Continuous digital hypothermia in the prevention and treatment of acute equine laminitis

A Knowledge Summary by

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KNOWLEDGE SUMMARY

PICO question

Does continuous digital hypothermia improve clinical outcome in equids with acute laminitis compared to supportive treatment alone?

Clinical bottom line

Category of research question

Treatment

The number and type of study designs reviewed

Six experimental randomised controlled trials and one multicentre retrospective case series were reviewed

Strength of evidence

Moderate

Outcomes reported

The outcomes reported were reduced severity of histopathological lamellar lesions in limbs treated with continuous digital hypothermia (CDH; initiated prior to or soon after the onset of experimentally induced acute laminitis) compared to limbs remaining at an ambient temperature in all five experimental studies where histology was performed. A significant reduction was observed in the prevalence or severity of clinical signs of laminitis in limbs treated with CDH compared to limbs remaining at an ambient temperature. In a single retrospective case series, significantly reduced prevalence of clinical laminitis was reported amongst animals receiving CDH compared to those that did not in a referral hospital population of animals treated for colitis

Conclusion

There is moderate evidence to support that CDH when used prior to or in the early stages of clinical signs, may reduce the severity and progression of lamellar lesions in acute laminitis and no evidence demonstrating that it improves clinical outcome compared to supportive treatment alone. Further research into the clinical outcome of equids treated for acute laminitis using CDH is warranted

How to apply this evidence in practice

The application of evidence into practice should take into account multiple factors, not limited to: individual clinical expertise, patient's circumstances and owners' values, country, location or clinic where you work, the individual case in front of you, the availability of therapies and resources.

Knowledge Summaries are a resource to help reinforce or inform decision making. They do not override the responsibility or judgement of the practitioner to do what is best for the animal in their care.

The evidence

Seven studies were found to provide evidence behind the use of continuous digital hypothermia (CDH) for the prevention or treatment of equine laminitis. Three of these are randomised, controlled, blinded, experimental studies with small sample sizes (Dern et al., 2018; Stokes et al., 2019; and van Eps et al., 2014). Another three studies were controlled, experimental studies but had further limitations due to not being fully blinded or randomised (van Eps et al., 2012; van Eps & Pollitt; 2004; and van Eps & Pollitt, 2009). Only one study included cases of laminitis induced by the euglycaemic hyperinsulinaemic clamp model (Stokes et al., 2019). The other five experimental studies used the oligofructose-induced laminitis model through alimentary carbohydrate overload, mimicking laminitis associated with sepsis.

Due to the experimental nature of these publications, they provide limited information regarding the clinical outcome of horses receiving CDH and supportive treatment, as opposed to supportive treatment alone. Only one study compared the outcomes of colitis cases that received prophylactic CDH with those that did not, in a referral hospital population (Kullmann et al., 2014). While including a larger sample size (n=130 horses) compared to the reviewed experimental studies, this observational retrospective case series provides low level evidence and is the only publication providing evidence of CDH in a clinical setting.

Summary of the evidence

Stokes et al. (2019)	
Population:	Clinically normal Standardbred geldings (mean age 6.4 ± 1.8 years). All horses had retired from racing within the preceding 4 weeks, and were reported to be sound, with no gross or radiographic abnormalities of the feet.
Sample size:	Eight horses

Intervention details:	• All horses were stabled for 1 week prior to study and fed lucerne (alfalfa) hay
	• All horses were confined to stocks (a crush) and received *ad libitum* access to lucerne hay and water
	• 72 hour experimental model:
	  ○ 24 hour control period initially, followed immediately by laminitis induction via euglycaemic hyperinsulinaemic clamp (EHC) model [intravenous bolus of insulin (45 mIU/kg bodyweight (bwt)] diluted in 50 ml of saline (0.9% Sodium Chloride (NaCl)) immediately followed by a continuous intravenous infusion of insulin [6 mIU/kg bwt/minute; concurrent continuous intravenous infusion of 50% glucose, with the administration rate adjusted to maintain euglycaemia (5 ± 1 mmol/L)]
	  ○ euthanasia performed after 48 hours of EHC
	• For each horse:
	  ○ random allocation of one forelimb per horse for CDH 30 minutes prior to commencing EHC using coin toss
	  ○ CDH forelimb continuously cooled for the remainder of the experiment via 50% ice and 50% water mixture, to a level just distal to the carpus via immersion within a rubber boot
	  ○ other forelimb left at ambient temperature

	• Post-euthanasia, dorsal lamellae of both forelimbs dissected from hoof and distal phalanx
	• Concurrent treatments:
	  ○ all horses received one dose of phenylbutazone (4 mg/kg bwt IV) between 52–58 hours when Obel Grade 1 laminitis (Obel, 1948) became clinically apparent
	• 5/8 horses received a second dose of phenylbutazone 8–10 hours later. Reasons for this were not given.
	• Histology, histomorphometry and immunohistochemistry
	  ○ histology performed by single blinded veterinary pathologist on randomised sections from the proximal, middle and distal regions of the forefeet; histological severity of each section was graded using a previously published 0–3 scale (Pollitt, 1996) based on basement membrane (BM) separation from the lamellae in increasing severity:
	  ▪ Grade 0: normal
	  ▪ Grade 1: mild changes (BM lifted to form teat shaped bubbles)
	  ▪ Grade 2: moderate changes (absence of BM at the tips of secondary dermal lamellae)
	  ▪ Grade 3: severe and extensive changes (complete separation of primary epidermal and primary dermal lamellae)
	  ○ histomorphometry performed by single blinded observer on randomised sections from the proximal, middle and distal regions and mean of 5 measurements of primary epidermal lamellae (PEL) length (measured from abaxial to axial margins) used for data analysis
	  ○ immunohistochemical staining of middle region sections for the cellular proliferation marker targeting protein for xenopus kinesin-like protein 2 (TPX2), and TPX2-positive cell count performed by single blinded observer with the mean cell counts from both the abaxial and axial regions of 5 PEL used for data analysis

