



Measuring hoof horn haemorrhage in heifers: A history

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ABSTRACT

Understanding the aetiology and pathogenesis of claw-horn disease (CHD) is essential for developing prevention/treatment programmes. Haemorrhages in the hoof horn (i.e. white line/sole haemorrhages) are an important part of the pathogenesis of CHD, being precursors to and predictors of lesions such as white-line disease and sole ulcer. Understanding haemorrhage development can provide useful information about the aetiology and pathogenesis of CHD. The development of hoof horn haemorrhages is best studied in cattle without previous claw-horn damage, as previous history of damage can markedly alter the hoof's response to stressors. Since the early 1990s, many prospective studies of the risk factors associated with CHD have been undertaken in late pregnant and early lactation heifers, which have a low risk of having had CHD but which are exposed to the same risk factors as lactating cows. Those studies have used a range of methods to assess hoof horn haemorrhages, with the principal focus, particularly initially (but also more recently), being on measuring lesion severity. However, as the science developed it became clear that measuring lesion extent was also important and that combining severity and extent in a single measure was the best approach to assess hoof horn haemorrhages. Studies of hoof horn haemorrhage in heifers have significantly increased our understanding of CHD, demonstrating the importance of housing and the relative lack of importance of post-calving nutrition. Most importantly, they have shown the importance of parturition as a risk factor for CHD, and how parturition interacts with other risk factors to accentuate their effect. The use of such studies has decreased in recent years, despite recent research showing that we still have much to learn from prospective studies of hoof horn haemorrhages in heifers.

Introduction

Worldwide, lameness is regarded as an important welfare issue in dairy cattle (von Keyserlingk et al., 2009; Čobanović and Magrin, 2023) and there has been a large amount of research focussed on understanding the aetiology and pathogenesis of lameness and of the risk factors associated with its development (e.g. Blowey, 2005; Barker et al., 2009; Browne et al., 2022). However, in many cases, particularly where claw-horn damage is the underlying pathology, lameness occurs at the end stage of a process of damage accumulation (Newsome et al., 2016), so studies which focus on lame animals are likely to be using data from animals where the initial damage that started the process occurred a considerable time previously. This is particularly important for prospective studies looking at the influence of factors on the risk of lameness (or development of lameness-associated changes), where the inclusion of study animals which have existing subclinical damage, or which have a previous history of lameness, could mask or alter the apparent response to the risk factors being tested (Randall et al., 2018).

Hence, a population of animals which have a low risk of previous disease or damage is ideal for prospective research into the aetiology and pathogenesis of lameness. As parturition, housing, lactation and lactation diets seem to be key risk factors for lameness (Knott et al., 2007), first lactation heifers would appear to be a useful group as (if analysis starts before calving) as this is a population which has not encountered at least three of those key risks. In addition, first lactation heifers are particularly useful, as, after calving, the environment and the nutritional regimes they are exposed to are the same as that of older cows, so studies can evaluate factors which are relevant to the whole farm situation not just heifers.

Having identified that heifers are an ideal population in which to study the development of lameness, it is then crucial to identify changes which are precursors to lameness (or predictors of future lameness). Risk factors can then be studied in relatively small groups of cattle on a single site, rather than studying the much larger numbers that would be required (often on multiple farms) if the principal outcome was detectable lameness. For claw horn disease, one key surrogate endpoint

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that has been identified is the presence and severity of haemorrhages in the hoof horn. The aim of this narrative review of the peer-reviewed literature is to look at the history of research into hoof horn haemorrhages in heifers, to critically assess key studies, and to show how measurement of hoof horn haemorrhage severity and extent in heifers has helped us to better understand the aetiology, and pathogenesis of claw horn disease and its associated risk factors in dairy cows.

What are hoof horn haemorrhages?

Hoof horn haemorrhages are visible haemorrhages in the hoof horn. They can be seen in the sole horn ('sole haemorrhages') close to, or in the horn which forms the white line ('white line haemorrhages'). For completeness, haemorrhage can also be seen in the wall horn ('wall haemorrhages'), although the latter are generally not included in studies of the development of 'hoof horn haemorrhages' with only the solar regions of the claw capsule being assessed. Irrespective of cause, sole and white line haemorrhages arise from the solar corium, which is the part of the dermis of the hoof which produces the hoof horn of the sole and the white line. Damage to the corium can result in blood escaping from the vessels of the corium, staining the surrounding horn, which when it comes into wear (usually 6 or more weeks later; [Ossent and Lischer, 1998](#)) can be seen as hoof horn haemorrhages. If the blood solely diffuses down the horn tubules, then the haemorrhages can be seen as 'paintbrush' haemorrhages ([Greenough, 1985](#)).

Hoof horn haemorrhages can be painful and associated with lameness ([Leach et al., 1998](#)). However, when they are being assessed in controlled studies of the aetiology and pathogenesis of lameness, the focus is not on whether they cause pain, but on their value as precursors to, or predictors of, more serious lesions or future lameness ([Leach et al., 1998](#)).

Greenough and Vermunt (1991): the first systematic evaluation of hoof horn haemorrhages in heifers (and older cows)

[Greenough and Vermunt \(1991\)](#) undertook the first peer-reviewed study to systematically evaluate and score hoof horn haemorrhages in heifers. Their justification for this study was that hoof horn haemorrhages were considered to be a sign of 'laminitis', which they linked to sole ulcer and white line disease. [Greenough and Vermunt \(1991\)](#) stated that their aim was to develop and validate a system for objectively evaluating the severity of hoof horn haemorrhages. In order to do this, they assessed hoof horn haemorrhages in all four claws of the hind feet at two monthly intervals from 4 months before expected date of calving to 2 months after in 30 cows (10 heifers, 10 second parity cows and 10 third parity). They used a 4 point scoring system: 0, no haemorrhages observed; 1, slight discolouration; 2, moderate haemorrhage; 3, severe haemorrhage; 4, exposed corium.

