapeutic agent to the scalp, and cellular uptake and metabolism are reduced when residual drug reaches the hair follicles.

The alternate pathophysiological theory for laminitis proposes that digital hypo-perfusion during the developmental stage leads to lamellar ischemia and necrosis. Profound, cold-induced vasoconstriction would seem contraindicated if digital hypo-perfusion was the primary mechanism behind the development of laminitis. However, despite a reduction in digital perfusion, the hypometabolic effect of cryotherapy could protect the lamellar tissue from ischaemic damage. Similarly, a profound cold-induced reduction in metabolism could protect the lamellar tissue from a lack of glucose (proposed as an initiator of lamellar separation in one study). Until the true pathophysiology of laminitis is discovered, the apparent resilience of the equine distal limb to prolonged, extreme cold may hold the key to successfully preventing the disease. Continuous distal limb cryotherapy during the developmental stage of laminitis has the potential to preserve the lamellar tissue until the systemic insult, occurring elsewhere in the body, has abated.

EFFICACY OF CONTINUOUS DISTAL LIMB CRYOTHERAPY FOR THE PREVENTION OF ACUTE LAMINITIS.

Experimental data : We have completed two controlled studies on the efficacy of cryotherapy for the prevention of laminitis. In the first study laminitis was induced in six horses using the oligofructose overload model. Each horse had one forelimb immersed in ice and water (mean temperature 0.5-1.7°C) for a 48 hour experimental period, achieving a mean internal hoof temperature of 3.5-0.9°C. All horses developed clinical and histological laminitis in one or more of the untreated limbs. The cooled limbs did not develop clinical laminitis and had significantly reduced lamellar histological damage. The study also showed significantly reduced up-regulation of lamellar MMP mRNA in the cooled limbs when compared with the untreated limbs. Although cryotherapy markedly reduced the severity of laminitis it did not completely prevent minor histological changes in 4 of the 6 horses.

In a second study cryotherapy was applied to all 4 limbs of 6 horses for 72 h. Laminitis was induced as before and the observation period was extended until 7 days post oligofructose dosing. The horses showed either no or very mild clinical signs of laminitis and histology of lamellar tissues taken 7 days post induction showed no laminitis. Control horses were lame at 7 days and had moderate to severe laminitis histopathology (van Eps *et al.* 2004).

Cryotherapy was instigated immediately following administration of the carbohydrate induction bolus in these studies. In a clinical case of grain overload or acute colitis such prompt initiation of cryotherapy may not be possible. It is unclear whether such a potent prophylactic effect would occur if cryotherapy was initiated later in the course of the disease when lameness was already present. Thus the potential of cryotherapy to prevent laminitis has been demonstrated and further clinical evaluation of the technique is justified.

Clinical data : Anecdotal evidence of the successful use of cryotherapy to prevent acute laminitis has surfaced following the initial evidence-based recommendations for its use. The authors have trialed continuous distal limb cryotherapy for the prevention of laminitis in 7 cases of

acute colitis (5 Thoroughbred geldings, 1 Thoroughbred colt and 1 Arab mare). All cases presented with fever (>39.5°C), profuse watery diarrhoea and signs of endotoxaemia and circulatory shock (injected mucous membranes with poor capillary refill time, rapid heart rate and depression). Only one horse had signs of laminitis before the initiation of cryotherapy. This horse had increased intensity of digital pulses in all four limbs, though lameness was not obvious. All cases were placed into a plastic tub with a rubber floor. Shoes, if present, were not removed. Water, then cubed ice, was added to the tub to submerge the fore and hind limbs. The level of ice and water was maintained at the upper third of the cannon bones. Approximately 100 kg of cubed ice was required to cool the water initially. Subsequently, 50 kg of ice was added at 4- to 8-hour intervals to maintain the temperature within the bath at less than 5°C.