Study design:	Randomised, controlled (within subject), blinded, experimental
Outcome studied:	• Pedometer count of each forelimb independently • Hoof temperature monitored using hoof wall thermistors attached to data logging devices • Lameness evaluation via Obel grading system (Obel, 1948) 48 hours post EHC initiation by videoing each horse walking in a straight line for 20 metres, evaluated by a blinded clinician experienced in evaluation of lameness due to laminitis
Main findings: (relevant to PICO question):	• All eight horses developed laminitis within 48 hours of EHC Pedometer counts: • significant increase in ambient limb pedometer count frequency during EHC (24–72 hours)

	• no significant change in pedometer count in CDH limbs throughout the experimental period
	• Weight shifting of ambient limb was clinically apparent from 52–58 hours (28–34 hours after commencing EHC)
	• Video analysis at 72 hours showed 6/8 horses lame at walk in the ambient limb (remaining two horses were not detectably lame at walk)
	Hoof wall temperature:
	• in the CDH limbs, temperature decreased after ice boot application (24–72 hours) (median 6.4°C; interquartile range (IQR) 6.0°–6.9°C)
	• in ambient limbs, temperature gradually increased throughout the experimental period (median 29.7°C; IQR 28.6°–31.1°C)
	Histology:
	• Secondary epidermal lamellae (SEL) elongation and disruption:
	  ○ severe, with Obel Grade 3 dermoepidermal separation in all ambient eight feet in ≥1 of the 3 section regions and none of the eight CDH feet
	  ○ compared to ambient feet, CDH sections were 98% less likely to exhibit Obel Grade 2 or 3 histopathological scores (odds ratio 0.02; 95% CI 0.001–0.365; p<0.01)
	• Total PEL length and non-keratinised PEL length
	  ○ significant increase in ambient feet compared to CDH feet (both p<0.01 for all 3 section regions)
	• Intense nuclear TPX2 expression
	  ○ frequent in epidermal basal cells in ambient feet and occasional in CDH feet
	• TPX2-positive cell counts
	  ○ significantly lower in the CDH feet compared with ambient feet (p<0.01)

Limitations:	• CDH was initiated at the onset of hyperinsulinaemia which limits the direct extrapolation of findings from this study to application in clinical settings • Horses were combined to stocks for the 72 hours experimental period. This lack of ambulation does not represent a standard clinical scenario • Whilst the contralateral limb was used as a control, there was no separate control group receiving only supportive treatment • Small sample size • Only young, healthy Standardbred horses included • Lameness evaluation was described as being blinded but the way this was ensured was not described. If the CDH limb was obviously wet when video recordings were made, this could have introduced bias into the lameness evaluation. • The dose rate and reason for 5/8 horses receiving a second dose of phenylbutazone was not reported • Lameness evaluation using video analysis is subjective

Dern et al. (2018)	
Population:	Clinically normal Standardbred geldings (aged 3–11 years). All horses were reported to be sound, with no gross or radiographic abnormalities of the feet.
Sample size:	15 horses: n=8 controls and n=7 horses administered oligofructose (OF)

Intervention details:	• Horses randomly assigned to control or OF group
	• All horses confined to stocks for the duration of the study with constant access to hay and water
	• In the OF group, laminitis induction achieved via bolus of 10 g/kg bwt OF (up to a maximum dose of 4.2 kg) dissolved in water and administered via nasogastric tube
	• CDH was initiated 12 hours post OF administration (OF group) or 12 hours after confinement in stocks (control group)
	• For each horse:
	  ○ random allocation of one forelimb per horse for CDH
	  ○ CDH forelimb placed in a rubber boot filled with 50% water and 50% ice to the level of the proximal metacarpus
	  ○ other forelimb left at ambient temperature
	• Euthanasia via pentobarbital sodium 36 hours after the beginning of the experimental period
	• The dorsal lamellae dissected from the hoof and distal phalanx, snap frozen or fixed in formalin and processed
	• Assessment was via real time quantitative PCR or light microscopy by blinded observers
	• Concurrent treatments:
	  ○ all horses received a single dose of phenylbutazone (4.4 mg/kg bwt IV) at onset of lameness signs (Obel Grade 1 lameness (Obel, 1948))

Study design:	Randomised, controlled, blinded experimental study

Outcome studied:	• Forelimb hoof wall thermistors and pedometer devices:
	  ○ hoof wall temperature
	  ○ frequency of weight shifting
	• Histological examination by two blinded observers, BM separation from the lamellae graded using a previously published 0–3 scale (Pollitt, 1996)
	• Primary study outcomes not directly relevant to PICO question (therefore not commented on further):
	  ○ lamellar mRNA concentrations of inflammatory mediators
	  ○ lamellar leukocyte numbers