In order to account for the greater clinical importance of higher haemorrhage scores, if haemorrhages were present the simple haemorrhage scores were adjusted using the formula 'severity score' = 2 (score⁻¹). No specific account was taken of the extent of lesions except that four zones were scored per claw (see [Fig. 1](#)). Amalgamated data were reported for each cow at each examination for white line haemorrhage severity (zones 1 and 2/3; [Fig. 1](#)) and sole haemorrhage severity (zones 4 and 5).

[Greenough and Vermunt \(1991\)](#) reported that the severity of the haemorrhages differed between the parity groups at each of the examinations but not overall. They concluded that their data showed that heifers had higher haemorrhage severity scores up to calving but after calving their severity scores decreased whereas those of the multiparous cows continued to increase. The study clearly demonstrated that scoring haemorrhages rather than just reporting their presence or absence was feasible. It therefore stands as a landmark study in the history of cattle lameness. Nevertheless, there are several issues with the paper. One crucial limitation is the choice of scoring scheme; the use of 0, 1, 2, 4,

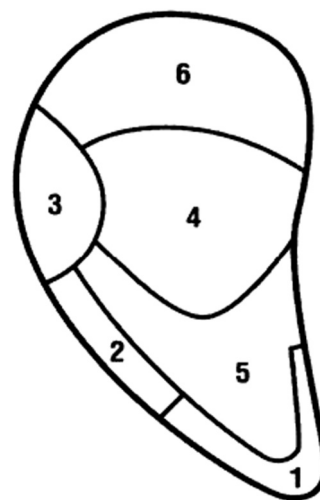


Fig. 1. Hoof map recommended for haemorrhage scoring ([Greenough and Vermunt, 1991](#)). Zones 1, 2 and 3 are considered 'white line', zones 4 and 5 'sole', and zone 6 'heel bulb'. *Note:* Most studies reviewed in this paper which scored individual zones initially scored zones 1–5 and subsequently combined them into white line or sole haemorrhage scores for further analysis (e.g. [Leach et al., 1998](#); [Livesey et al., 1998](#)). [Greenough and Vermunt \(1991\)](#) scored zones 2 and 3 as a single zone, so their combined white line score was derived from only two scores. [Frankena et al. \(1992\)](#) scored all 6 zones and analysed presence/absence of haemorrhages across the claw rather than reporting white line or sole scores.

and 8 as severity scores resulted in a dataset that is not amenable to transformation even when scores are summed. This means that even with modern statistical systems, non-parametric techniques remain the most suitable technique for assessing the data, limiting the power of the analysis (which is already low as there were only 10 animals per parity group) and severely limiting the ability to assess interactions (for example whether the change with time was different between groups).

Nevertheless, although the statistical limitations are important, the main issue with the paper was that despite their stated aim of validating their method, [Greenough and Vermunt \(1991\)](#) did not actually validate their assessment. Validation of a technique is based on identifying that a technique is reliable and applicable. No assessment of reliability was undertaken in this study. In particular, the reproducibility and repeatability of the technique – whether the same scores would be given if two separate assessors scored the cow at the same time or if the same assessor scored the same cow twice – were not assessed.

Thus the conclusions by [Greenough and Vermunt \(1991\)](#) have to be tempered by the lack of validation of the technique, particularly the conversion of the simple 1–4 score to the transformed severity score, and by the limited statistical analysis of the data. Nevertheless, despite these issues, it must again be stressed that this was an historic ground-breaking paper and provided a template for the design of future studies aimed at controlled evaluation of potential risk factors for lameness.

Frankena et al. (1992): presence or absence of hoof horn haemorrhages in young heifers

Following-on from the conclusion by [Greenough and Vermunt \(1991\)](#) that causative factors responsible for sole haemorrhages were present before mid-pregnancy, [Frankena et al. \(1992\)](#) undertook a cross-sectional study of sole haemorrhages in heifers between 2.5 and 12 months old, scoring all six zones (see [Fig. 1](#)) on all four claws of both hind limbs. Their scoring system was very similar to that used by [Greenough and Vermunt \(1991\)](#) except that a category of slight haemorrhage was added between slight discoloration and moderate haemorrhage (scores 1 and 2, respectively in [Greenough and Vermunt,](#)

1991). However, in contrast to the use of a severity score by Greenough and Vermunt (1991), Frankena et al. (1992) simply identified whether a heifer had a least one hoof horn haemorrhage on any claw that could be described as slight or worse. They were thus able to analyse their data using logistic regression and show, using data from 1078 calves from 117 farms, that age, environment and feeding were all important factors determining whether a calf had slight or worse hoof horn haemorrhages. With regard to age, prevalence increased from 10 % to 70 % between 2.5 and 12 months of age [odds ratio (OR) 1.2 for each month increase in age], while keeping heifers on straw yards as opposed to concrete slats reduced the odds of sole haemorrhage by over 5 times (OR=0.18). Interestingly, both feeding hay (as opposed to other forages) and feeding concentrates (vs not) decreased the odds of sole haemorrhages (OR = 0.53 and 0.44, respectively).

This was a large-scale study, which showed that it was feasible to collect detailed haemorrhage score data on a large number of calves on multiple farms, (although as a cross-sectional study there was only one measurement per calf). As the analysis was focused at a presence/absence level, it lacked the ability to determine how the factors they identified as associated with hoof horn haemorrhages influenced the severity of those lesions. This is a significant limitation because their hypothesis was that recording and scoring haemorrhages was useful because the clinical significance of hoof horn haemorrhages is related to their severity. Unlike Greenough and Vermunt (1991), Frankena et al. (1992) did address the issue of repeatability of haemorrhage scoring, but rather than a systematic analysis they stated that “*variation between observers with regard to scoring the severity of sole haemorrhages is thought to be small, because at least two of the three observers were present at each examination*” and claimed that that this procedure led to a uniform diagnosis.

