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a cross-sectional study

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Decision tree analysis of clinical data to aid diagnostic reasoning for equine laminitis: a cross-sectional study.

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Abstract

The objective of this cross-sectional study was to compare the prevalence of selected clinical signs in laminitis cases and non-laminitic but lame controls to evaluate their capability to discriminate laminitis from other causes of lameness. Participating veterinary practitioners completed a checklist of laminitis-associated clinical signs identified by literature review. Cases were defined as horses/ponies with veterinary-diagnosed, clinically apparent laminitis; controls were horses/ponies with any lameness other than laminitis. Associations were tested by logistic regression with adjusted odds ratios (OR) and 95% confidence intervals, with veterinary practice as an *a priori* fixed effect. Multivariable analysis using graphical classification tree-based statistical models linked laminitis prevalence with specific combinations of clinical signs. Data were collected for 588 cases and 201 controls. Five clinical signs had a difference in prevalence of greater than +50%: 'reluctance to walk' (OR 4.4, 'short, stilted gait at walk' (OR 9.4), 'difficulty turning' (OR 16.9), 'shifting weight' (OR 17.7) and 'increased digital pulse' (OR 13.2) (all $P<0.001$). 'Bilateral forelimb lameness' was the best discriminator; 92% of animals with this clinical sign had laminitis (OR 40.5, $P<0.001$). If, in addition, horses/ponies had an 'increased digital pulse', 99% were identified as laminitis. 'Presence of a flat/convex sole' also significantly enhanced clinical diagnosis discrimination (OR 15.5, $P<0.001$). This is the first epidemiological laminitis study to use decision-tree analysis, providing the first evidence-base for evaluating clinical signs to differentially diagnose laminitis from other causes of lameness. Improved evaluation of the clinical signs displayed by laminitic animals examined by first-opinion practitioners will lead to equine welfare improvements.

Introduction

Equine laminitis is a painful disease of the foot that affects equidae worldwide (Mellor and others 2001; Wylie and others 2011). The insidious nature of the disease and potential for unrelenting pain often necessitates euthanasia of the affected animal on welfare grounds (Hunt 1993; Menzies-Gow and others 2010b). Effective diagnosis is necessary to allow prompt instigation of palliative and therapeutic treatments, to maximise recovery prospects.

In equine medicine, 'laminitis' is used to describe animals presenting with pain localised to the lamellar region of the foot, with or without concurrent solar pain under the distal margin of the distal phalanx (Stashak 2002). There are no universally accepted gold-standard techniques for the detection and quantification of the four stages of laminitis (Eustace 2010; Herthel and Hood 1999; Hunt and Wharton 2010; Menzies-Gow and others 2010c; Swanson 1999). Acute laminitis arises with the development of clinical signs appreciable as changes in the normal stance and gait of the animal (Baxter 1994; Coffman and Garner 1972; Swanson 1999). Acute laminitis either progresses to the subacute form or to the chronic form of the disease. The subacute stage can either persist, develop to chronic laminitis, or lead to complete recovery. Development of chronic laminitis usually results in a cycle of recurrent episodes (Hood 1999). The terminology used to describe chronic laminitis is extremely variable (Parks and Mair 2009), but is often taken to describe progression from acute laminitis to failure of the SADP resulting in dislocation of the DP following detachment of the hoof wall (Grosenbaugh and others 1999).

Laminitis is necessarily commonly diagnosed solely on the presence of a combination of characteristic clinical signs (Baxter 1994; Vinuela-Fernandez *et al.* 2011a). Diagnostic challenges are compounded by the multifactorial aetiology of the disease, which can arise as a consequence of systemic inflammatory disease, endocrine disease or abnormal weight/load bearing which may initiate distinct pathophysiological processes as reviewed by Eades (2010). However, the common feature of all cases of laminitis is the induction of pathological changes within the SADP, resulting in overt foot pain and clinical signs related to lameness (Baxter 1994; Budras and others 2009a; Budras and others 2009b).

Despite the perceived importance there is remarkably little evidence-based data regarding the clinical presentation of laminitis (Eustace 2010; Hunt and Wharton 2010; Mellor and others 2001; Wylie and others 2013a), adding to inherent difficulties in establishing accurate diagnosis of laminitis due to the non-specific nature of clinical signs and the absence of robust case definitions. Furthermore, there is no general agreement regarding standardised criteria to diagnose laminitis or to classify affected animals based on the phase of disease progression and/or disease aetiology (Parks and Mair 2009; Rohrbach and others 1995). The debilitating consequences of laminitis do, however, require prompt veterinary intervention and accurate diagnosis is therefore essential.

All the factors outlined above complicate the overall challenge of diagnostic reasoning based on clinical signs, presenting the veterinary clinician with a challenge to diagnose laminitis differentially from other forms of orthopaedic disorder. Therefore, the aim of this study was to compare the prevalence of selected clinical signs in laminitis and non-laminitis lameness cases in order to evaluate the capabilities of clinical signs to differentially diagnose laminitis from other causes of lameness. The study is presented considering recommendations of the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement (von Elm and others 2007).

Materials and Methods

Data were collected from two groups:

Group A

A convenience sample of five veterinary institutions (two referral centres, two large first-opinion and referral equine hospitals and a first-opinion mixed practice) were visited and invited to provide data for this study. In addition, veterinary practices (n=93) that were interested in participating in a parallel epidemiological investigation of equine laminitis, were contacted by telephone or email and invited to provide data on clinical signs of lameness (of any origin) for the study reported here.

A literature review was conducted to identify previously suggested clinical signs of laminitis and differential diagnoses. The resultant list was reviewed by expert equine clinicians in selected referral hospitals and laminitis researchers, and a 'lameness reporting form' (LM) (Supplementary Information Item 1) was designed to gather information on laminitis-relevant clinical signs from both laminitic (cases) and non-laminitic lame (controls) horses.

Part one of the LM gathered case identifying information with five subsequent sections recording whether clinical signs pertaining to the foot, stance and lameness irregularities (clinical signs) were present, absent or had not been assessed. Part two of the LM allowed practitioners to record their diagnosis as free text and to select specific diagnostic techniques used to confirm the diagnosis from six tick-box options. A free-text comments section was also included for any additional information pertinent to confirmation of the diagnosis.

Participating practitioners were asked to complete a LM for equine lameness of any origin seen between February-April 2009, and January 2010-May 2011, with the second phase of data collection initiated to increase numbers for analysis. Completed forms were returned by post using supplied reply-paid envelopes. Upon arrival LMs were divided into two groups for analysis: one group containing reported laminitis cases and another containing all animals for which the primary cause of lameness was not laminitis (controls).

Group B

Following this development phase, a 'laminitis reporting form' (LRF) was finalised (Supplementary Information Item 2) as previously described (Wylie and others 2013a). As for the LM, the LRF consisted of five distinct sections on lameness, stance characteristics, feet affected and observed laminitis-related acute and chronic clinical signs. Based on the data collected from animals in Group A, some modifications to the form were made, hence for the purposes of this study only those clinical signs which were reported for both groups were compared. No further clinical data were recorded for the purposes of this study.

A LRF was completed for any case of laminitis, defined as a horse or pony with veterinary-diagnosed, clinically apparent laminitis (i.e. an active episode of laminitis), attended by one of the participating practitioners (Wylie and others 2013a). In animals with recurring laminitis, an episode of veterinary-diagnosed active laminitis was defined as new if the animal had returned to its previous/normal level of soundness and had not received analgesic medication for 14 days or more between episodes (Wylie and others 2013a). However, for the purposes of this study only the first episode of laminitis was included. Practices were asked to complete the LRF for all eligible cases occurring from May 2009 to April 2011.

Statistical analysis

To increase the numbers for data analysis, Groups A and B were combined. Multiple different clinical signs were categorised (present, not present or not assessed) under the following five sections:

(1) Lameness: recumbency, refusal to move unless forced, reluctance to walk, lame at walk, lame at trot, short stilted gait at walk, short stilted gait at trot, difficulty turning

(2) Stance: shifting weight, front feet placed in front of body, reluctance to lift foot

(3) Feet affected: bilateral front feet, bilateral hind feet or all four feet

(4) Acute clinical signs: increased digital pulse, increased hoof temperature, pain on sole pressure

(5) Chronic clinical signs: Coronary band swelling, coronary band depression, divergent growth rings, change in hoof wall angle, wall separation, flat/convex sole, widened white line, pink crescent dorsal to frog, sole prolapse

Initial examination, coding of data and descriptive analyses were conducted using Microsoft Excel (Excel 2003, Microsoft). The prevalence (including corresponding 95% confidence intervals [CI]) of each clinical sign, excluding records where the sign was not assessed, in both case and control animals and the between-group differences in prevalence of presence of clinical sign were determined. Associations between each clinical sign and case or control status were tested using logistic regression models reporting adjusted odds ratios (OR) taking into account veterinary practice as a fixed effect, with 95% confidence intervals (CI), and Wald test P-values. All analyses were conducted in R Statistical Package (version 3.1.2 © 2014 The R Foundation for Statistical Computing) using the 'epicalc' and 'tree' packages. Statistical significance was set at a value of $P<0.05$.