Main findings: (relevant to PICO question):	• All seven horses in the OF group developed laminitis (Obel Grade 1 lameness (Obel, 1948)) within 24 hours of OF administration Hoof wall surface temperature (median and interquartile range): • significant decrease in hoof wall temperature compared to ambient from 12–36 hours within both OF and control

	groups (p<0.05) ○ OF limb: CDH 6.07°C (5.40°–6.64°C) vs 82°C (26.71°–29.05°C) ○ control limbs: CDH 5.06°C (4.83°–5.92°C) vs ambient 24.67°C (23.25°–26.06°C) ○ no significant difference in temperature between CDH limbs of the control and OF groups or between ambient limbs of the control and OF groups • Within the OF group: ○ pedometer count showed an increase in limb movement of ambient limbs, peaking at 18 hours, where it was significantly greater than CDH limbs (p<0.05) ○ median histological scores of the middle lamellar sections were significantly lower in CDH limbs (0; IQR 0–0) compared to ambient limbs (2; IQR 1.5–2.5)
Limitations:	• Method of randomisation not reported • Small sample size • Title states that CDH was initiated at a 'clinically relevant time point', but it was initiated at 12 hours post OF administration before lameness was apparent in all but one horse • Only Standardbred horses included • Lameness was assessed at one time point only during the 36 hours study period, which was prior to instigating CDH therapy

Kullmann et al. (2014)	
Population:	Horses ≥2 years old admitted to two university hospitals between January 2002–August 2012, diagnosed with colitis, enterocolitis or typhlocolitis (45 mares, 78 geldings, seven intact males)
Sample size:	130 horses: 82 from University A and 48 from University B
Intervention details:	• Inclusion criteria: ○ diagnosis of colitis, enterocolitis or typhlocolitis plus ≥2 of: fever, tachycardia, tachypnoea, leukocytosis or leukopenia ○ horses included whether or not they received CDH ○ the horses not receiving CDH formed a control group • Exclusion criteria: ○ Horses receiving intermittent digital hypothermia (feet placed intermittently (every 2–6 hours) in ice or ice applied as bags or rectal sleeves wrapped around the hooves) ○ horses admitted to hospital with acute or chronic laminitis or if diagnosis of laminitis at time of hospital admission ○ horses <2 years old as considered to have decreased risk of acute laminitis

	○ draught, pony or miniature breed or equine metabolic syndrome diagnosed due to increased risk of acute laminitis • Treatment data collected included use of CDH (cryotherapy performed on both forelimbs) • CDH protocol: submerge distal limbs in ice to level just proximal to metacarpophalangeal joint for a minimum of 48 hours using 5 L fluid bags attached to limb via duct tape. Bags filled with ice every 2 hours or more frequently if necessary • Treatment group: 69 horses received CDH • Control group: 61 horses did not receive CDH
Study design:	Multicentre retrospective case series
Outcome studied:	• Development of laminitis ○ diagnosis of laminitis based on Obel lameness grade (Obel, 1948), increased digital pulses, abnormal posture and inability or unwillingness to move • Long-term outcome on horses that developed laminitis and discharged from hospital included telephone conversations with owners, trainers and referring veterinary surgeon
Main findings: (relevant to PICO question):	27/130 horses (21%) developed laminitis, 103/130 horses (79%) did not • 7/69 (10%) horses with CDH developed laminitis, 3/7 (43%) were euthanised prior to hospital discharge due to laminitis • 20/61 (33%) horses with no CDH developed laminitis, 11/20 (55%) were euthanised prior to hospital discharge due to laminitis • Horses were less likely to develop laminitis if they received CDH compared with horses that did not receive CDH (odds ratio 0.14; p=0.003). • Hospital site was significantly associated with development of laminitis. Bias may have been introduced in the reporting of Potomac Horse Fever (PHF) ○ 15/27 (55%) of horses with laminitis tested positive for PHF ○ 25/48 (52%) of horses at University B colitis cases were PHF positive, compared to 25/82 (30%) of University A colitis cases ○ only 89/130 (68%) of horses in the study were tested for PHF so the variable was not included in the regression analysis • 114/130 (88%) horses discharged from hospital • 16/130 horses (12%) were euthanised in hospital; of which 14/16 were euthanised due to laminitis. 2/103 horses without laminitis were euthanised due to severity of the gastrointestinal disease and poor response to therapy
Limitations:	• Only horses that developed laminitis were followed up after hospital discharge • Ponies, miniature and draught breeds were excluded from the study

	• Retrospective study over two sites • Possible some horses may have been admitted to study with subtle signs of laminitis masked on hospital admission due to receiving analgesia prior to referral • No discussion as to why clinical decision was made to use CDH or not in each case, which could have introduced bias via clinician decision-making • No data on initiation time point of CDH • CDH was initiated for a minimum of 48 hours but whether any horses with CDH continued after this time point is not reported

van Eps et al. (2014)	
Population:	Clinically normal Standardbred geldings (aged 3–11 years). All horses were reported to be sound, with no gross or radiographic abnormalities of the feet.
Sample size:	Eight horses