Bergsten (1993): repeatability of haemorrhage scoring from photographs

Bergsten (1993) was the first peer-reviewed study to focus on the repeatability of haemorrhage scoring. As part of a study which standardised the photographing and scoring of hoof horn haemorrhages, interdigital dermatitis and heel horn erosion, the repeatability of the assessment for sole haemorrhages was determined using 1400 slides from 350 cows, with each slide showing both digits of a single foot. The haemorrhage scoring system used was similar to that used by Frankena et al. (1992) except that it took account of extent as well as intensity of the haemorrhages: 0, no haemorrhages; 1, slight haemorrhages in a small area; 2, slight haemorrhages in a large area or moderate haemorrhages in a small area; 3, moderate haemorrhages in a large area; 4, severe haemorrhages in a small area; and 5, severe haemorrhages in a large area or sole ulcer. Additionally, results were only recorded at the claw level (i.e. only one result per claw) and then added to create a score for all claws of an individual cow, for claws in front feet only and for claws in hind feet only. The slides used in Bergsten (1993) had been scored in a previous analysis (Bergsten, 1988) and the results of the two analyses were compared. In both cases, the digits were scored by the same two observers (who agreed on the score for each digit), but the second scoring was blinded to the first. The results showed very strong agreement between the two scoring events, with, at the digit level (2800 scores), 1668 (83.4 %) of the evaluations having the same score on both occasions, while only 11 (0.4 %) differed by a score of more than 1. Clearly the use of two scorers (who have to agree on the score for each digit) is likely to reduce disagreement, but even taking that into account, the data reported by Bergsten (1993) do strongly support the suggestion by Frankena et al. (1992) that the high repeatability of haemorrhage scoring results in consistent scoring.

Further studies using the Greenough and Vermunt system

Leonard et al. (1994) used Greenough and Vermunt’s scoring system

(with severity score transformation) to assess the impact on hoof horn haemorrhages in heifers of two different cubicle systems. Spring-calving heifers were housed in late autumn (November) and their hoof horn haemorrhages (from all claws of all limbs) scored alongside measurements of behaviour. Leonard et al. (1994) reported that, during housing, hoof horn haemorrhages were either mild or absent (median score 0). Although haemorrhage score increased in both groups during the period between housing and calving, after calving the hoof horn haemorrhages continued to increase in severity (unlike the effect reported by Greenough and Vermunt (1991)). Leonard et al. (1994) found a difference in hoof horn haemorrhage score between the two cubicle types, which they suspected may have been due to differences in lying behaviour; however at the individual cow level, lying behaviour was only correlated with hoof horn haemorrhage score in one of the cubicle houses and then at only one of the four timepoints. This study again highlighted the issue of the reduced power of a scoring system which produces non-parametric results thereby precluding an analysis of interactions in a dataset with repeated measures.

The study of Vermunt and Greenough (1995) was a follow-up to their 1991 publication, and focussed on the development of haemorrhages in heifers before calving as well as changes after calving. Measurements were made from 13 months of age to 2 months after calving, with study animals randomly allocated to one of two environments: 1) housed permanently in a cubicle house with concrete passageways; or 2) outdoors on a dry lot. These treatments were applied from when heifers were diagnosed as pregnant and continued until 2 weeks prior to their expected calving date, when heifers were transferred to individual straw-bedded calving pens. After calving, all heifers were kept in the cubicle house. The scoring system they used was a 0–5 system, which was similar to their first scoring system with the addition of ‘marked haemorrhage’ as an additional score inserted between moderate and severe. Most importantly, rather than create a severity score for each hoof, a zone-specific severity score was created by summing the haemorrhage score for that zone for each of the eight claws. This allowed the haemorrhage score to be analysed using a repeat measures ANOVA which could evaluate the interaction between housing and time. Vermunt and Greenough (1995) reported that 77 % of their heifers had some degree of haemorrhage at 13 months of age and that haemorrhages were present during pregnancy and were higher in animals reared in cubicles than those housed on the dry lot. Contrary to their previous study, they found that haemorrhages in most hoof zones were much more severe after calving, but there was no difference between the two pre-calving treatments on the change in haemorrhage score after calving.

The differences between the scoring systems used by the two Vermunt and Greenough studies highlight the lack, at the time of the study, of an accepted scoring system for haemorrhages and the limited evidence of repeatability and agreement for any of the scoring systems.

Leach and colleagues: towards a more objective scoring system

Leach et al. (1998) focussed on the issues of repeatability and agreement by assessing lesion scoring using a variety of scoring systems. To do this they combined a visual assessment of lesion score alongside measurement of lesion size. Their visual appearance score was based on the colour and density of the haemorrhage, with lesion size being measured using digital image analysis. Five lesion score categories were created:

- 1) total number of lesions;
- 2) sum of severity scores for each zone;
- 3) sum of adjusted severity scores (using the same transformation as Greenough and Vermunt, 1991);
- 4) sum of the areas of sole lesions and lengths of white line lesions expressed as a percentage of claw area and white line length; and,

5) combined score multiplying severity \times size [i.e. a score for each individual lesion was created by multiplying its adjusted severity score (category 3) by its area/length (category 4). These individual lesion scores were then summed to create a combined score].