Multivariable analysis was carried out using a multi-factorial classification - tree-based statistical models (hereafter ‘tree models’) (Clark and Pregibon 1997). This analytical technique was chosen due to the unbalanced dataset with potentially different combinations of factors present in different horses. The analysis consisted of determining a binary division of the clinical signs prevalence data (laminitis *vs.* non-laminitis lameness), such that there is the largest difference in terms of prevalence of laminitis *vs.* non-laminitis lameness for those two subsets of data. One subset of animals with a specific clinical sign is first considered (e.g. those with ‘bilateral forelimb lameness’) and the binary division in terms of any of the other clinical signs resulting in the largest difference in prevalence of laminitis is determined. The other subset is then considered (e.g. those with no ‘bilateral forelimb lameness’) and again the clinical signs for which binary division gives the largest difference in prevalence of laminitis *vs.* non-laminitis lameness is determined. The different “branches” of the tree are independent of each other in terms of what binary partitions are presented. This binary partitioning is continued for smaller and smaller subsets of data until no differentiation in terms of prevalence is possible. The trees are then ‘pruned’ to exclude very small differentiations based on a few horses. The analysis is presented in graphical form allowing easy comprehension of the grouping of clinical signs giving the largest differences in prevalence in the data. Univariable comparisons of the distribution of clinical signs for particular subsets identified in the trees were then carried out as per the association between clinical signs and case/controls status described above.

Five separate preliminary tree models were produced for the following characteristics to represent the features of clinically active laminitis recorded: i) lameness, ii) stance, iii) feet affected, iv) acute signs only and iv) acute and chronic signs. ’Lame at trot’ and ‘short stilted gait at trot’ were excluded from the lameness tree model due to large numbers of missing data where these signs had not been assessed (missing for 55.0% and 49.4% of observations, respectively).

After consideration of the five preliminary trees, those variables identified in each preliminary tree as being the greatest differentiators in terms of laminitis were analysed together to form two combined tree models: (i) a combined model of lameness, stance characteristics, feet affected and observed laminitis-related acute clinical signs to reflect active episodes of laminitis in horses with no evidence of chronic laminitis, and (ii) a combined model of lameness, stance characteristics, feet affected and observed laminitis-related acute and chronic clinical signs to reflect active episodes of laminitis in horses with evidence of previous SADP failure (chronic laminitis).

Results

Recruitment

Group A

All five veterinary establishments visited agreed to provide data for this study. In addition, 25 first-opinion veterinary practices agreed to participate, of which 14 (46.7%) contributed data to the study. Lameness forms were provided for 238 unique horses/ponies: 89 (37.4%) from referral practices and 149 (62.6%) from first-opinion practices. Thirty-seven animals (15.5%) were diagnosed by veterinary practitioners as laminitis cases and 201 (84.5%) were diagnosed with non-laminitis lameness. Other causes of lameness included, but were not restricted to, proximal suspensory desmitis (n=40, 17.3%), foot abscesses (n=22, 9.5%) and fractures (n=16, 6.9%). Overall, 73 (30.7%: CI 24.8, 36.5) Group A animals were diagnosed on the basis of clinical signs without further diagnostic procedures (cases 32.4%: CI 17.3, 47.5, controls 30.3%: CI 24.0, 36.7) and 155 (65.1%: CI 59.1, 71.2) animals were diagnosed using multiple diagnostic modalities (cases 62.2%: CI 46.5, 77.8, controls 65.7%: CI 59.1, 72.2). Stated diagnostic techniques used to investigate lameness in the laminitic cases included clinical examination (94.6%: CI 87.3, 100), radiography (64.9%: CI 49.5, 80.2), regional anaesthesia (nerve blocks) (13.5%: CI 2.5, 24.5), surgical/post-mortem findings (13.5%: CI 2.5, 24.5) and blood testing for concurrent predisposing metabolic conditions (8.1%: CI 0.01, 16.9).

Group B

The recruitment of cases is described in detail in Wylie et al. (2013a). In brief, LRFs were received for 551 unique horses/ponies from 30 first-opinion veterinary practices over the two-year period.

Clinical signs

The prevalence of the presence of each clinical sign in laminitis cases and non-laminitis lame controls, excluding records where the sign was not assessed, and difference in prevalence between the two groups are provided in Table 1. The overall prevalence of specific clinical signs ranged from 2.7% (CI 1.5, 3.9) for 'sole prolapse' (number assessed = 706) to 85.0% (CI 81.4, 88.7) for 'lame at trot' (number assessed = 367). The difference in prevalence between cases and controls ranged from -14.1% for 'lame at trot' (sign more common in controls) to +71.9% for 'short stilted gait at walk' (found more often in cases than controls). There were five clinical signs with a difference in prevalence of greater than +50%: three lameness-related signs ('reluctance to walk', 'short, stilted gait at walk' and 'difficulty turning'), one stance-related sign ('shifting weight') and one acute clinical sign ('increased digital pulse').

The logistic regression results are provided in Table 2. For each clinical sign there was a statistically significant increase in the odds of occurrence in the laminitis (cases) group, with the exception of 'recumbent', 'lame at trot' and 'coronary band swelling' for which there was no significant difference (P>0.05). No odds ratio could be calculated for 'coronary band depression' or 'sole prolapse' because no animals in the control group showed these clinical signs.

The preliminary tree models are provided in Supplementary Information Item 3. Consideration of the lameness tree identified the best discriminator as 'short stilted gait at walk'; 93.1% (CI 90.6, 95.5) of animals with that clinical sign had laminitis; 94.1% (CI 91.6, 96.5) of animals with both 'short stilted gait at walk' and 'difficulty turning' had laminitis. Of the 219 animals that did not have a 'short stilted gait at walk', only 27.9% (CI 21.9, 33.8) had laminitis – however, if they had 'difficulty turning' 59.7% (CI 48.0, 71.5) had laminitis. For animals where both these clinical signs were absent, if they were 'reluctant to walk' 40.0% (CI 15.2, 64.8) had laminitis.

The best discriminator in the stance tree was 'shifting weight'; 98.1% (CI 96.6, 99.6) of animals with that clinical sign had laminitis. In animals that were not 'shifting weight', 'front feet placed in front of the body' identified 94.2% (CI 89.2, 99.1) as laminitis cases.

In the 'acute clinical signs' tree, 91.0% (CI 88.5, 93.5) of animals with 'increased digital pulses' had laminitis, and 'pain on sole pressure' in the absence of 'increased digital pulses' identified 69.0% (CI 52.1, 85.8) as cases of laminitis.

The best discriminator in the 'acute and chronic clinical signs' tree was 'increased digital pulses'; 91.0% (CI 88.4, 93.5) of animals with that clinical sign had laminitis, and the additional presence of 'divergent growth rings' identified 100% as laminitis cases.

The tree diagram combining categories of clinical signs for acute laminitis with lameness, stance and feet is provided in Figure 1. Presence of 'lameness in both forelimbs' was the best discriminator, with 93.1% (CI 90.7, 95.5) of animals with this clinical sign belonging to the laminitis group. Additional presence of an 'increased digital pulse' improved diagnostic accuracy to 99% (CI 97.9, 100) ($P<0.001$). A 'bilateral forelimb lameness' with no 'increase in digital pulse', yet presence of a 'short stilted gait at walk' identified 100% of animals as laminitis cases, however statistical analysis of this sub-group and the presence of 'shifting weight' was not possible due to small numbers of animals with these signs. The presence of 'pain on sole pressure' was not statistically associated with improved clinical discrimination ($P=0.30$).

The overall tree diagram considering both acute and chronic laminitis clinical signs with lameness, stance and feet is provided in Figure 2. Presence of 'lameness in both forelimbs' was again the best discriminator; 92% of animals with this clinical sign had laminitis ($P<0.001$). The additional presence of 'increased digital pulses' improved this to 99% of cases ($P<0.001$). Presence of a 'flat/convex sole' also provided improved clinical discrimination ($P=0.002$). It was not possible to assess statistical significance for 'short stilted gait at walk', or 'shifting weight', again because of the small numbers of animals with these signs.

Discussion

This is the first study comparing the prevalence of veterinary-recognised clinical signs in laminitis and other causes of lameness to evaluate the capabilities of discrimination for differential diagnosis.