Intervention details:	• All horses housed and fed in stables for 4 weeks prior to the experiment
	• Laminitis induction via bolus of 10 g/kg OF dissolved in water and administered via nasogastric tube
	• Horse confined to stocks and monitored for lameness every 4 hours beginning 12 hours after bolus of OF
	• Two investigators were required to recognise and agree on Obel Grade 2 lameness (Obel, 1948) to initiate CDH and perineural anaesthesia
	• CDH was initiated 12 hours post OF administration (OF group) or 12 hours after confinement in stocks (control group)
	• For each horse:
	  ○ random allocation of one forelimb per horse for CDH
	  ○ CDH forelimb placed in a rubber boot filled with 50% water and 50% ice to the level of the proximal metacarpus
	  ○ other forelimb left at ambient temperature
	• Euthanasia via pentobarbital sodium 36 hours after the beginning of the experimental period
	• All four hooves on each horse examined histologically, (total of eight CDH forelimbs, plus eight control forelimbs and 16 hindlimbs left at ambient temperature)
	• Concurrent treatments:
	  ○ immediately on recognition of lameness, analgesia was initiated via a single phenylbutazone injection (8 mg/kg bwt IV)
	  ○ bilateral forelimb continuous perineural analgesia (palmar nerve block) via catheter placement with 2 ml 2% mepivacaine hydrochloride hourly
	  ○ intermittent perineural analgesia of hindlimbs if a subjective increase in weight shifting was noted

Study design:	Randomised, controlled (within subject), blinded, experimental study

Outcome studied:	• Pedometers taped to antebrachium and recorded every hour, monitoring step count of individual limbs
	• Forelimb hoof temperature monitored via thermistors attached to the hoof surface
	• Histological examination by two blinded observers was based on basement membrane (BM) separation from the lamellae in increasing severity:
	  ○ Grade 0: normal
	  ○ Grade 1: mild changes
	  ○ Grade 2: moderate changes
	  ○ Grade 3: severe and extensive changes
	  ○ Grade 4: complete physical separation of lamellar epidermis from dermis, with no association between epidermal and dermal tissues on the section

Main findings: (relevant to PICO question):	• All eight horses developed Obel Grade 2 lameness within 17–21 hours of OF administration • Pedometer data demonstrated increased frequency of limb movement in the ambient temperature limbs compared to the cooled limbs after the initiation of peripheral nerve blocks and CDH • After initiation of the perineural analgesia and CDH, the CDH treated limb pedometer count frequency was significantly decreased at 5–14, 17 and 22 hours compared with the onset of lameness (0 hours) • Median hoof wall surface temperature was 7.1°C for the CDH feet and 30.2°C for the ambient feet Histology: • Median histological scores significantly greater in the ambient limbs (proximal 2.8 [IQR 2.5–4]; middle 3.5 [IQR 2–4]; distal 2.5 [IQR 2–3.8]) compared to the CDH limbs (proximal 0.5 [IQR 0.5–1.4]; middle 1 [IQR 0.6–1]; distal 0.75 [IQR 0.5–1] • CDH initiated at the onset of lameness reduced the severity of lamellar injury • Complete physical separation of dermal and epidermal lamellae in four ambient temperature feet which was not observed in any of the CDH feet
Limitations:	• Analgesia via continuous peripheral nerve block is not common practice in many clinical situations – this was done as a humane consideration in this experimental study • The study was only continued for 36 hours after the onset of lameness so it is unclear whether the laminitis lesions in the CDH feet may have progressed after discontinuation of the cooling • Small sample size • Only Standardbred horses included

van Eps et al. (2012)	
Population:	Clinically normal Standardbred horses (10 geldings and four mares; aged 4–11 years). All horses were reported to be sound, with no gross or radiographic abnormalities of the feet.
Sample size:	14 horses

Intervention details:	• Horse confined to stocks
	• Laminitis induction via bolus of 10 g/kg OF dissolved in water and administered via nasogastric tube
	• For each horse:
	  ○ CDH forelimb placed in a rubber boot filled with 50% water and 50% ice to a level just below the carpus
	  ○ other forelimb left at ambient temperature
	• Experiment 1 (eight horses): Euthanised 24 hours after OF bolus (with no lameness) and tissues collected
	• Experiment 2 (six horses): Euthanised immediately upon recognition of Obel Grade 1 lameness between 20–28 hours post OF bolus
	• One horse from Experiment 1 became lame at 20 hours and was euthanised immediately so included in Experiment 2 meaning Experiments 1 and 2 both include seven horses
	• Euthanasia immediately post anaesthetic induction with immediate harvesting of dorsal lamellae snap frozen in liquid nitrogen
	• Control lamellar tissue from a previous study used for comparison

Study design:	Controlled experimental study
Outcome studied:	• Onset of Obel Grade 1 lameness (Obel, 1948) • Forelimb hoof temperature monitored via thermistors attached to the hoof surface • Pedometer readings from Experiment 2 to monitor weight shifting

Main findings: (relevant to PICO question):	• Pedometer counts significantly higher in ambient limbs compared to CDH limbs at 18 and 20 hours, compared to 2 hours time point ($p<0.05$)
	• In all cases, the pedometer data showed an increase in count frequency in ambient temperature limbs 2–4 hours prior to visual recognition of weight shifting behaviour
	• Hoof wall surface temperature (mean ± standard error):
	  ○ CDH limbs 4.2° ± 52°C
	  ○ ambient limbs 23.1° ± 1.4°C