All lesion scores could be recorded as totals or divided into white line or sole scores. Leach et al. (1998) concluded that although their severity scores were subjective, they were sufficiently repeatable within and between observers to be recommended for use, with Spearman rank correlations of ≥ 0.83 from scoring of photographs. The correlation was lower for comparisons in the live cow ($p = 0.69$), but Leach et al. (1998) suggested that the necessity to clean the foot between such comparisons reduced the association and that measurements in the live cow were actually likely to be more reliable than those based on photographs as detail and colour was lost in photographs.

Leach et al. (1998) concluded that the most useful haemorrhage score combined severity and size, but that, as mild lesions tended to be larger than the more severe ones, a transformed severity score (as per Greenough and Vermunt) needed to be used in the calculation of such a score as not transforming the severity score would result in the over-representation of mild lesions. In addition, they showed that the results of a single claw (right hind outer claw) were a reasonable proxy for the results for all claws, especially in regard to sole haemorrhage score, meaning that significantly fewer measurements needed to be taken to assess hoof horn haemorrhages than had been used previously. Finally, they found only a poor to moderate correlation between scores for white line lesions and sole lesions and thus concluded that these regions should be scored separately.

The method developed by Leach et al. (1998) was used in an observational study on first-calving heifers from 4 weeks before calving to 32 weeks after (Leach et al., 1997). Their analysis found that there was a different pattern between the haemorrhages in the white line and those in the sole, with white line lesion extent peaking at 4 weeks after calving and severity at 9 weeks after calving, whereas sole lesions increased in both severity and extent up to 14 weeks after calving. Leach et al. (1997) suggested that the combined effects of calving, housing and introduction to the lactation diet resulted in insults to the corium of the claw which led to the development of hoof horn haemorrhages. They proposed that the white line and sole lesions they saw represented different stages in the same pathological syndrome, with the difference in timing reflecting local differences in horn composition and net growth. This study combined with the validation reported by Leach et al. (1998) established hoof horn haemorrhage scoring as a useful technique for frequent monitoring of hoof horn health and formed the basis of most subsequent studies of hoof horn haemorrhages in heifers. The main issue with the study was the use of non-parametric analysis, which limited the ability to identify interactions in the repeated measures dataset.

The study which, perhaps, best showcased the value of systematic collection of validated hoof horn haemorrhage data in heifers was that by Le Fevre et al. (2001) who analysed 4 years' worth of hoof haemorrhage data, collected as per Leach et al. (1998) on the SAC Crichton Royal Dairy Farm. By studying correlations between hoof horn haemorrhage measures across claws and zones, they were able to significantly increase our understanding of claw horn lameness. Firstly, they showed that hoof horn haemorrhages were highest in the claws that were most commonly affected by claw horn lameness, i.e. principally the lateral hind claw but also the medial front claw, strongly supporting the hypothesis that hoof horn haemorrhages reflected the initial stages of claw horn lesion development. However, they also showed that the differences between the lateral hind claw and the medial front claw were much greater for sole haemorrhages than white line haemorrhages, again supporting the conclusion that white line and sole haemorrhages are different entities with both shared and distinct risk factors. This was further confirmed by the very limited correlation between white line and sole lesions on the same claw ($r < 0.12$). Le Fevre et al. (2001) concluded that their analysis strongly suggested that "the recording of all

claw horn lesions causing lameness as a single class is an over-simplification which should if possible be avoided".

Le Fevre et al. (2001) also evaluated the correlation in individual claws between hoof horn haemorrhage severity, extent and a score which weighted extent by severity. They found almost complete correlation between the three measures except on the lateral hind claws where correlation was still strong but much lower ($r = 0.76$ for sole lesions and $r = 0.88$ for white line lesions on right hind foot). The reason for this was that more severe lesions were generally smaller than less severe lesions, and the lateral hind claws had the most severe lesions. However, as a significant proportion of small lesions were not severe, Le Fevre et al. (2001) concluded that the weighted score [as recommended by Leach et al. (1998)] was the most appropriate measure to use.

Based on their analysis, Le Fevre et al. (2001) concluded that hoof horn haemorrhages were principally biomechanical in origin, i.e. that they resulted from the concussive effects of exposure to hard or uneven surfaces, rather than nutritional in origin ('laminitis').

Livesey and colleagues: testing the importance of nutrition and housing

Livesey et al. (1998) was the first of a series of studies evaluating the impact of housing and nutrition on the development of hoof horn haemorrhage in heifers. These studies used a simplified version of the system validated by Leach et al. (1998) (refer Table 1). Each zone was individually scored by multiplying the severity and the extent scores, and then the sum of the zone scores in both hind feet was used to create a total score for that zone. Zone scores were combined into a white line score (zones 1, 2, 3; refer Fig. 1) and a sole haemorrhage score (zones 4 and 5). In contrast to Leach et al. (1998), no transformation of the severity score was used.

Livesey et al. (1998) studied haemorrhages from about 6 weeks before to 12 weeks after calving in 40 primiparous Holstein heifers in a 2×2 factorial design with straw yard housing vs cubicle housing and 60 % concentrate by dry matter vs 30 % concentrate by dry matter.

Haemorrhage scores were analysed using an analysis of variance (ANOVA), which allowed the effects of interactions between nutrition and housing to be analysed. Livesey et al. (1998) found that in heifers kept on straw yards after calving, white line haemorrhage scores decreased, whereas in those on cubicle yards, white line haemorrhage scores increased. The pattern was different for sole haemorrhages, which increased in all groups after calving. They found no main effect of diet on white-line haemorrhages, but at 6 weeks after calving, cubicle-housed heifers on the 60 % concentrate diet had a higher haemorrhage score than straw yarded heifers. For sole haemorrhages, the effect of diet at 6 weeks after calving was the same as for white-line haemorrhages; with higher proportion of concentrates, there was an increased haemorrhage score but only in cubicle-housed heifers. However, at 12 weeks after calving their analysis identified an effect of increased concentrates on haemorrhage score in both housing groups.