A wide range of clinical signs were displayed by the laminitic cases, in agreement with previous reviews (Baxter 1994; Eustace 2010; Hunt and Wharton 2010; Swanson 1999). There were no individual, or combinations of, clinical signs present in every case. The clinical signs that were considered to be the most useful on the basis of this work were three features of lameness investigation ('reluctance to walk', 'short, stilted gait at walk' and 'difficulty turning'), one feature of stance ('shifting weight') and an 'increased digital pulse'. All these signs had a difference in prevalence of over 50% between active laminitis cases (signs more prevalent) and non-laminitic lame horses (signs less prevalent). As the clinical details forms were designed to gather information on laminitis, it may be expected there was a statistically significant difference in the distribution of many of the clinical signs between laminitis cases and non-laminitis lameness controls. For the purposes of this study it was considered important to focus only on the lameness-associated clinical signs for two main reasons. Firstly, because regardless of the underlying pathological process of laminitis, the common feature of all cases of laminitis is the induction of pathological changes within the SADP, resulting in overt foot pain and clinical signs related to lameness (Baxter 1994; Budras and others 2009a; Budras and others 2009b; Eades 2010), and as a consequence previous epidemiological studies of laminitis have used only lameness-associated clinical signs as their case inclusion/exclusion criteria (Alford and others 2001; Dorn and others 1975; Hood and others 1994; Menzies-Gow and others 2010a; Parsons and others 2007; Slater and others 1995). Secondly, to keep the amount of work required by the veterinary surgeons to a minimum to enhance compliance. Collection of data regarding systemic clinical signs would have increased the amount of work required by the participating veterinary practitioners, and it was considered that their presence would aid the diagnosis of the underlying, predisposing condition rather than laminitis directly. Nevertheless, it is acknowledged that as part of the diagnostic process veterinarians will use the animal's history and other clinical features in making their diagnosis. As such, collection of additional clinical data in future studies would be useful to improve the current decision trees, as well as to generate further trees pertaining to, for example, signs of colic.

Currently, visual assessment of lameness is a highly subjective process. Many kinetic and kinematic methods for objectively assessing lameness have been reviewed previously (Hood and others 2001; Keegan 2010), and it is possible that these may prove to be more reliable than visual assessment alone in the future (Dyson

2011). Further evaluation of techniques to evaluate stance and gait characteristics of lame animals may result in a more objective method of diagnosing and/or scoring laminitis, as well as other reasons for lameness. Recently developed techniques allow assessment of horse movement without impeding the use of the animal, and may have a role in evidence-based assessment of lameness in horses in veterinary practice in the future (Dyson 2011; Keegan 2010; Pfau and others 2007). There was no statistically significant difference in prevalence of 'lameness at trot' between cases and controls, and this variable was not included in the tree analysis due to large number of laminitic cases that were not assessed at trot. The high level of missing data is likely to reflect the appropriate reluctance of veterinary surgeons to trot suspect laminitis cases on welfare grounds and so as not to exacerbate lamellar pathology, and the common use of intrasynovial anaesthesia for diagnosis of other lamenesses commonly evaluated at the trot.

Two clinical signs – 'coronary band depression' and 'prolapsed sole' - were pathognomonic for laminitis in this study, . were only found in 13.6% and 3.7% of cases, respectively. Both these signs can indicate disease progression to chronic phase laminitis (i.e. SADP failure and distal phalanx dislocation within the hoof); therefore these signs would not be expected to be present in acute cases, unless they were also suffering from concurrent pathology such as chronic seedy toe/white line disease or severe club feet (Kuwano and others 1999). These results may help veterinary practitioners prioritise where to begin their clinical examination of an active laminitis case, as primary inspection of the sole and coronary band would prevent the animal undergoing lameness evaluation which could precipitate further SADP damage/failure.

Two overall combined trees were generated to reflect the two clinical scenarios of active laminitis, one consisting of clinical signs considered to occur in the acute phase of the disease, and one that also contained data reflective of lamellar damage and displacement of the SADP. In both scenarios, the presence of a bilateral lameness was the most useful discriminator, followed by the presence of increased digital pulses. Whilst these clinical presentations are not specific for laminitis, this work provides an evidence-base for case diagnosis and future epidemiological case definitions.

This work did not provide evidence for some commonly cited clinical signs of diagnostic importance. In particular, 'front feet in front of the body', taken to represent the classic 'laminitis stance', was found in less than half of the diagnosed active laminitis cases, and did not prove to be a useful discriminator. Therefore, despite much anecdotal publicity of this visibly apparent clinical sign (Stashak 2002; Swanson 1999), veterinarians, researchers and owners should be careful to avoid relying on its presence for making a diagnosis of laminitis [40].

The use of clinical recording forms based on evidence-based recommendations may help veterinary practitioners structure their clinical examination of an active laminitis case. However, in medical practice well-validated diagnostic algorithms tools are underused (Pearson and others 1994). For example, a simple predictor based on seven clinical signs for ischaemia in humans was only used in 2.8% of cases (Corey and Merenstein 1987). The clinical usefulness of developing such a technique would need to be established by a survey of first-opinion practitioners to decide whether such a tool would provide useful assistance in laminitis diagnosis in the field.

The limitations of this study include diagnosis by a number of different veterinary clinicians, which may have different levels of experience. To take this into account veterinary practice was included in the generation of the odds ratio estimates, however, misclassification bias may still occur, although this would have tended to shift the odds ratios towards non-significant. Similarly, as it is not possible to obtain a definitive diagnosis of active laminitis in an observational epidemiological study there was the potential for misclassification of cases and controls. For this reason, veterinary recordings of the clinical signs observed was used, as described in Wylie et al., (Wylie and others 2013a, b) and misclassification would have again reduced the ability to detect significant differences rather than produce anomalous significant differences. Inclusion of data in the tree models required the animals to have data for each included variable, resulting in smaller numbers of contributing individuals as the trees became more complex. Consequently, although the variables retained high statistical significance, smaller contributing sample sizes led to larger confidence intervals around prevalence point estimates and the need therefore for some caution in their interpretation.

It is acknowledged that there may be some bias in the data if veterinary practitioners did not accurately detail the clinical signs which they observed and perhaps listed clinical signs that they anticipated to reflect their diagnosis. Furthermore, it would be interesting to collect greater numbers of control animals to conduct the analyses between specific control lamenesses, such as forelimb foot pain only, to highlight more subtle differences between presenting pathologies.

In conclusion, separate clinical signs were compared between laminitis and non-laminitis cases of lameness, and no individual sign was present in every case of laminitis. The clinical signs which best indicated a case of laminitis were characteristic of the chronic phase of the disease only. Improved evaluation of the clinical signs displayed by laminitic animals examined by first-opinion practitioners will lead to equine welfare improvements, as the best recoveries occur in animals undergoing intensive treatment within several hours of the appearance of the disease (Redden 1986). Future consensus on a basic disease definition may permit future systematic review and meta-analysis of epidemiological investigations collecting similar information in different locations worldwide.

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Table 1: Prevalence and 95% confidence intervals (CI) for each clinical sign in both laminitis cases and non-laminitis lameness controls, excluding records where the sign was not assessed, and the percentage of horses that were assessed with corresponding difference in prevalence.

Clinical signs		Cases (n=588)					Controls (n=201)					Overall (n=789)		
		Present (n)	Absent (n)	Prevalence (%)	LCI (%)	UCI (%)	Present (n)	Absent (n)	Prevalence (%)	LCI (%)	UCI (%)	Number assessed	Percentage assessed (%)	Difference in prevalence (%)
Lameness	Recumbent	24	479	4.8	2.9	6.6	1	191	0.5	0.0	1.5	695	88.1	+4.3
	Refusal to move unless forced	148	361	29.1	25.1	33.0	14	180	7.2	3.6	10.9	703	89.1	+21.9
	Reluctance walk	395	155	71.8	68.1	75.6	38	157	19.5	13.9	25.1	745	94.4	+52.3
	Lame walk	409	95	81.2	77.7	84.6	76	122	38.4	31.6	45.2	702	89.0	+42.8
	Lame trot	152	42	78.4	72.6	84.2	160	13	92.5	88.6	96.4	367	46.5	-14.1
	Short stilted walk	446	66	87.1	84.2	90.0	29	162	15.2	10.1	20.3	703	89.1	+71.9
	Short stilted trot	125	55	69.4	62.7	76.2	53	119	30.8	23.9	37.7	352	44.6	+38.6
	Difficulty turning	456	47	90.7	88.1	93.2	52	137	27.5	21.2	33.9	692	87.7	+63.1
Stance	Shifting weight	316	256	55.2	51.2	59.3	7	188	3.6	1.0	6.2	767	97.2	+51.7
	Front feet in front	250	317	44.1	40.0	48.2	6	190	3.1	0.7	5.5	763	96.7	+41.0
	Reluctance lift foot	300	269	52.7	48.6	56.8	24	169	12.4	7.8	17.1	762	96.6	+40.3
Feet Affected	Bilateral fore	538	44	92.4	90.3	94.6	32	152	17.4	11.9	22.9	766	97.1	+71.7
	Bilateral hind	244	323	43.0	39.0	47.1	25	156	13.8	8.8	18.8	748	94.8	+28.3
	All four feet	234	348	40.2	36.2	44.2	5	193	2.5	0.3	4.7	780	98.9	+39.5
Acute	Increased digital pulse	520	50	91.2	88.9	93.6	45	150	23.1	17.2	29.0	765	97.0	+68.2
	Increased hoof temperature	324	218	59.8	55.7	63.9	30	164	15.5	10.4	20.6	736	93.3	+44.3
	Pain sole pressure	263	271	49.3	45.0	53.5	35	149	19.0	13.4	24.7	718	91.0	+30.2
Chronic	Coronary band swelling	27	505	5.1	3.2	6.9	6	186	3.1	0.7	5.6	724	91.8	+2.0
	Coronary band depression	73	462	13.6	10.7	16.6	0	192	0.0	0.0	0.0	727	92.1	+13.6
	Divergent growth rings	148	378	28.1	24.3	32.0	3	190	1.6	0.0	3.3	719	91.1	+26.6