Limitations:	• Selection bias may have been introduced as study does not mention whether CDH limb was randomly selected or how selection of horses into different experimental groups was achieved • Control tissue for histology was not from this study although had been harvested in an identical fashion • Only Standardbred horses were included • Small sample size

van Eps & Pollitt (2009)	
Population:	Clinically normal Standardbred horses (14 geldings, four mares). All horses were reported to be sound, with no gross abnormalities of the feet.
Sample size:	18 horses
Intervention details:	• Horses allocated randomly into three groups of six • All horses housed and fed in stables for 4 weeks prior to the experiment • Group 1: CDH controls • Group 2: laminitis induction by OF method and CDH treatment for 72 hours beginning immediately after induction dose of OF • Group 3: laminitis induction by OF and no CDH • Laminitis induction via bolus of 10 g/kg OF dissolved in water and administered via nasogastric tube. 10% of the induction dose of OF given daily in feed for 3 days prior to administration of bolus dose • CDH for Groups 1 and 2 administered via a wooden water bath with a rubber mat surrounded by stocks. Water added to a level just below the carpus and cooled and recirculated at 1°C using a refrigeration pump and heat exchanger. Cubed ice was added initially to reduce the temperature rapidly • Forelimb internal hoof temperature recorded continuously for Groups 1 and 2 as described by van Eps & Pollitt (2004) • Group 3 were cross-tied on rubber mats for 72 hours to try to standardise conditions (Groups 1 and 2 were confined to the water bath) but did have a limited lameness evaluation every 12 hours during the initial 72 hour period. This consisted of walking the horse in a circle in both directions within the stable. • After the 72 hour period, all horses were released into stables for the remainder of the 7 day experimental period and examined at 12 hour intervals • Concurrent treatments: ○ Horses with Obel Grade 3 or 4 lameness (Obel, 1948) were given mixture of phenylbutazone (4.5 mg/kg bwt IV) and sodium salicylate (1.2 mg/kg bwt IV) at every 12 hour examination until lameness was Obel Grade 2 or less • A final lameness exam at 168 hours was video recorded, all footage randomised and evaluated by blinded clinicians experienced in lameness evaluation • Lateral radiographs taken of each fore foot prior to the experiment and just prior to euthanasia • All horses were euthanised after final lameness evaluation
Study design:	Controlled experimental study
Outcome studied:	• Continuous recording of forelimb internal hoof temperature in Groups 1 and 2

	• Lateral radiographs of each foot to assess changes during study and between groups • Histology of dorsal hoof lamellae to measure lamellar length
Main findings: (relevant to PICO question):	Clinical parameters • Heart rate significantly higher in Groups 2 and 3 vs Group 1 and significantly higher in Group 3 between 44–60 hours, vs Group 2 • Clinical signs of laminitis first noted in Group 3 at 24 hours (4/6), and 36 hours (2/6). No signs noted in Groups 1 and 2 Lameness: • Horses in Groups 1 and 2 were judged to be non-lame (by a single observer) at all time periods between 72 hours and 7 days. • Median Obel Grade lameness scores at 7 days were significantly less in Group 1 (range 0–1) and Group 2 (range 0–2), compared with Group 3 (range 2.5–4) • No significant difference in Obel lameness scores between Groups 1 and 2 Forelimb internal hoof temperature: • There was no significant difference between mean internal hoof temperatures of Group 2 (3.8â° ±6â°C) and Group 1 (3.9â° ± 0.9â°C) at any time point Radiographs: • Rotation of the distal phalanx relative to the dorsal hoof wall was not detected in any radiographs • A small but significant increase in the dorsal hoof wall to distal phalanx distance was noted in Group 3 at 7 days, compared to their baseline radiographs • The laminitis preventive effect of distal limb CDH lasted beyond the 72 hours of application
Limitations:	• The authors of the study noted the design of wooden water bath was cumbersome, difficult to maintain and required constant supervision. This would not be a practical method of CDH in clinical practice • There was no internal hoof temperature monitoring for Group 3. Depending on ambient temperature at the time of the study this could have meant results for Group 3 would have been significantly different to Groups 1 and 2. • Bias may have been introduced in Group 3 by allowing some movement where the horses in the water bath had none for 72 hours • Only Standardbred horses included • Small sample size