Although Livesey et al. (1998) used an ANOVA, this did not account for the repeat measures nature of the data. Their analysis therefore assumes that the results of an individual heifer at one timepoint is independent of the results at another timepoint which is not consistent with the longitudinal pattern seen with hoof horn haemorrhages (Offer et al., 2000). Thus for this review the data were reanalysed using a repeated measures generalised linear model with a log link (to normalise the

Table 1
Scoring system for the severity and extent of hoof haemorrhages (from Livesey et al., 1998).

Score	Severity	Extent
1	Slight discolouration	<10 % of surface area
2	Moderate haemorrhage	10–50 % of surface area
3	Severe haemorrhage	>50 % of surface area

residuals) (all analyses used SPSS version 23, IBM, USA).

For sole haemorrhage, the reanalysis found that the only significant effects were time ($P < 0.001$) and housing ($P = 0.008$). Overall, there was an increase in sole haemorrhage score with time; sole haemorrhage scores at weeks 6 and 12 post-partum were both higher than the score pre-calving (mean back transformed difference 6.0 and 9.8, respectively; $P \leq 0.001$). The score at week 12 was higher than that at week 6, but this difference was not significant at the 5 % level ($P = 0.21$). Overall, heifers housed in cubicles had significantly higher sole haemorrhage scores than those housed in straw yards (mean difference 3.7; $P < 0.001$). Diet was not significant either as a main effect ($P = 0.13$) or as an interaction ($P \geq 0.096$).

For white line haemorrhage, all the main effects and interactions had a P -value > 0.53 , except for housing ($P = 0.01$) and the interaction between housing and time ($P = 0.059$). For the former, cattle housed in cubicles had higher white line haemorrhage scores than those housed in a straw yard (mean difference 11.6; $P < 0.001$). For the latter, whereas there was no evidence of a significant change in white line haemorrhage score after calving in heifers kept in straw yards (mean difference in score compared to calving score was -4.6 and -5.6 , at week 6 and week 12, respectively; $P > 0.14$); in heifers housed in cubicles, mean white line haemorrhage scores were greater at 6 and 12 weeks post-partum than prior to calving (mean difference 8.0 and 12.6, respectively; $P < 0.004$).

This re-analysis has strongly confirmed two key findings from the original analysis: the impact of housing and the difference in response of sole and white line haemorrhages to calving, housing and diet. The main difference is the apparent absence of an effect of diet. This does not mean that there was no effect of diet on hoof horn haemorrhages in this study (or in general) but suggests that the original finding may have been an artefact of the statistical method.

The second study in the series (Livesey et al., 2003) had treatment groups which were more similar than in the first study. All the study heifers were housed in a cubicle yard after calving (the difference was the bedding used in the cubicles – straw vs wood shavings), and both study diets were 50 % concentrate diets with the test diet replacing starch carbohydrate with sugar beet pulp. The authors identified no differences between the two groups in the development of hoof horn haemorrhages, even though the cattle fed the high starch diet had lower milk fat production, indicating that there were differences between the groups in regard to rumen fermentation. In this study (and the subsequent one) the statistical analysis used repeat measures ANOVA with square root transformation to normalise the residuals.

The third study in the series (Laven and Livesey, 2004) used a 3×2 factorial design with three housing treatments (straw yards, cubicles with thin mats, cubicles with thicker mattresses) and two dietary treatments (15 g/d of additional rumen protected methionine vs no additional methionine). Haemorrhage scoring started approximately 3 weeks before calving and continued for 26 weeks post-calving. Laven and Livesey (2004), consistent with Livesey et al. (1998), found that housing heifers on straw yards rather than in cubicles significantly reduced both white-line and sole haemorrhages. However, although the use of mattresses rather than mats was associated with increased lying times and less severe hock lesions, Laven and Livesey (2004) were not able to identify a difference in haemorrhage score between heifers on mattresses and those on mats. Further, although additional methionine significantly increased milk yield, clearly showing that the concentration of methionine in the base diet was not optimal, there was no evidence that supplementation reduced hoof horn haemorrhages.

The use of hoof horn haemorrhage scoring in these studies allowed the effect of housing and diet to be studied in a focussed controlled manner, and clearly showed the association between housing and hoof horn damage as well as the relationship between parturition and haemorrhage score. The studies also showed the relative lack of impact of diet on hoof horn haemorrhages (especially when the repeated measures nature of the data was built into the models). The lack of association between diet and hoof horn haemorrhages was further emphasised by an

analysis (Laven et al., 2004) of 2 years of haemorrhage score data in combination with measurement of the concentrations/activities of six acute phase reactants: albumin, fibrinogen, haptoglobin, seromuicoid, iron and caeruloplasmin. This analysis found no clear relationships between any of the acute phase reactants and sole or white line haemorrhages. Laven et al. (2004) concluded the development of the hoof horn haemorrhages observed in the first two years of the study were not primarily caused by endotoxemia (contradicting the laminitis hypothesis whereby endotoxins released in response to subclinical acidosis caused changes in the blood supply to the foot initiating degenerative changes; Nocek, 1997).

Webster and colleagues: unlocking the importance of parturition

In Webster (2001), John Webster used hoof horn haemorrhage scoring to investigate the role of housing (cubicle vs straw yards) and feeding (wet vs dry forage) around parturition. He used a 2×2 factorial design and measured hoof horn haemorrhages (using the system set out in Leach et al. (1998) from 4 weeks before to 24 weeks after calving. Consistent with Livesey et al. (1998), he found that housing heifers in straw yards significantly decreased the post-parturient rise in both white line and sole haemorrhage score. He also showed that this effect of straw yards persisted for at least 24 weeks after calving, even though hoof horn haemorrhage scores in cubicle-housed heifers decreased significantly between 16 and 24 weeks post-partum. Lower forage dry matter was associated with increased sole haemorrhages but only in heifers kept in cubicles. Webster stated that his results strongly suggested that “systemic factors associated with calving and the onset of lactation have a central role in the aetiology of [claw horn lesions]” but that the impact of these external factors is modified by external stresses to the foot (principally environmental stresses).