Change hoof wall angle	129	383	25.2	21.4	29.0	7	186	3.6	1.0	6.3	705	89.4	+21.6
Wall separation	71	445	13.8	10.8	16.7	2	184	1.1	0.0	2.6	702	89.0	+12.7
Flat/convex sole	232	291	44.4	40.1	48.6	9	180	4.8	1.7	7.8	712	90.2	+39.6
Widened white line	133	368	26.6	22.7	30.4	8	176	4.4	1.4	7.3	685	86.8	+22.2
Pink crescent	46	464	9.0	6.5	11.5	1	189	0.5	0.0	1.6	700	88.7	+8.5
Sole prolapse	19	498	3.7	2.1	5.3	0	189	0.0	0.0	0.0	706	89.5	+3.7

Table 2: Odds ratios and 95% confidence intervals (CI), with corresponding Wald *P*-values, for each clinical sign in laminitis cases compared to non-laminitis lameness controls. ORs are adjusted for the effect of veterinary practice.

Clinical Signs		Number	Adjusted Odds Ratio	95% Confidence Interval	Wald P-value
Lameness	Recumbent	695	5.1	0.5, 51.4	0.17
	Refusal to move unless forced	703	3.5	1.6, 7.7	0.002
	Reluctance walk	745	4.4	2.2, 8.6	<0.001
	Lame walk	702	2.2	1.0, 4.7	0.04
	Lame trot	367	0.3	0.0, 2.6	0.29
	Short stilted walk	703	9.4	4.5, 19.6	<0.001
	Short stilted trot	352	3.9	1.6, 9.6	0.003
	Difficulty turning	692	16.9	7.0, 40.8	<0.001
Stance	Shifting weight	767	17.7	6.8, 45.6	<0.001
	Front feet in front	763	24.5	7.9, 75.9	<0.001
	Reluctance lift foot	762	4.0	1.9, 8.1	<0.001
Feet Affected	Bilateral fore	766	40.5	16.3, 100.9	<0.001
	Bilateral hind	748	21.3	7.7, 59.1	<0.001
	All four feet	780	96.3	22.1, 419.8	<0.001
Acute	Increased digital pulse	765	13.2	6.0, 29.3	<0.001
	Increased hoof temperature	736	5.7	2.8, 11.5	<0.001
	Pain sole pressure	718	2.7	1.4, 5.3	0.005
Chronic	Coronary band swelling	727	1.1	0.3, 3.9	0.88
	Coronary band depression	724	NA	NA	NA
	Divergent growth rings	719	96.3	17.1, 542.8	<0.001
	Change hoof wall angle	705	21.1	6.3, 71.0	<0.001
	Wall separation	702	58.5	5.1, 672.8	<0.001
	Flat/convex sole	712	15.5	5.9, 40.5	<0.001
	Widened white line	685	17.3	5.5, 54.5	<0.001
	Pink crescent	700	16.5	2.0, 136.5	0.009
	Sole prolapse	706	NA	NA	NA

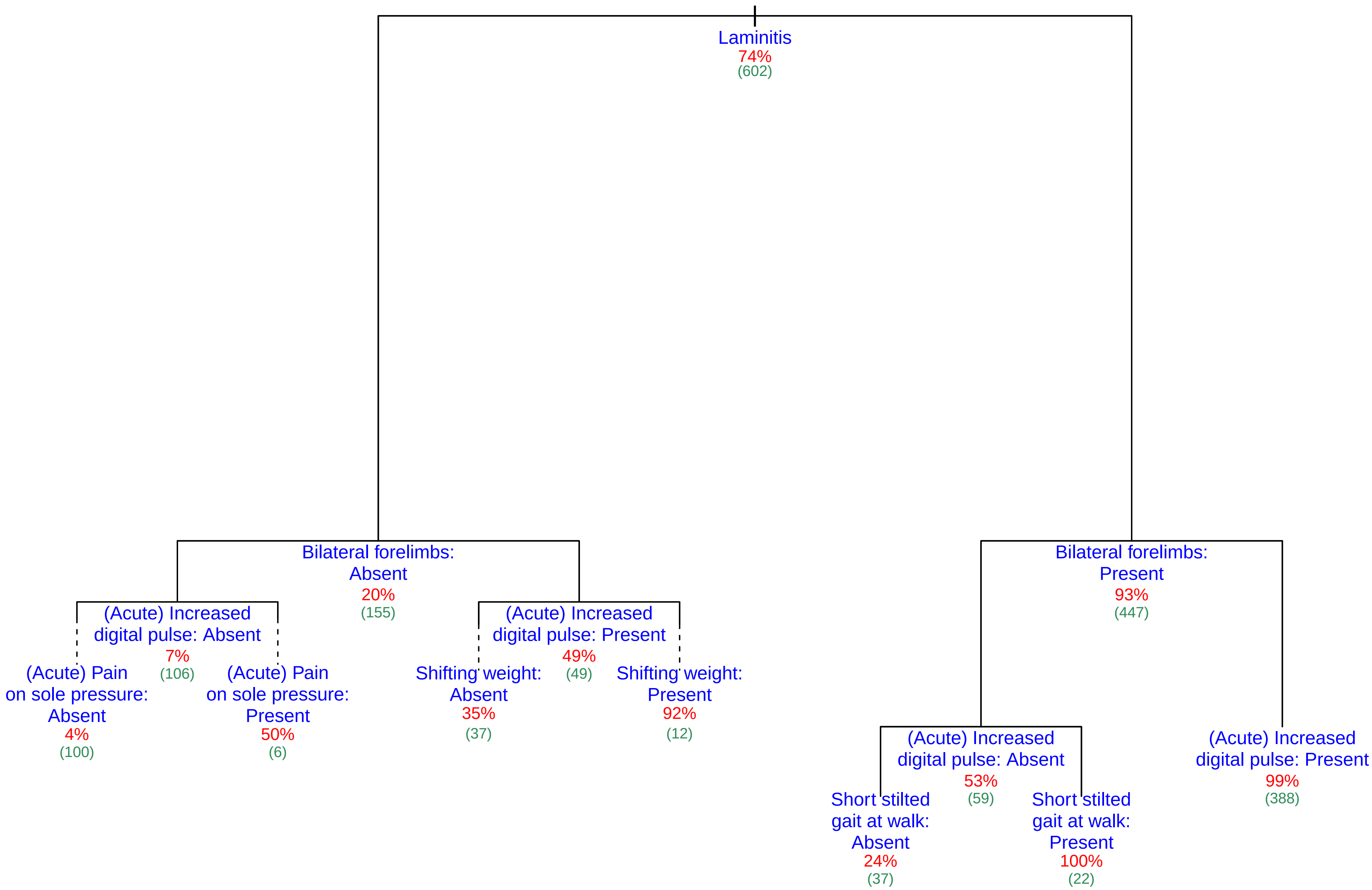
Figure 1: Tree diagram of the occurrence of laminitis for combinations of lameness, stance, feet affected, and acute laminitis clinical signs. Data were from 586 horses/ponies for which information on each clinical sign was described, of which 74% had laminitis. The percentage at the end of each branch are the occurrence rates of laminitis in those horses/ponies with that particular combination of clinical signs, and the value in brackets the number of horses/ponies of that particular combination of clinical signs.