van Eps & Pollitt (2004)	
Population:	Mature, clinically normal Standardbred horses (six geldings, four mares). All horses were reported to be sound, with no gross abnormalities of the feet.
Sample size:	10 animals: four control animals (three geldings, one mare), and six case animals (three geldings, three mares)
Intervention details:	• Four control horses were immediately euthanised via barbiturate overdose to use as controls for the matrix metalloproteinase-2 (MMP-2) mRNA analysis. It is unclear from the study whether they received the same care prior to the experiment • Six case subjects housed and fed in stables for 3 weeks prior to experiment • Induction of laminitis via OF method: 10 g/kg of OF administered in 4 L of water via nasogastric intubation • Horses confined to stocks for 48 hours with free access to food and water • Left forelimb placed in rubber boot with 50% ice and 50% water to just below the carpus • Forelimb hoof temperature monitored continuously via thermistor probes inserted into predrilled hole within dorsal hoof wall. Hindlimb hoof temperature monitored 2 hourly via infrared scanning device • Euthanasia via barbiturate overdose post lameness evaluation at 48 hours • Sections of dorsal hoof lamellae removed for histological examination by four blinded evaluators using the Pollitt (1996) scoring system. The median score was used for each limb • Lameness evaluation 2 hours prior to OF bolus and after removal of the ice boot at 48 hours
Study design:	Controlled experimental study
Outcome studied:	• Hoof temperature monitored via thermistors • Observation of clinical signs of laminitis (increased digital pulse amplitude, incessant shifting of weight and lameness measured by Obel Grade) • Severity of laminitis (histological evaluation)
Main findings: (relevant to PICO question):	• Mean hoof temperature of CDH treated limbs (Group 1: 3.9° ± 9°C, Group 2: 3.8° ± 0.6°C) was significantly less than all the ambient limbs by 2 hours • Lameness evaluations revealed all horses were obviously lame in the ambient forelimb at walk. Lameness was not observed in the CDH limb of any horse Histology: • Histological scores of the ambient forefeet (median scores 1–3) were significantly greater than that of the CDH forefeet (median scores 0–0.5) ($p<0.05$) • Detachment of the BM from the secondary epidermal lamellae was present in all ambient feet except hind feet of

	one subject • Detachment of the BM was not seen in any of the CDH feet
Limitations:	• All horses had the left forelimb CDH treated • Lameness evaluation was not blinded • Different devices were used to measure temperature in forelimbs and hindlimbs • Only Standardbred horses included • Small sample size

Appraisal, application and reflection

Laminitis is a common problem affecting equids seen in practice (Wylie et al., 2011). It is often debilitating and can cause severe morbidity and mortality. Acute laminitis may occur as a complication of various primary systemic diseases (van Eps, 2010), excessive unilateral weight bearing (Wylie et al., 2015) or, most commonly as a consequence of hyperinsulinaemia (de Laat et al., 2010; and Patterson-Kane et al., 2018).

The seven papers summarised all contribute some evidence towards the question of whether CDH can help improve clinical outcome in cases of acute laminitis. All the experimental studies deal with continuous digital hypothermia, and horses receiving intermittent digital hypothermia in the case series by Kullmann et al. (2014) were excluded.

The study by van Eps & Pollitt (2009) reported that CDH, when performed for 72 hours on six horses that had not undergone laminitis induction, did not produce any significant lameness or other ill-effects. The horses appeared to tolerate CDH well, although distal limb oedema was reported in all 18 CDH treated horses, which resolved by 7 days following cessation of treatment (van Eps & Pollitt, 2009). A hypothesis provided by the study's authors was that this was a consequence of the cryotherapy in conjunction with restricted ambulation for 72 hours. All other included studies reported using CDH for a minimum of 36 hours with no significant side-effects, demonstrating that CDH is safe to use in horses.

Most of the experimental studies included appear to build on the work of van Eps & Pollitt (2004), refining the experimental model to often include randomisation and blinding of lameness exams which should serve to increase the quality of evidence. These experimental studies have limitations with regards to generalisability towards general clinical practice as the CDH was initiated prior to or immediately after induction of laminitis both by the oligofructose (OF) method (van Eps & Pollitt, 2004; van Eps & Pollitt 2009; and van Eps et al., 2012) and the euglycaemic hyperinsulinaemic clamp (EHC) method (Stokes et al., 2019). However, results obtained using the OF method support the prophylactic use of CDH in clinical cases considered to be at risk of sepsis-associated laminitis (van Eps & Pollitt, 2004; van Eps & Pollitt 2009; and van Eps et al., 2012). In the study by Dern et al. (2018), CHD was initiated 12 hours after induction of laminitis, however this was still before all but one of the horses showed any signs of lameness. In the van Eps et al. (2014) study CDH was initiated in one forelimb only after the onset of lameness was agreed upon by two investigators, providing evaluation of the protective effects of CDH at a more clinically relevant time point, and the most robust evidence relating to clinical practice of all the experimental studies. Although still limited by the small sample size, this study provides evidence of a therapeutic effect of CDH when applied after the onset of clinically apparent laminitis.

Stokes et al. (2019) was the only experimental study of insulin-induced laminitis. The main limitation to clinical application in this study is that CDH was initiated at the time of laminitis induction, before recognition of clinical laminitis. However, the results of the study indicate that overall the protective effects of CDH were similar to those noted in the OF studies, with a reduction in the severity of laminitis, both clinically and histologically (Stokes et al. 2019). Further work would be needed to evaluate whether these protective effects extend beyond the CDH period as shown in the OF method by van Eps & Pollitt (2009).

As a retrospective analysis of clinical records, Kullmann et al. (2014) falls much lower on the hierarchy of evidence but was included here as it provides evidence of CDH being used in a clinically relevant scenario. In this case series, CDH was performed by submerging the limbs in ice just proximal to the metacarpophalangeal joint, as opposed to the level of the proximal metacarpus which was used in all other studies. There were many limitations to this study which must be borne in mind, particularly surrounding the recording and standardisation of why CDH treatment was initiated, how the cases were selected for CDH treatment, the lack of information regarding when CDH treatment was commenced, and whether CDH continued in any cases beyond the minimum 48 hours specified. The criteria stated that horses admitted to the hospital with acute or chronic laminitis or diagnosed with laminitis at the time of admission were excluded, so an assumption is made that CDH was started after they had arrived at hospital – similar to the timing used in the experimental study by van Eps et al. (2014). The authors note however that lameness at time of admission may have been masked in some cases by the administration of analgesics by the referring vet (Kullmann et al. 2014).