This hypothesis was further tested in a study (Webster, 2002) which measured hoof horn haemorrhages from 4 weeks before to 24 weeks after calving in two heifer groups: 1) heifers housed in cubicles for the whole period and 2) heifers housed in straw yards from 4 weeks before to 8 weeks after calving and then in cubicles for the remainder of the study period. Both groups were fed the same pre-calving and post-calving diets. Interestingly, in contrast to his previous study and that of Livesey et al. (1998), Webster (2002) reported no significant effect of housing in straw yards on white line haemorrhage score with haemorrhage scores peaking 4–8 weeks after calving and then decreasing in both treatment groups. In contrast, sole haemorrhages peaked later in both groups (8 weeks for straw yard heifers and 12 weeks for cubicle heifers) and were much higher in the cubicle group. Transfer from straw yards to cubicles, 8 weeks after calving was not associated with a rise in either sole or white line haemorrhages. These data strongly support the conclusion by le Fevre et al. (2001) that white line and sole disease are different entities. Their pathogenesis is related (for example, both Livesey et al. (1998) and Webster (2001) found that straw yards reduced both white line and sole haemorrhage score), but other unknown factors may influence one type of haemorrhage only [explaining why Webster (2002) failed to find the benefit of straw yards on white line haemorrhages].

Webster (2002) concluded that his observations provided “strong support for the hypothesis that the primary insult to the structural integrity of the foot is systemic and associated with physiological changes occurring around the time of calving and in early lactation”. However, he also conceded that the increases in haemorrhage score could be related to the nutritional adjustments associated with parturition rather than the inevitable physiological events that accompany parturition.

Webster’s subsequent study set out to specifically test the relative importance of nutrition and parturition in the development of hoof horn haemorrhages. That study (Knott et al., 2007) is perhaps the most important study of hoof horn haemorrhages in heifers. The study evaluated hoof horn haemorrhages in two-year-old heifers ($n = 48$) assigned to different combinations of three different interventions: housing

(cubicles vs straw yards), calving (inseminated vs not-inseminated) and feed (high forage vs concentrate). The combinations are summarised in Table 2.

Hoof horn haemorrhages were evaluated at five timepoints (for calving heifers this was over the period from approximately 4 weeks before calving to 12 weeks after). As heifers were slaughtered at intervals during the study period in order to collect material for biomechanical, biochemical and histological analysis, hoof horn haemorrhages were scored only at the start of the study and then in the week prior to slaughter. As in Webster's previous studies, hoof horn haemorrhages were scored as per Leach et al. (1998) and cumulative scores for the severity and extent of all haemorrhages were presented (sole and white line were not separated).

Despite the small numbers, and haemorrhage score being collapsed from four timepoints to two (early being 2 weeks before calving/diet change and 4 weeks after; and late being 8 and 12 weeks after calving/diet change) Knott et al. (2007) were able to demonstrate significant effects of housing ($P = 0.0234$), calving ($P = 0.0015$) and time ($P < 0.0001$) on hoof horn haemorrhages. Similar effects were not reported for feed. The revolutionary aspect of this study was linking hoof horn haemorrhages to changes in the hoof. Building on their previous research into biomechanical and histopathological changes in the hoof around calving (Tarlton et al., 2002), Knott et al. (2007) were able to show that the changes in hoof horn haemorrhages were associated with changes in the support structures of the hoof and that the factors associated with hoof horn haemorrhage score (i.e. housing, calving and time) were also associated with changes in the support structures. In particular, they were able to show that parturition and housing had additive deleterious effects on the supportive capacity of the corium. They also found that the lack of an effect of concentrate feeding on hoof horn haemorrhages was reflected in the lack of an effect of concentrate feeding on the support structures of the hoof.

The results from Knott et al. (2007) add to previous studies that showed parturition was associated with the development of claw horn lesions, to demonstrate how parturition causes those lesions and significantly increases the risk of claw horn disease. Combined with the findings by Tarlton et al. (2002) that the changes associated with parturition in the suspensory apparatus were consistent with fibrogenic changes in the corium as opposed to inflammatory changes within the laminae, they demonstrated that the development of claw horn lesions is, at least initially, principally a biomechanical rather than an inflammatory metabolic process. Knott et al. (2007) concluded that the key causes of the haemorrhages they saw were the combined effects of the mechanical stresses associated with housing and the loss of connective tissue resilience associated with parturition. In regard to nutrition, they stated that diet is seldom a major lameness risk factor "for heifers fed well balanced lactation diets containing sufficient fibre and formulated to avoid ruminal acidosis". This is a major change away from the nutrition-first hypothesis exemplified in articles such as Nocek (1997).

The hoof horn haemorrhage research undertaken by Webster and colleagues at the University of Bristol clearly established the importance

of parturition in the development of claw horn lesions. Indeed, it is now commonly accepted that parturition is a key driver of lameness in dairy cows. Similarly, Newsome et al. (2016) in their summary of the sequence of events involved in the development of claw horn lesions, include parturition alongside the environment and hoof overgrowth as key factors leading to inappropriate forces on the germinal epithelium of the sole. However, the conclusions by Knott et al. (2007) that claw horn lesion development is not primarily inflammatory and that nutrition plays a limited role in their development have had less impact. Despite Newsome et al. (2016) excluding nutrition and inflammation from their summary of claw lesion development (except in minor roles – body fat mobilisation for nutrition and digital cushion fat utilisation for inflammation), there is still a belief among some scientists that nutrition is important in the development of claw horn lesions (e.g. Passos et al., 2023; Van Saun, 2023). This is exemplified by the continued use of the term 'laminitis' even though the research by Webster and colleagues (and other published research such as Lischer et al., 2002) clearly showed that the principal change leading to claw horn lesions is not inflammation of the lamellae but a degenerative change of the corium (i.e. coriosis).