All horses were treated (while in the cold bath) with intravenous polyionic fluids and plasma, antibiotics, NSAIDs and activated charcoal and paraffin oil by nasogastric tube. Lucerne hay and water were provided *ad libitum*. The cases were monitored constantly and remained in the cold bath for a minimum of 72 hours. All horses tolerated the cold bath well, without attempting to escape. The decision to remove the horses from the cold bath after the 72-hour period was based on resolution of clinical signs. Each horse was removed when the rectal temperature stabilized below 38.5°C, the manure was formed, and the mucous membranes returned to normal color. Five of the horses were removed at, or shortly after, 72 hours. The remaining 2 horses were removed from the bath at approximately 96 hours. None of the horses were lame on removal from the cold bath; however, all had increased intensity of digital pulses in all four limbs for the ensuing 24 hours. Variable distal limb oedema was also present. One horse that had signs of incipient laminitis before commencement of cryotherapy was mildly lame between 12 and 24 hours after removal from the cold bath. The lameness disappeared over the subsequent 10 days of hospitalization and radiographs of this horse revealed no displacement of the distal phalanx within the hoof capsule. It is unclear whether cryotherapy reduced the severity or had no effect on the development of laminitis in this case.

The remaining 6 horses were sound throughout the hospitalization period, and no lameness was detected on subsequent re-examinations 4 to 6 weeks later. All horses have returned to athletic activity, reportedly at previous levels. At the time of publication, three of the Thoroughbred horses have won metropolitan races since discharge. After examination of hospital records, the authors estimate the incidence of acute laminitis in previous similar cases of acute colitis (that were not treated with cryotherapy) to be 40 to 50%. Although these are very limited numbers, the authors believe the prophylactic use of continuous distal limb cryotherapy in similar cases at risk of developing laminitis is worthy of further clinical evaluation.

APPLICATION METHODS

Any means by which the distal limbs can be continually exposed to temperatures of 0 to 5°C is acceptable. The cooling method should include the hoof and its solar surface. We suggest cooling the limb up to the top of the cannon, as this appears to result in more effective cooling of the lamellar region. Cooling just the feet is not enough. Ice and water immersion is effective, practical and inexpensive. Commercial cryotherapy cuff devices could be modified to include the hoof, though this is practically difficult. These devices are usually designed for compression as well as cooling. The effects of prolonged compression on the equine distal limb are currently unknown. The authors have had experience with a range of boots and tubs for ice and water immersion.

We have found that the use of a tub, 200 cm long, 80 cm wide and 50 cm high, most practical for prolonged, continuous application of cryotherapy to all four limbs. A water-tight door at one end for ease of access, and a rubber floor are suggested. Temporary or permanent stocks, together with cross-tying the head may assist in keeping the horse stationary. A refrigerated pump, re-circulating water at around 2°C, can reduce or replace the requirement for ice. Overall, vigilance should be exercised to maintain immersion temperatures below 5°C to maximize the protective effect.

Continuous distal limb cryotherapy shows considerable promise as a technique for preventing acute laminitis. The authors continue to evaluate cryotherapy in clinical cases at risk of developing laminitis, and welcome correspondence from others engaged in similar pursuits. Currently the most challenging aspect of cryotherapy in the clinical situation is the identification of cases that will develop laminitis, and subsequently deciding when to initiate and cease cryotherapy in these cases. A biological marker to identify horses at imminent risk of developing laminitis is needed. Such a marker would define the clinically silent developmental phase of laminitis in individual cases, and greatly improve the potential for prevention of the acute disease. Undoubtedly genetic markers exist for the early identification of horses developing laminitis. Up-regulation of MMP-2 mRNA early in the acute phase of laminitis has been demonstrated in lamellar tissue. If this process begins during the developmental phase of laminitis, particularly within the blood, skin, ergot or chestnut tissue, a diagnostic potential exists. The eventual discovery of the exact pathophysiology of laminitis will surely lead to effective and direct methods of prevention and therapy. In the meantime, the apparent resilience of the equine distal limb to prolonged, extreme cold can be harnessed and may hold the key to successfully preventing the disease.

MANAGEMENT PRACTICES TO AVOID PASTURE ASSOCIATED LAMINITIS

There is now strong circumstantial evidence that fructan in the hindgut of horses may trigger laminitis. Horses can ingest fructan rich pasture rapidly in amounts exceeding

(Longland and Byrdy 2006) that used to induce experimental laminitis (van Eps and Pollitt 2006).

Owners of horses predisposed to laminitis should develop strategies to reduce risk. Most horse owners in New Zealand and Australia are committed to pasture feeding regimens throughout the year so a combination of both pasture and horse management practices need to be considered. The aim is to reduce the concentrations of water soluble carbohydrate (WSC) in pasture and to prevent its consumption by the grazing horse.