The histopathology results from all the studies must be used to try to extrapolate clinical outcome given that all of the horses in the experimental studies were euthanised before clinical outcome could be assessed. All studies where histological examination was undertaken (Stokes et al., 2019; Dern et al., 2018; van Eps et al., 2014; van Eps & Pollitt, 2009; and van Eps & Pollitt, 2004) reported significant reduction in histological scores for laminitic feet treated with CDH compared to untreated laminitic feet. As histological changes due to laminitis are assumed to be non-reversible and the CDH reduced histological lesion progression, the significant difference observed due to the CDH may subsequently improve clinical outcome. Clinical significance of the different histology gradings is still unknown and a major limitation to these studies is the severity of laminitis caused by the induction models. In many natural cases, the onset of laminitis is insidious and may not be spotted until well after the time period where CDH was initiated in these studies. All of the horses studied also had no history of laminitis and it would be interesting to compare effects of CDH on horses with pre-existing chronic laminitis.

The study by van Eps & Pollitt (2009) provides the best evidence that the protective effects of CDH in acute laminitis continue after the CDH is discontinued. Horses were subjected to 72 hours of CDH immediately following laminitis induction before being euthanised 7 days after induction. Results of blinded Obel lameness grading just prior to euthanasia showed no significant difference in control horses in Group 1 (no laminitis induction but CDH performed) and horses in Group 2 (laminitis induction and CDH). However, Obel lameness scores in Group 3 (laminitis induction but no CDH) were significantly higher than in either Group 1 or 2. Histopathology revealed significant lamellar changes in Group 3, mild changes in Group 2 and no lamellar changes in Group 1 (van Eps & Pollitt, 2009).

CDH resulted in a decrease in clinical signs of laminitis (decreased pedometer count (weight shifting)) in the cooled forelimb compared to the uncooled forelimb and compared to the pedometer count prior to initiation of CDH (van Eps et al., 2014). Euthanasia was performed after 36 hours of CDH and histological evaluation identified complete physical separation of dermal lamellae from epidermal lamellae in 4/8 uncooled feet, compared to 0/8 cooled feet (van Eps et al., 2014). At the proximal, middle and distal dorsal lamellar sections the median histological scores were significantly decreased in the CDH feet, compared to the uncooled feet (van Eps et al., 2014). The van Eps et al. (2012) study also reported an increase in pedometer count frequency in ambient temperature limbs 2-4 hours prior to a subjective visual recognition of weight shifting behaviour. Given that one limb was encased in a cumbersome ice boot, the subjective data may be subject to significant bias but the objective pedometer data may prove useful for further research into clinically relevant time periods to initiate CDH. Reduced frequency of weight shifting was also reported in CDH treated limbs compared to ambient temperature limbs in the two other experimental studies where this was measured objectively using pedometers (Dern et al., 2018; Stokes et al., 2019). Additionally, reduced severity of lameness, as assessed by Obel Lameness Grades (van Eps & Pollitt, 2009), and a reduced prevalence of lameness (van Eps & Pollitt, 2004) were reported for CDH treated limbs compared to ambient temperature limbs.

Given the PICO question related to CDH and supportive treatment compared to supportive treatment alone, it should be noted that there are other forms of supportive treatment in the management of laminitis than NSAIDs. Commonly foot supports or corrective foot trimming, deep bedding, dietary management, and other pharmaceuticals such as paracetamol or acepromazine are often employed in the treatment of laminitis. Due to the experimental nature of six of the studies, including these variables within the studies would have been difficult to achieve but this should be borne in mind when thinking about the applicability to general clinical practice.

Further research is required to determine whether the conclusions from the experimental studies included in this Knowledge Summary are applicable to all equidae and underlying causes of laminitis. It is reasonable to assume that these findings would translate to other large size breeds but future work needs to be done to evaluate the effectiveness of CDH in clinical situations, in equids other than Standardbred horses such as ponies or donkeys, and on the effectiveness in animals with a previous history of laminitis, or a history of equine metabolic syndrome and/or pituitary pars intermedia dysfunction. Prospective cohort studies of clinical cases utilising strict inclusion criteria could help evaluate application of CDH for treatment of acute laminitis in clinical practice.

In conclusion, there is moderate evidence demonstrating that CDH does reduce the severity and/or frequency of clinical signs of pain, such as weight shifting and lameness in experimentally induced laminitis in Standardbred horses. Histological examination of the lamellae in cases of experimentally induced laminitis demonstrates that CDH reduces epithelial inflammatory events and protects against lamellar separation, especially if initiated before clinical signs are apparent. There is weak evidence to show that CDH provides protective effects in true clinical situations and a lack of evidence to show it improves clinical outcome compared to supportive treatment alone.