The publication of Knott et al. (2007) seems to have been a high point in the use of hoof horn haemorrhage scoring to understand the aetiopathogenesis of lameness, as the use of hoof horn haemorrhage scoring in heifers has been much less prevalent since that study. In part this may be a switch away in lameness research from small scale studies looking at specific risk factors in heifers to studies on lameness detection and treatment and the impact of lameness on behaviour. Furthermore, although multiple studies published after Knott et al. (2007) have evaluated hoof horn haemorrhages in heifers, few of these have used a haemorrhage scoring system which utilised both severity and extent, such as that developed by Leach et al. (1998) or even a simplified version (such as that used by Livesey et al., 1998).

Measuring hoof horn haemorrhage severity but not extent

Bergsten et al. (2015) reported the results of a study that had started in 2004, before the publication of Knott et al. (2007). They evaluated the impact of rearing environment and post-calving environment on the severity of sole and white line haemorrhages (alongside other measures of claw and leg health). They found that pre-calving, heifers reared in cubicles with concrete alleyways had a higher prevalence of sole haemorrhages than heifers reared in straw yards, and that those haemorrhages were more severe. The effect of rearing environment was similar for white line haemorrhages, although the prevalence of white line haemorrhages was lower than that of sole haemorrhages. After calving, heifers were randomly assigned to either cubicles with rubber slats or cubicles with concrete slats (using a crossover design). Irrespective of whether heifers were kept on rubber or concrete slats after calving, haemorrhage prevalence and severity were higher after calving than in the rearing phase, with heifers on the concrete slats having the most haemorrhages. Heifers reared in cubicles with concrete alleyways and kept after calving in cubicles with concrete slats had a lower prevalence of severe sole or white line haemorrhages than first-calving heifers on concrete slats that had been reared in straw yards. However, these differences were not statistically significant and, in addition, the observed difference between the two rearing groups was much less than the difference between rubber and concrete slats. Thus, these results do not support a conclusion that rearing environment has a major impact of hoof horn haemorrhages after calving. The use of a measurement of extent as well as severity might have been useful in this case as it would, potentially, have increased the difference between the two rearing groups. It is unclear why extent was not measured in this study as Bergsten had used a combined measure in a previous study (Bergsten, 1993).

In a retrospective study, Randall et al. (2016) evaluated the severity of hoof horn haemorrhages present around first calving on future

Table 2

Heifer groups (ordered by housing, pregnancy status, main feed) across the two years of the study undertaken by Knott et al. (2007).

Period	Group 1 ^a	Group 2 ^b	Group 3 ^c
Year 1	Straw, calving, concentrate (n=12)	Straw, not pregnant, concentrate (n=8)	Straw, not pregnant, forage only (n=4)
Year 2	Cubicle, calving, concentrate (n=12)	Cubicle, not pregnant, concentrate (n=9)	Cubicle, not pregnant, forage only (n=3)

^a Group 1 cows were housed in their allocated house from at least 4 weeks before calving and fed forage before calving and concentrate diet after calving.

^b Group 2, cows were fed the forage diet for four weeks after housing and then fed the concentrate diet.

^c Group 3 cows were fed forage diet only.

lameness risk. They scored white line and sole haemorrhages separately, using a scoring system for severity that was very similar to that used by Leach et al. (1998), but with no transformation of the severity score to account for the extent of the lesions. Similar to previous studies, they reported that by 0–2 months post-calving, the great majority of heifers had some degree of white line or sole haemorrhage (75 and 80 %, respectively), with haemorrhage severity increasing over time from pre-calving to 2–4 months post calving. In their dataset, after calving, sole haemorrhage score was much higher on average than white line haemorrhage score.

Unsurprisingly, given the connection between hoof horn haemorrhages and clinical lameness established by previous studies, Randall et al. (2016) reported that compared to heifers with no or very mild white line haemorrhages (score 0–1), heifers with relatively severe white line haemorrhages (score 3–4) at 2–4 months post calving had significantly increased risk of subsequent lameness, as did those with relatively severe sole haemorrhages (score 4–8). However, Randall et al. (2016) also reported that a white line haemorrhage of 2–4 in the pre-calving period was associated with a decreased risk of future lameness (compared to a score of 0 or 1 at that time) as was a sole haemorrhage score of 2 at 2–4 weeks post-calving (again compared to a score of 0 or 1 at that time). The authors suggested that their results indicated that a mild insult around the time of first calving may be protective for future lameness, and that this may occur because the response to the mild insult results in adaptive changes in the hoof which make it more biomechanically resilient. As all the heifers were kept after calving in a cubicle house, it is likely that the benefit of mild to moderate haemorrhages applies only to heifers kept in an environment which produces significant mechanical stress on the hoof. It would thus be interesting to replicate this study in a less “stressful” environment, such as a straw yard or pasture. It would also be interesting to look at how lesion extent affects the association, as it is plausible that a mild lesion that is greater in extent will produce more protective adaptive changes than a lesion of similar severity but lesser extent.