PASTURE FACTORS

Some pasture species are notorious fructan accumulators (they are selected and bred for this) and if possible should not be fed to horses. The WSC content of grass can reach 56% of its total dry matter (DM) of which fructan can be 44%. Grass that is actively growing tends to store less WSC. Maintaining soil moisture and fertility and keeping grass short by mowing or grazing encourages leaf growth and WSC consumption (Watts and Chatterton 2004). WSC accumulation in grass is driven by photosynthesis and takes time to occur. It peaks in the afternoon and early evening and high WSC intake can be avoided by allowing grazing only in the early morning. Likewise pasture shaded by tree-lines and windbreaks accumulates less WSC and susceptible horses can be strip grazed behind electric fences in these areas. Some horse managers will poison selected paddocks to eliminate pasture altogether and at time of high risk keep their horses on these "dry lots". Times of risk are conditions of high light intensity and low ground temperatures such as in spring and autumn and particular care is indicated at these times. Under these conditions photosynthesis and WSC production is relentless but growth and metabolism is slow; hence WSC accumulation. Using a cash flow analogy; the bank balance is greatly in credit – cash income exceeds expenditure. Drought or periods of low soil moisture may also drive WSC accumulation and even dry looking pasture can have a high WSC concentration. Drought breaking rain can also be a trap. WSC accumulated in subsoil roots during dry times is rapidly mobilized to new shoots and many a pony has foundered on insignificant looking pasture after rain. Another trap is slashed or heavily grazed pasture or stubble after harvesting. Most of the WSC of grass is stored, not in the green leaves, but in the lower, pale green stems that as a WSC reservoir. Grass that has gone to seed in summer is usually low in overall WSC content in its leafy tissues but could still pose a risk from the starch in the seeds. A yield of starch from the seed of perennial ryegrass has been estimated at 360kg/ha per growing season (Longland and Byrdy 2006). Horses will selectively strip seed from standing pasture and could conceivably consume sufficient starch to trigger laminitis from hindgut fermentation.

HORSE FACTORS

Grazing muzzles have been successfully used to limit grass and thus WSC intake by horses at pasture. The hole in the muzzle limits intake and confines it to leafy tops that are lower in WSC content. When horses and ponies have no access to pasture and are yarded or confined to dry lots what are they to be fed? The usual solution is grass or forage hay. However the haymaking process may not always reduce WSC and sometimes the most innocent looking hay may have dangerous WSC levels. If possible choose hay made from mature seeded, pasture made in summer. Hay could still be dangerous if harvested during periods of plant stress such as autumn and spring. Analysis of the WSC content of such hay is warranted but not always practical. Fortunately soaking hay in fresh water leaches out WSC (but not starch) and reduces the WSC content significantly. Sixty minutes of soaking and draining removed an average of 31% of the soluble sugars from 15 hay samples (Watts and Chatterton 2004).

Pony breeds in particular are prone to obesity and insulin resistance and obese individuals are at high risk of developing laminitis. The diet of obese individuals can be modified so that energy is derived from fat and fibre rather than from high glycaemic sources. Owners should monitor the body weight and learn to condition score their horses aiming for more optimum weights. Insulin resistance can be reversed by weight reduction and regular exercise.

"Founderguard" is an antibiotic formulation that can be fed to horses and ponies at pasture and when present in the hindgut limits the proliferation of *Streptococcus bovis*. When 'predosed' it may control hindgut carbohydrate fermentation to levels that prevent serious laminitis.

CONCLUSIONS

The skills and knowledge of veterinary clinicians is sorely tested by laminitis. Many a horse is lost even in the hands of the most eminent practitioner or surgeon. Laminitis is a great leveller and its development brings undone the work of many a veterinarian after heroic efforts have been made to rectify serious medical and surgical emergencies. We persist with therapies that never really work and do so because we are bereft of genuine knowledge about their true efficacy. Vasodilator, anticoagulant and anti-inflammatory therapy have been administered for decades without significantly changing the outcome; laminitis, seemingly with a mind of its own, inexorably leads to crippling lameness in our patients. Laminitis research is difficult, expensive and sometimes heartbreaking. We cling to strongly held opinions, rather than facts, for far too long after evidence invalidates their soundness. However two laminitis facts have recently emerged that will make a difference. Firstly, cryotherapy is a proven preventive if applied during the developmental phase of laminitis. The challenge is being proactive and medically smart enough to predict which ill horses may develop laminitis in the next few hours and to instigate long term cryotherapy now! Tubs and boots full of ice and water are messy and difficult to maintain. A simpler, easy to apply, system of delivering ice cold water to the legs and feet of horses is needed and would help popularise distal limb cryotherapy. The second fact is the new discovery that laminitis is linked directly to