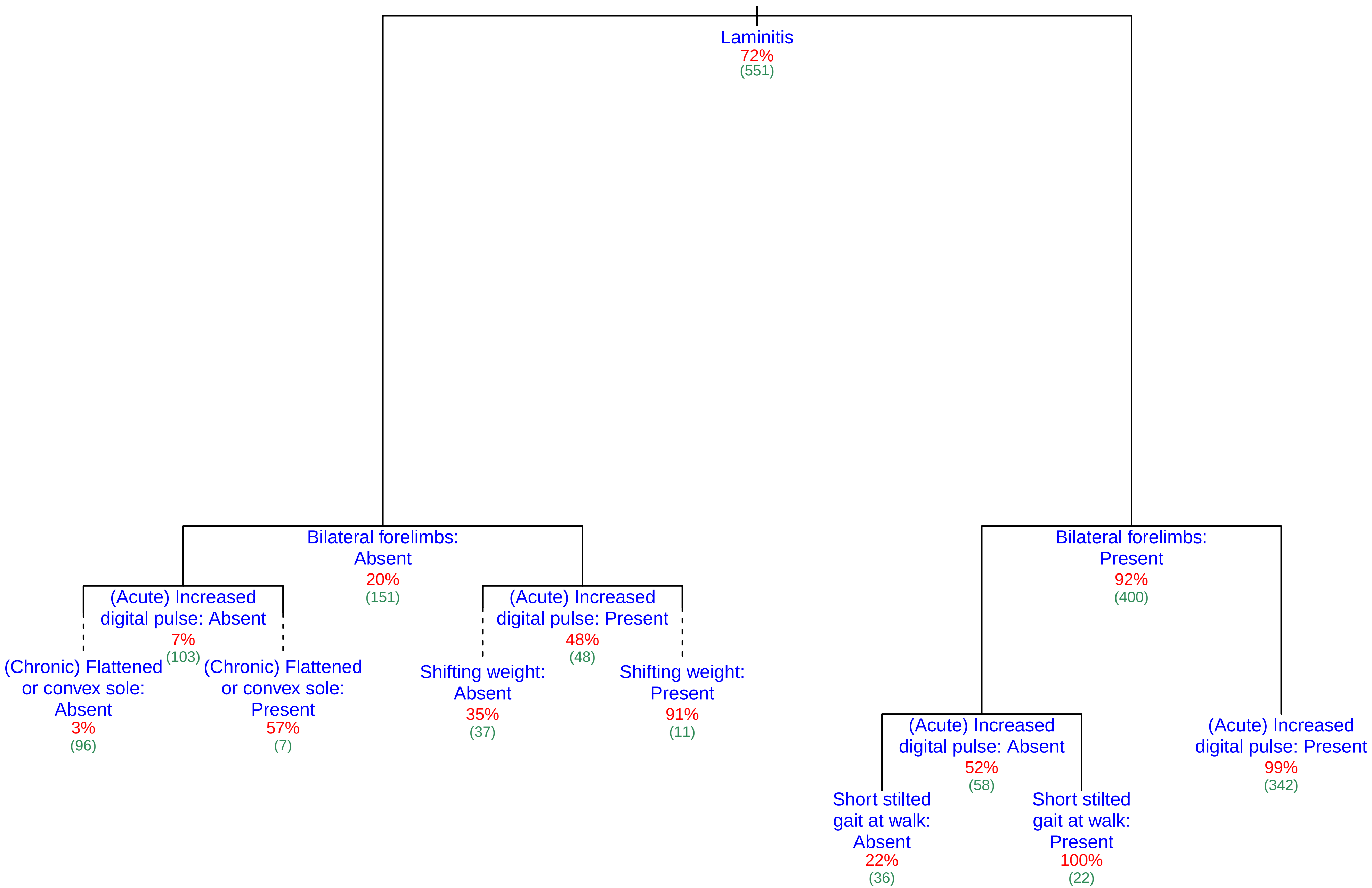
Figure 2: Overall tree diagram of the occurrence of laminitis for combinations of lameness, stance, feet affected, acute and chronic laminitis clinical signs. Data were from 551 horses/ponies for which information on each clinical sign was described, of which 72% had laminitis. The percentage at the end of each branch are the occurrence rates of laminitis in those horses/ponies with that particular combination of clinical signs, and the value in brackets the number of horses/ponies of that particular combination of clinical signs.

Supplementary Information Item 1: Lameness reporting form (LM) used to investigate the clinical signs of laminitis in Group A recruiting both cases and controls.

Supplementary Information Item 2: Laminitis reporting form (LRF) used to investigate the clinical signs of laminitis in Group B recruiting cases only.

Supplementary Information Item 3: Preliminary Tree models of the occurrence of laminitis for combinations of lameness, stance, feet affected, acute and chronic laminitis clinical signs.


Laminitis
74%
(602)
Bilateral forelimbs: Absent
20%
(155)
(Acute) Increased digital pulse: Absent
7%
(106)
(Acute) Pain on sole pressure: Absent
4%
(100)
(Acute) Pain on sole pressure: Present
50%
(6)
(Acute) Increased digital pulse: Present
49%
(49)
Shifting weight: Absent
35%
(37)
Shifting weight: Present
92%
(12)
Bilateral forelimbs: Present
93%
(447)
(Acute) Increased digital pulse: Absent
53%
(59)
Short stilted gait at walk: Absent
24%
(37)
Short stilted gait at walk: Present
100%
(22)
(Acute) Increased digital pulse: Present
99%
(388)


Laminitis
72%
(551)
Bilateral forelimbs: Absent
20%
(151)
(Acute) Increased digital pulse: Absent
7%
(103)
(Chronic) Flattened or convex sole: Absent
3%
(96)
(Chronic) Flattened or convex sole: Present
57%
(7)
(Acute) Increased digital pulse: Present
48%
(48)
Shifting weight: Absent
35%
(37)
Shifting weight: Present
91%
(11)
Bilateral forelimbs: Present
92%
(400)
(Acute) Increased digital pulse: Absent
52%
(58)
Short stilted gait at walk: Absent
22%
(36)
Short stilted gait at walk: Present
100%
(22)
(Acute) Increased digital pulse: Present
99%
(342)

Supplementary Information Item 1: Lameness reporting form (LM) used to investigate the clinical signs of laminitis in Group A recruiting both cases and controls.

LAMENESS REPORTING FORM 1

Name of horse/pony: _

Surname of owner/Case I.D: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Date of clinical examination: [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

Lameness	**Assessment** *(please circle 1 option per line)*
Recumbent	Yes / No / Didn't assess
Refusal to move unless forced	Yes / No / Didn't assess
Reluctance to walk	Yes / No / Didn't assess
Lame at walk	Yes / No / Didn't assess
Lame at trot	Yes / No / Didn't assess
Short, stilted gait at walk	Yes / No / Didn't assess
Short, stilted gait at trot	Yes / No / Didn't assess
Difficulty turning	Yes / No / Didn't assess

Stance	**Assessment** *(please circle 1 option per line)*
Shifting weight	Yes / No / Didn't assess
Front feet placed in front of body	Yes / No / Didn't assess
Front feet placed underneath body	Yes / No / Didn't assess
Square stance	Yes / No / Didn't assess
Reluctance for a foot to be lifted	Yes / No / Didn't assess

Feet affected	**Assessment** *(please circle 1 option per line)*	**Most severely affected foot/feet** *(please tick all that apply)*
Right fore	Yes / No / Didn't assess	
Left fore	Yes / No / Didn't assess	
Right hind	Yes / No / Didn't assess	
Left hind	Yes / No / Didn't assess	

Clinical signs of the most severely affected foot/feet	**Assessment** *(please circle 1 option per line)*
Increased digital pulse	Yes / No / Didn't assess
Increased hoof temperature	Yes / No / Didn't assess
Decreased hoof temperature	Yes / No / Didn't assess
Pain on sole pressure	Yes / No / Didn't assess
Coronary band swelling	Yes / No / Didn't assess
Coronary band depression	Yes / No / Didn't assess

Clinical signs of the most severely affected foot/feet	**Assessment** *(please circle 1 option per line)*
Divergent growth rings (wider at heels)	Yes / No / Didn't assess
Change in dorsal hoof wall angle	Yes / No / Didn't assess
Wall separation	Yes / No / Didn't assess
Flattened or convex sole	Yes / No / Didn't assess
Widened white line	Yes / No / Didn't assess
Pink crescent dorsal to frog	Yes / No / Didn't assess
Prolapsed sole	Yes / No / Didn't assess

For office use only:

Animal Health Trust 1361

AHT Reference: [ ][ ][ ][ ] [ ][ ]

Supplementary Information Item 2: Laminitis reporting form (LRF) used to investigate the clinical signs of laminitis in Group B (cases only).

AHT Animal Health Trust

ANIMAL HEALTH TRUST
LAMINITIS REPORTING FORM

WorldHorseWelfare

1. Name of horse/pony: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

2. Surname of owner/Case ID: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

3. Date of clinical examination: ☐☐ / ☐☐ / ☐☐☐☐ (d d / m m / y y y y)

4. Lameness	Assessment *(please **cross** one option per line)* YES	NO	DIDN'T ASSESS
Recumbent	☐	☐	☐
Refusal to move unless forced	☐	☐	☐
Reluctance to walk	☐	☐	☐
Lame at walk	☐	☐	☐
Lame at trot	☐	☐	☐
Short, stilted gait at walk	☐	☐	☐
Short, stilted gait at trot	☐	☐	☐
Difficulty turning	☐	☐	☐

5. Stance	Assessment *(please **cross** one option per line)* YES	NO	DIDN'T ASSESS
Shifting weight	☐	☐	☐
Leg trembling	☐	☐	☐
Front feet placed in front of body	☐	☐	☐
Hind feet placed underneath body	☐	☐	☐
Reluctance for a foot to be lifted	☐	☐	☐