Kofler et al. (2023) also measured sole haemorrhage severity but not extent in an experimental group of 24 heifers. The heifers were all fed a post-partum diet designed to produce subacute ruminal acidosis (SARA) (based on 60 % concentrates, principally barley). At the end of the feeding period, the heifers were divided into three approximately equal sized groups based on the proportion of study days in which the heifer had had SARA. Kofler et al. (2023) recorded sole haemorrhage score (geometric severity score) alongside other lesions and evaluated the effect of proportion of days with SARA (light, moderate or severe) and sole haemorrhage score and lameness. Interestingly, although they reported a decreased odds of lameness for cows categorised as having light SARA compared to severe SARA (lameness incidence 3.2 % vs 14.3 %, respectively), they did not find a significant impact of SARA category on sole haemorrhage severity (or on other lesion prevalence/severity). This finding is the opposite of the rationale put forward for measuring hoof horn haemorrhages, which is that they are more sensitive at identifying lameness risk factors than recording lameness. This rationale has been demonstrated to be correct by most of the papers highlighted in this review. In contrast, Kofler et al. (2023) found that the effects of SARA on lameness were detected by locomotion scoring but not by changes in hoof horn haemorrhages. The lack of an effect of SARA on hoof horn haemorrhage severity and on the prevalence or severity of other hoof horn lesions suggest that the increased locomotion score identified by Kofler et al. (2023) may not be associated with claw horn damage. Further research is required to test this suggestion.

The three studies reviewed in this section are examples of recent reports of heifer studies where haemorrhage severity has been measured but there has been no modification based on lesion extent. Haemorrhage severity unmodified by extent has also been recorded in studies of mixed age cows (e.g. Miguel-Pacheco et al., 2017; Newsome et al., 2017; Li et al., 2023). Indeed, Miguel-Pacheco et al. (2017) reported unmodified severity scores for white line and sole haemorrhages in newly lame cows

(lame for <2 weeks), and evaluated their effect on the likelihood of recovery after therapeutic hoof trimming. They found that the presence of white line haemorrhages reduced the likelihood of recovery [odds ratio (OR) 0.14; 95 % confidence interval (95 % CI) 0.04–0.55] but, as most of their lesions were not severe, they were not able to report an effect of lesion severity. However they reported that, if a cow had white line haemorrhage, its recovery was positively associated with the length of the lesion (OR 1.05, 95 % CI 1.00–1.09). Miguel-Pacheco et al. (2017) commented that this may be because longer lesions were generally milder and stated that the haemorrhage scoring system that they used (Leach et al., 1998) was “based on the appearance of the lesions rather than size”. This is incorrect since, as described earlier, Leach et al. (1998) explicitly measured lesion size alongside their visual assessment of sole and white line haemorrhages, made exactly the point about the inverse relationship between lesion severity and lesion size made by Miguel-Pacheco et al. (2017), and stated that they believed lesion size was an important measure. They also stated that if lesion severity alone was going to be used, it should be weighted to give “an appropriate degree of variation between scores and emphasis to serious lesions”. Despite this most of the studies which have recorded haemorrhage severity in either heifers or cows since the publication of Knott et al. (2007) have not used a weighted severity score (with only a few, such as Kofler et al., 2023, doing so).

Conclusions

Hoof horn haemorrhages have been systematically studied in heifers as a surrogate endpoint for claw horn lameness since the early 1990s. This approach relies on hoof horn haemorrhages being an effective predictor of (or a precursor to) clinical claw horn lameness, and, also, increased severity of hoof horn haemorrhages being associated with an increased risk of lameness. Both claims are consistent with our current understanding of the development of claw horn lameness. Studies of hoof horn haemorrhages do not depend on whether hoof horn haemorrhages cause clinical lameness, indeed their main advantage is that we can see differences in hoof horn haemorrhage severity without producing clinical lameness (i.e. we can investigate lameness without inducing it).

Identifying how to measure the severity of hoof horn haemorrhages was a major part of the early studies, with the conclusion that combining severity and extent was the best method of determining hoof horn haemorrhage severity. Furthermore, as a single assessment of hoof horn haemorrhages is a single time point in a progressive disease and we can only ever observe the surface of the sole in the live animal (and thus cannot necessarily distinguish between a single short duration insult and continued damage). Multiple assessments of hoof horn haemorrhages are required to properly understand the impact of potential risk factors on hoof horn haemorrhages. Combining effective measurement of hoof horn haemorrhage severity with multiple measurements of those haemorrhages in heifers has played a significant role in our understanding of the pathogenesis of claw horn lesions, from understanding the timing of lesion appearance and identifying the differences in development between white line and sole lesions, through to demonstrating the crucial role of parturition and environment (and their interaction) and the limited importance of nutrition. There are still areas [as shown by Kofler et al. (2023)], where we lack information on hoof horn haemorrhage development that may be addressed in further studies of hoof horn haemorrhages in heifers. These include the association between SARA and hoof horn haemorrhages, and the key risk factors driving haemorrhage development at pasture. Nevertheless, the model used in most of the studies described in this review, i.e. identifying risk factors associated with lameness and comparing the effect of their presence/absence on haemorrhage development in a small group of heifers under controlled conditions, is now much less common.

It is unclear why this is the case. In part it may be because of changes in government funding with fewer bigger grants driving large scale

projects, rather than multiple smaller scale projects. Another possibility is that the increased accessibility of big datasets has meant that researchers have focused on examining those datasets, and that small scale lameness research has become less popular. It is often relatively cheap to analyse a pre-existing large data set (in many cases just requiring a PhD student and a computer), whereas small group studies do require significant funding (principally animal-related costs) and require bespoke research facilities as they are very difficult to undertake on commercial farms. Nonetheless, even though they are currently out of favour, we believe that hoof horn haemorrhage studies in heifers are still needed to properly understand the development of claw horn lesions in dairy cows and thereby optimise our control of lameness.

CRedit authorship contribution statement

Richard Laven: Writing – review & editing, Writing – original draft, Conceptualization. **Linda Laven:** Writing – review & editing.

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