hyperinsulinaemia. Monitoring plasma insulin is relatively easy and veterinarians now have the power to explain insulin resistance and endocrinopathic laminitis to their clients and offer them a way to reverse the situation for their horses. Diet and exercise can return plasma insulin to normality and if lamellar pathology is not too advanced reduce the grade of lameness due to laminitis.

The pace of laminitis research is quickening and as new knowledge is discovered concomitant technological advances in drug development and delivery will further loosen the morbid grip that laminitis has had on progress in effective treatment.

ETHICS STATEMENT

All experiments on horses, conducted by AELRU, are approved by The University of Queensland Animal Ethics Committee (constituted as per the National Health and Medical Research Councils "*Australian code of practice for the care and use of animals for scientific purposes"* which is embedded in the "*Queensland animal care and protection Act 2001")* and all horses under experimentation are inspected by an Animal Welfare Officer.

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REFERENCES

ALLEN, D., JR., CLARK, E.S., MOORE, J.N. AND PRASSE, K.W. (1990) Evaluation of equine digital Starling forces and hemodynamics during early laminitis. *Am J Vet Res* **51**, 1930-1934.

BARTON, M.H., COLLATOS, C. AND MOORE, J.N. (1996) Endotoxin induced expression of tumour necrosis factor, tissue factor and plasminogen activator inhibitor activity by peritoneal macrophages. *Equine Vet J.* **28**, 382-389.

BELKNAP, J.K., GIGUÈRE, S., PETTIGREW, A., COCHRAN, A.M., VAN EPS, A.W. AND POLLITT, C.C. (2006) Lamellar proinflammatory cytokine expression patterns at the developmental stage and at the onset of lameness: innate vs. adaptive immune response. *Equine vet.J.* **38**, doi:10.2746/042516407X042155406).

COCHRAN, A., LIN, H.C., YIN, C., BLACK, S.H. AND BELKNAP, J.K. (2006) Ischemia-related genes are not expressed in the developmental stage of Black Walnut extract induced laminitis. *Equine vet.J.* **38** (in press).

CROSER, E.L. AND POLLITT, C.C. (2006) Acute laminitis: descriptive evaluation of serial hoof biopsies. In: *52nd Annual Convention of the American Association of Equine Practitioners.*, San Antonio, Texas. pp 542-546.

DARADKA, M. AND POLLITT, C.C. (2004) Epidermal cell proliferation in the equine hoof wall. *Equine vet. J.* **36**.

FRENCH, K.R. AND POLLITT, C.C. (2004a) Equine laminitis: cleavage of laminin5 (L5) associated with basement membrane dysadhesion. *Equine vet. J.* **36**, 242-247.

FRENCH, K.R. AND POLLITT, C.C. (2004b) Equine laminitis: glucose deprivation and MMP activation induce dermo-epidermal separation in vitro. *Equine Vet. J.* **36**, 261-266.

Hood, D. (2004) The hoof project.

HOOD, D.M. (1999) The pathophysiology of developmental and acute laminitis. *Veterinary Clinics of North America - Equine Practice* **15**, 321-343.

HUNT, R.J., ALLEN, D. AND MOORE, J.N. (1990) Effect of endotoxin administration on equine digital hemodynamics and starling forces. *Am J Vet Res.* **51**, 1703-1707.

KYAW-TANNER, M. AND POLLITT, C.C. (2004) Equine laminitis: increased transcription of matrix metalloproteinase-2 (MMP-2) occurs during the developmental phase. *Equine vet. J.* **36**.

LONGLAND, A.C. AND BYRDY, B.M. (2006) Pasture Nonstructural Carbohydrates and Equine Laminitis. *J. Nutr.* **136**, 2099S-2102S.