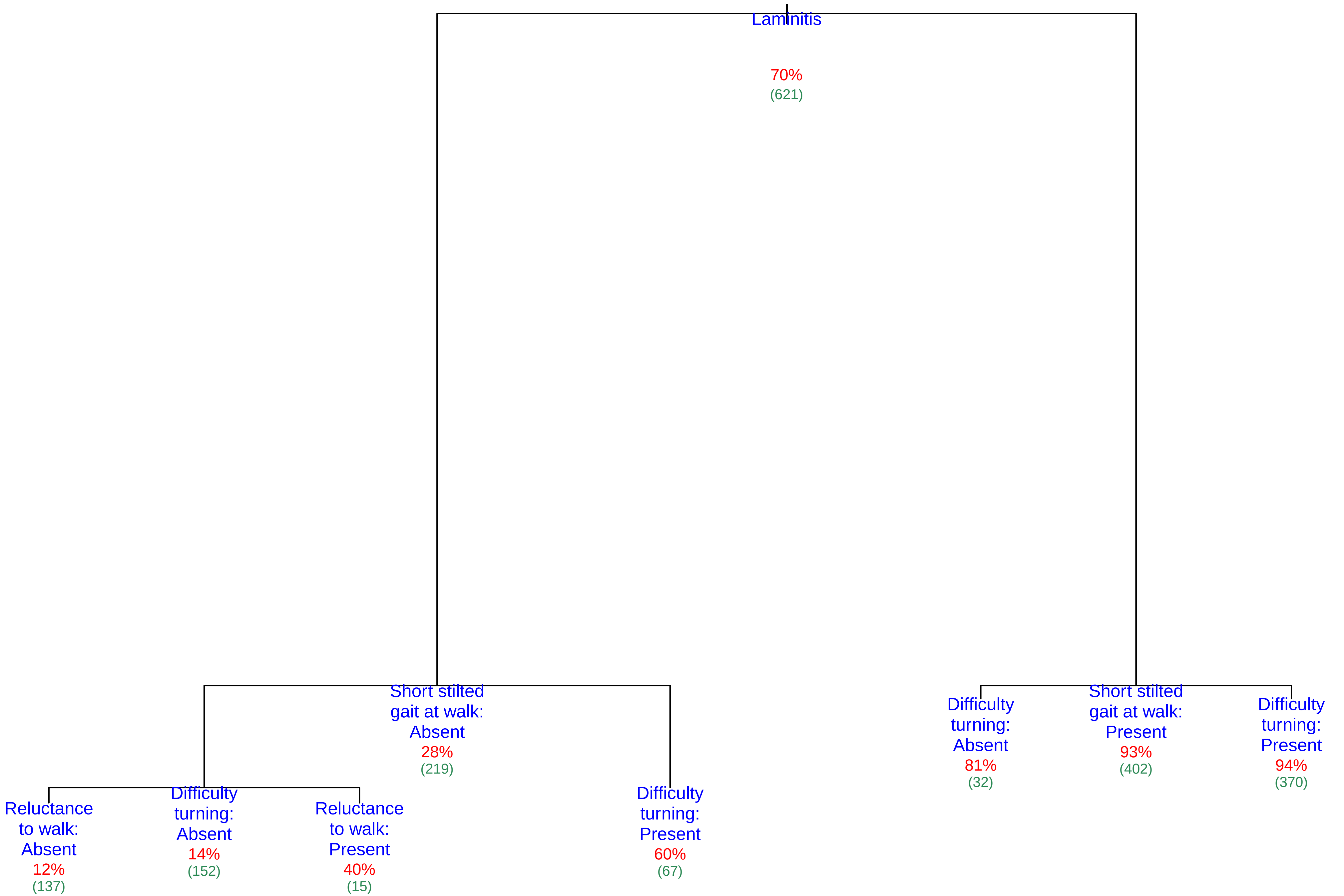
6. Feet affected	Affected *(please **cross** one option per line)* YES	NO	DIDN'T ASSESS	Most severely affected foot (or feet if bilaterally affected) *(please **cross** all that apply)*
Right fore	☐	☐	☐	☐
Left fore	☐	☐	☐	☐
Right hind	☐	☐	☐	☐
Left hind	☐	☐	☐	☐

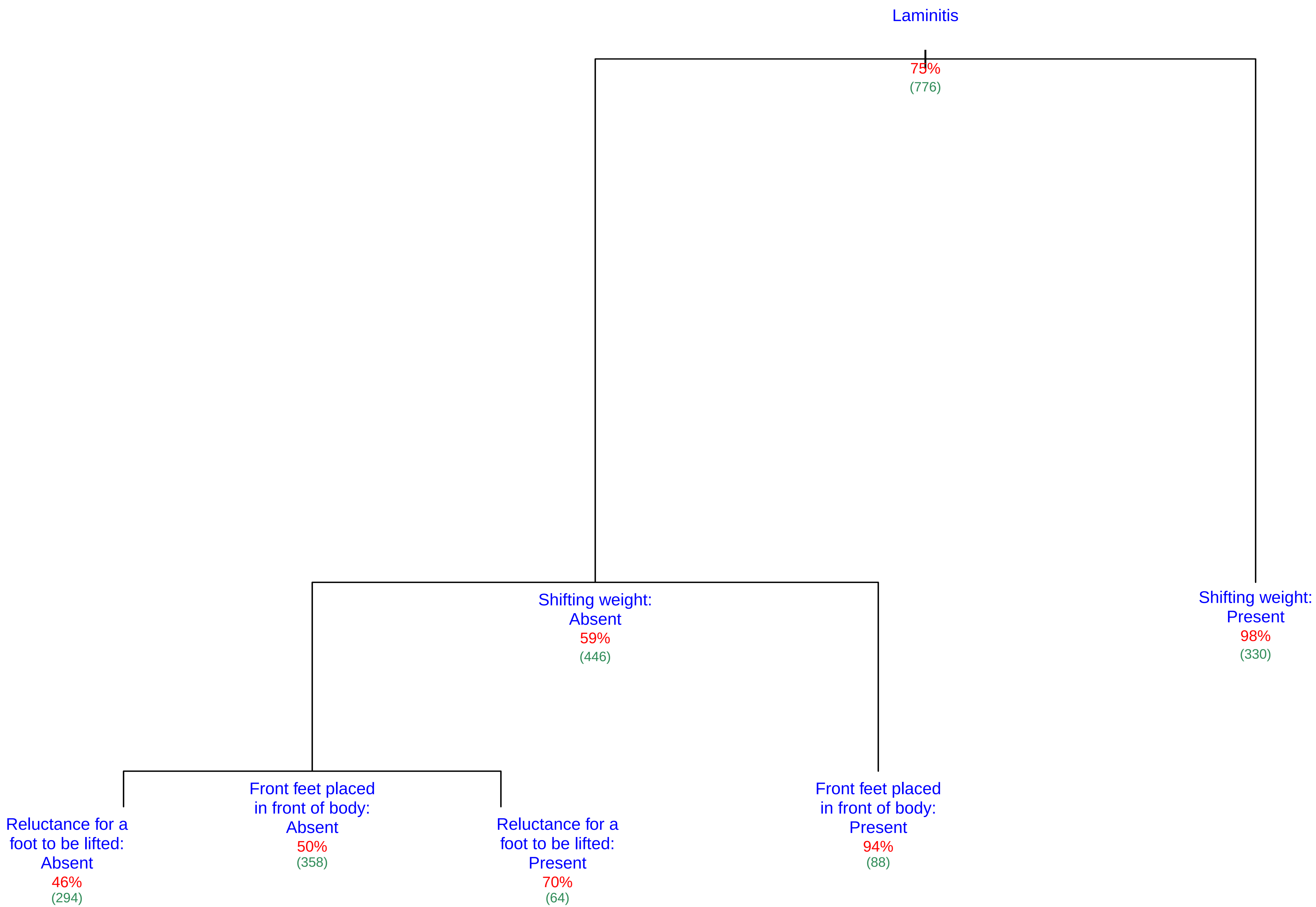
7. Clinical signs of the **most** severely affected foot/feet	Assessment *(please **cross** one option per line)* YES	NO	DIDN'T ASSESS
Increased digital pulse	☐	☐	☐
Increased hoof temperature	☐	☐	☐
Focal sole pain in front of frog	☐	☐	☐
Generalised dorsal hoof wall pain	☐	☐	☐
Coronary band swelling	☐	☐	☐
Coronary band depression	☐	☐	☐