Methodology Section

Search Strategy	
Databases searched and dates covered:	PubMed accessed via the NCBI website (1910–2019) CAB Abstracts (1977–2019)
Search terms:	Search terms used in both databases: Laminitis AND (hypothermia OR cold OR cryotherapy)
Dates searches performed:	26 Aug 2019

Exclusion / Inclusion Criteria	
Exclusion:	• Review, conference proceedings, book chapter, non-peer reviewed publication • Not in the English language • Could not be sourced • Additionally studies where evaluation of the effect of distal limb cryotherapy on clinical and histological signs of laminitis was not the primary focus were excluded
Inclusion:	Studies relating to the use of cryotherapy to treat acute laminitis in equids, regardless of cause of laminitis, where clinical and/or histological signs were the primary outcome measure(s)

Search Outcome						
Database	**Number of results**	**Excluded – did not address the PICO question**	**Excluded – review / book chapter / non-peer reviewed publication**	**Excluded – not in English language**	**Excluded – could not be sourced**	**Total relevant papers**
PubMed	31	19	5	0	0	7
CAB Abstracts	68	23	28	8	2	7
Total relevant papers when duplicates removed						**7**

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. de Laat, M.A., McGowan, C.M., Sillence, M.N. and Pollitt, C.C. (2010). Equine laminitis: Induced by 48 hr hyperinsulinaemia in Standardbred horses. *Equine Veterinary Journal*. 42(2), 129–135. DOI: http://dx.doi.org/10.2746/042516409X475779
2. Dern, K., van Eps, A., Wittum, T., Watts, M., Pollitt, C. and Belknap, J. (2018). Effect of continuous digital hypothermia on lamellar inflammatory signaling when applied at a clinically-relevant time point in the oligofructose laminitis model. *Journal of Veterinary Internal Medicine*. 32(1), 450–458. DOI: https://dx.doi.org/10.1111/jvim.15027
3. Kullmann, A., Holcombe, S.J., Hurcome, S.D., Roessner, H.A., Hauptman, J.G., Geor, R.J. and Belknap, J. (2014). Prophylactic digital cryotherapy is associated with decreased incidence of laminitis in horses diagnosed with colitis. *Equine Veterinary Journal*. 46(5), 554–559. DOI: https://doi.org/10.1111/evj.12156
4. Obel, N. (1948). Studies of the Histopathology of Acute Laminitis. Almgvist and Wilcsells Bottrykeri Ab Uppsala (Thesis).
5. Patterson-Kane, J.C., Karikoski, N.P. and McGowan, C.M. (2018). Paradigm shifts in understanding equine laminitis. *The Veterinary Journal*. 231, 33–40. DOI: http://dx.doi.org/10.1016/j.tvjl.2017.11.011
6. Pollitt, C.C. (1996). Basement membrane pathology: A feature of acute equine laminitis. *Equine Veterinary Journal*. 28(1), 38–46. DOI: https://dx.doi.org/10.1111/j.2042-3306.1996.tb01588.x
7. Stokes, S.M., Belknap, J.K., Engiles, J.B., Stefanovski, D., Bertin, F.R., Medina-Torres, C.E., Horn, R. and van Eps, A.W. (2019). Continuous digital hypothermia prevents lamellar failure in the euglycaemic hyperinsulinaemic clamp model of equine laminitis. *Equine Veterinary Journal*. 51(5), 658–664. DOI: https://doi.org/10.1111/evj.13072
8. van Eps, A.W. and Pollitt, C.C. (2004). Equine laminitis: cryotherapy reduces the severity of the acute lesion. *Equine Veterinary Journal*. 36(3), 255–260. DOI: https://doi.org/10.2746/0425164044877107

9. van Eps, A.W. and Pollitt, C.C. (2009). Equine laminitis model: Cryotherapy reduces the severity of lesions evaluated seven days after induction with oligofructose. *Equine Veterinary Journal*. 41(8), 741–746. DOI: https://doi.org/10.2746/042516409X434116
10. van Eps AW. (2010). Therapeutic hypothermia (cryotherapy) to prevent and treat acute laminitis. *Veterinary Clinics of North American Equine Practice*. 26(1), 125–33. DOI: https://doi.org/10.1016/j.cveq.2010.01.002
11. van Eps, A.W., Leise, B.S., Watts, M., Pollitt, C.C. and Belknap, J.K. (2012). Digital hypothermia inhibits early lamellar inflammatory signalling in the oligofructose laminitis model. *Equine Veterinary Journal*. 44(1), 120–124. DOI: https://doi.org/10.1111/j.2042-3306.2011.00416.x
12. van Eps, A.W., Pollitt, C.C., Underwood, C., Medina-Torres, C.E., Goodwin, W.A. and Belknap, J.K. (2014). Continuous digital hypothermia initiated after the onset of lameness prevents lamellar failure in the oligofructose laminitis model. *Equine Veterinary Journal*. 46(5), 625–630. DOI: https://doi.org/10.1111/evj.12180
13. Wylie, C.E., Collins, S.N., Verheyen, K.L., and Newton, J.R. (2011). Frequency of equine laminitis: a systematic review with quality appraisal of published evidence. *The Veterinary Journal*. 189(3), 248–256. DOI: https://doi.org/10.1016/j.tvjl.2011.04.014
14. Wylie, C.E., Newton, J.R., Bathe, A.P., and Payne, R.J. (2015). Prevalence of supporting limb laminitis in a UK equine practice and referral hospital setting between 2005 and 2013: implications for future epidemiological studies. *Veterinary Record*. 176(3), 72. DOI: https://doi.org/10.1136/vr.102426