MILINOVICH, G.J., TROTT, D., BURRELL, P.C., CROSER, E.L., AL JASSIM, R.A.M., MORTON, J.K., VAN EPS, A.M. AND POLLITT, C.C. (2007) Fluorescence in situ hybridization analysis of hindgut bacteria associated with the development of equine laminitis. *Environmental Microbiology doi : 10.1111/j.1462-2920.2007.01327.x*.

MILINOVICH, G.J., TROTT, D.J., BURRELL, P.C., VANEPS, A.W., THOEFNER, M.B., BLACKALL, L.L., ALJASSIM, R.A.M., MORTON, J.M. AND POLLITT, C.C. (2006) Changes in equine hindgut bacterial populations during oligofructose induced laminitis. *Environ. Microbiol.* **8**, 885–898.

MUNGALL, B.A., KYAW TANNER, M. AND POLLITT, C.C. (2001) In vitro evidence for a bacterial pathogenesis of equine laminitis. *Vet Microbiol* **79**, 209-223.

NOURIAN, A.R., BALDWIN, G.I., VAN EPS, A.W. AND POLLITT, C.C. (2007) Equine laminitis: ultrastructural lesions detected 24-30 hours after induction with oligofructose. *Equine vet. J. doi: 10.2746/042516407X177448*

OBEL, N. (1948) *Studies of the Histopathology of Acute Laminitis.*, Almgvist and Wilcsells Bottrykeri Ab Uppsala (Thesis).

PIRILÄ, E. (2003) *Expression and role of matrix metalloproteinases and the laminin-5 gamma-2 chain in wound healing and cell migration*, University of Helsinki, Helsinki.

POLLITT, C.C. (1996) Basement membrane pathology: a feature of acute equine laminitis. *Equine Vet J* **28**, 38-46.

POLLITT, C.C. AND DAVIES, C.T. (1998) Equine laminitis: its development coincides with increased sublamellar blood flow. *Equine Veterinary Journal Supplement* **26**, 125-132.

POLLITT, C.C., KYAW-TANNER, M., FRENCH, K.R., VAN EPS, A.R., HENDRIKZ, J.R. AND DARADKA, M. (2003) Equine Laminitis: In-depth. In: *American Association of Equine Practitioners 49th Annual Convention*, New Orleans, Louisiana U.S.A. pp 21 - 25.

POLLITT, C.C., PASS, M.A. AND POLLITT, S. (1998) Batimastat (BB-94) inhibits matrix metalloproteinases of equine laminitis. *Equine veterinary journal. Supplement* **26**, 119-124.

PRASSE, K.W., ALLEN, D., JR., MOORE, J.N. AND DUNCAN, A. (1990) Evaluation of coagulation and fibrinolysis during the prodromal stages of carbohydrate-induced acute laminitis in horses. *Am J Vet Res* **51**, 1950-1955.

ROACH, D.M., FITRIDGE, R.A., LAWS, P.E., MILLARD, S.H., VARELIAS, A. AND COWLED, P.A. (2002) Up-regulation of MMP-2 and MMP-9 Leads to Degradation of Type IV Collagen During Skeletal Muscle Reperfusion Injury; Protection by the MMP Inhibitor, Doxycycline. *Eur. J. Vasc. Endovasc. Surg.* **23**, 260-269.

VAN EPS, A.W. AND POLLITT, C.C. (2004) Equine laminitis: cryotherapy prevents development of the acute lesion. *Equine vet. J.* **36**, 255-260.

VAN EPS, A.W. AND POLLITT, C.C. (2006) Equine laminitis induced with oligofructose. *Equine Vet J* **38**, 203-208.

VAN EPS, A.W., WALTERS, L.J., BALDWIN, G.I., MCGARRY, M. AND POLLITT, C.C. (2004) Distal Limb Cryotherapy for the Prevention of Acute Laminitis. *Clinical Techniques in Equine Practice* **3**, 64-70.

WATTS, K.A. AND CHATTERTON, N.J. (2004) A review of factors affecting carbohydrate levels in forage. *J. Equine Vet. Sci.* **24**, 84-86.

WEISS, D.J., GEOR, R.J., JOHNSTON, G. AND TRENT, A.M. (1994) Microvascular thrombosis associated with onset of acute laminitis in ponies. *Am J Vet Res* **55**, 606-612.