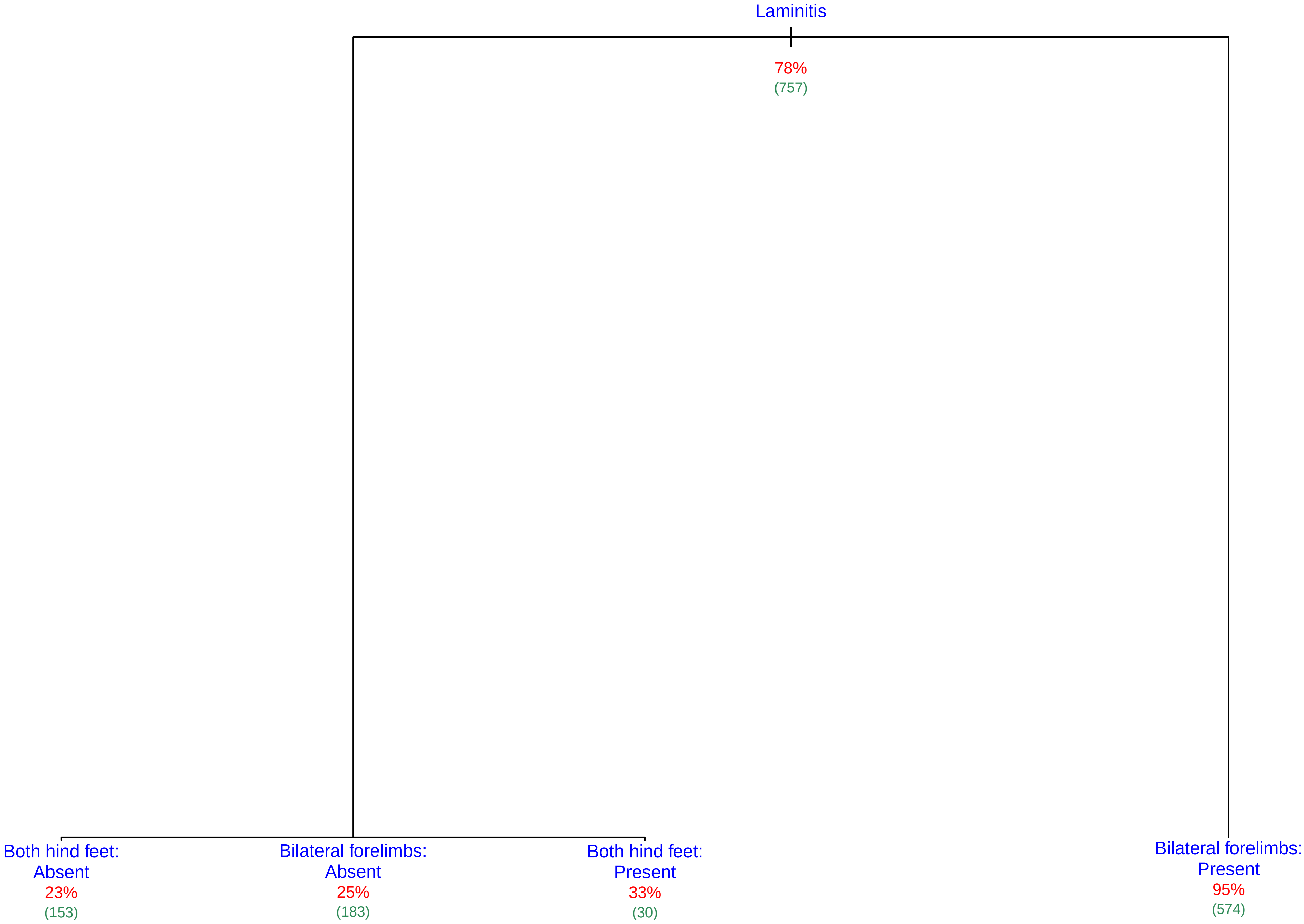
8. Clinical signs of the **most** severely affected foot/feet	Assessment *(please **cross** one option per line)* YES	NO	DIDN'T ASSESS
Divergent growth rings (wider at heels)	☐	☐	☐
Deviation in dorsal hoof wall angle	☐	☐	☐
Wall separation	☐	☐	☐
Flat sole	☐	☐	☐
Convex sole	☐	☐	☐
Abnormally wide white line	☐	☐	☐
Pink crescent/bruising in front of frog	☐	☐	☐
Prolapsed sole	☐	☐	☐

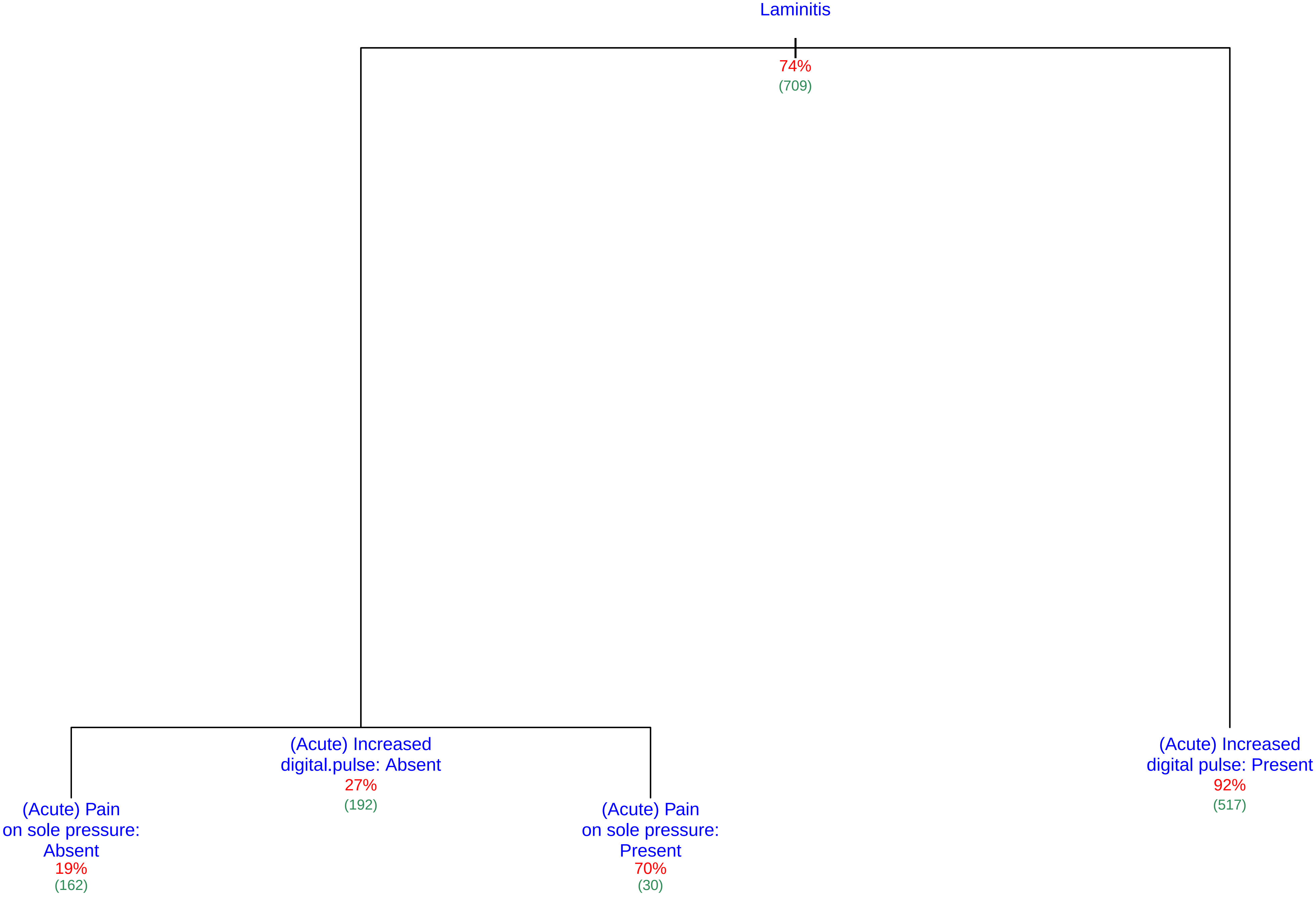
Please remember to complete and return BOTH sides of this form. 2317

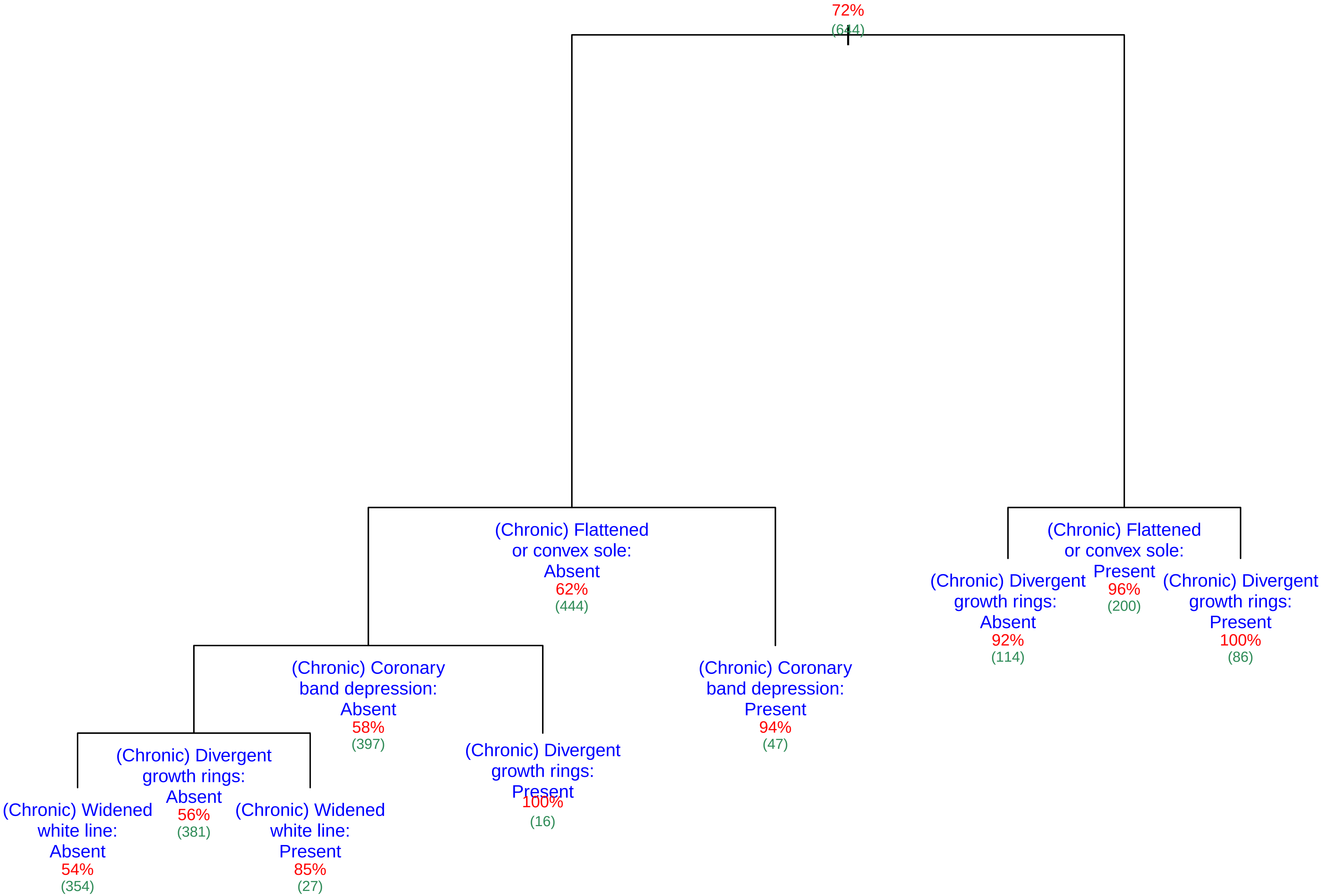
For office use only: P.I.D: ☐☐☐☐ Q.I.D: ☐☐☐☐ H.I.D: ☐☐☐☐


Laminitis
70%
(621)
Short stilted gait at walk: Absent
28%
(219)
Short stilted gait at walk: Present
93%
(402)
Difficulty turning: Absent
14%
(152)
Difficulty turning: Present
60%
(67)
Difficulty turning: Absent
81%
(32)
Difficulty turning: Present
94%
(370)
Reluctance to walk: Absent
12%
(137)
Reluctance to walk: Present
40%
(15)

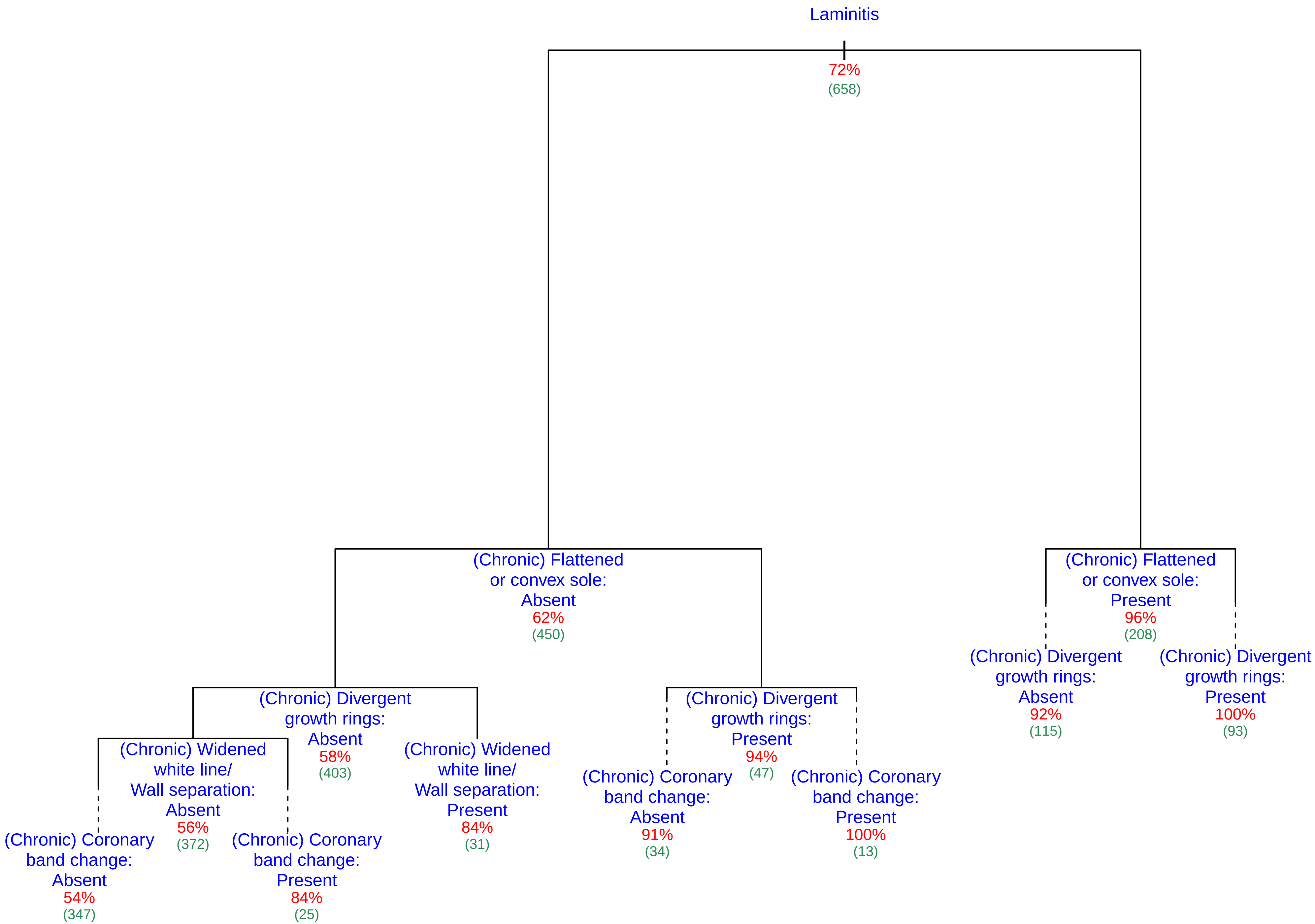

Laminitis
75%
(776)
Shifting weight:
Absent
59%
(446)
Shifting weight:
Present
98%
(330)
Front feet placed
in front of body:
Absent
50%
(358)
Front feet placed
in front of body:
Present
94%
(88)
Reluctance for a
foot to be lifted:
Absent
46%
(294)
Reluctance for a
foot to be lifted:
Present
70%
(64)


Laminitis
78%
(757)
Both hind feet:
Absent
23%
(153)
Bilateral forelimbs:
Absent
25%
(183)
Both hind feet:
Present
33%
(30)
Bilateral forelimbs:
Present
95%
(574)


Laminitis
74%
(709)
(Acute) Increased
digital.pulse: Absent
27%
(192)
(Acute) Increased
digital pulse: Present
92%
(517)
(Acute) Pain
on sole pressure:
Absent
19%
(162)
(Acute) Pain
on sole pressure:
Present
70%
(30)



72%
(644)
(Chronic) Flattened or convex sole: Absent
62%
(444)
(Chronic) Flattened or convex sole: Present
96%
(200)
(Chronic) Coronary band depression: Absent
58%
(397)
(Chronic) Coronary band depression: Present
94%
(47)
(Chronic) Divergent growth rings: Absent
92%
(114)
(Chronic) Divergent growth rings: Present
100%
(86)
(Chronic) Divergent growth rings: Absent
56%
(381)
(Chronic) Divergent growth rings: Present
100%
(16)
(Chronic) Widened white line: Absent
54%
(354)
(Chronic) Widened white line: Present
85%
(27)


Laminitis
72%
(658)
(Chronic) Flattened or convex sole: Absent
62%
(450)
(Chronic) Flattened or convex sole: Present
96%
(208)
(Chronic) Divergent growth rings: Absent
58%
(403)
(Chronic) Divergent growth rings: Present
94%
(47)
(Chronic) Divergent growth rings: Absent
92%
(115)
(Chronic) Divergent growth rings: Present
100%
(93)
(Chronic) Widened white line/ Wall separation: Absent
56%
(372)
(Chronic) Widened white line/ Wall separation: Present
84%
(31)
(Chronic) Coronary band change: Absent
91%
(34)
(Chronic) Coronary band change: Present
100%
(13)
(Chronic) Coronary band change: Absent
54%
(347)
(Chronic) Coronary band change: Present
84%
(25)