Cross sectional study to identify prevalence of and factors associated with laminitis in UK donkeys

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**Summary**

**Background**: Laminitis causes lameness in donkeys, but its prevalence and factors associated with disease remain uncertain.

**Objectives**: To determine the prevalence of and identify factors associated with laminitis in donkeys.

**Study design**: Retrospective cross-sectional study.

**Methods:** All donkeys at the Donkey Sanctuary, UK October 2015 to March 2019 were included. For animals that had laminitis during this period, age, sex, weight, body condition score, and the onset date and type of each episode (first or recurrent, acute or chronic) were recorded. Additionally, management data, foot lesion score, endocrine data, other medical conditions, and occurrence of foot trimming, surgical procedures, diagnostic imaging, behavioural modification therapy or movement between farms within the month prior were noted. Controls were animals that did not experience laminitis during this period and similar data were recorded. Multivariable logistic regression modelling assessed differences between the control group and laminitis outcome groups (first, all laminitis, acute and chronic episodes).

**Results:** Altogether, 707 animals were included; 364 were control animals; 343 had a first episode of laminitis during the study period, of which 200/343 had no further episodes and 143/343 had recurrent episodes resulting in a total of 512 laminitis episodes. ; and the period prevalence was 48.5% over 42 months. Overall, 180/512 (35%) laminitis episodes were acute and 332/512 (65%) chronic. Compared to control animals, the laminitic outcome groups were significantly (P<0.05) more likely to be younger (first episode), less likely to get extra feed (all four groups) or have an additional medical problem (first episode), and less likely to have undergone dental work, movement, imaging (all four groups) or surgery (first; all laminitis, chronic episodes) in the month preceding the episode.

**Main limitations:** These results may not be applicable to the wider donkey population.

**Conclusions:** Laminitis commonly affects donkeys, but factors associated with laminitis differ from horses.

**Introduction**

Laminitis is a common and painful condition of the equine foot and laminitis in horses and ponies has been the focus of a great deal of research recently. However, there is limited published information relating to the condition in donkeys.

Hoof disease and lameness are highly prevalent in donkeys in both developing and developed countries [1]. With respect to developed countries, *post mortem* data from 1444 aged donkeys in a United Kingdom (UK) sanctuary over a 7-year period found that foot disorders were present in nearly half (44.8%) of cases [2]. In addition, an assessment of 2500 donkeys kept at the same UK Sanctuary found that 27.2% presented with foot lameness [1]. Whilst the main cause of the lameness was foot abscesses (10.7%), other causes of foot lameness included acute (2.7%) and chronic (2.8%) laminitis [1]. In a second questionnaire study, 4% of UK donkeys on loan to foster carers from the same UK sanctuary were reported to have suffered from laminitis [3].

Endocrinopathic laminitis is the commonest form of laminitis in horses and ponies [4]. Donkeys in developed countries often have many of the risk factors associated with endocrinopathic laminitis in horses and ponies due to food being abundant and minimal need to perform heavy work or physical activity. These risk factors include obesity, insulin dysregulation (ID), access to good quality grazing, inappropriate feed and pituitary pars intermedia dysfunction (PPID) [1]. However, it is yet to be determined whether endocrinopathic laminitis is the commonest form in donkeys and which factors are associated with laminitis in donkeys regardless of form. Thus, the aim of this study was to determine the prevalence of and factors associated with laminitis in a single large herd of donkeys resident at a UK sanctuary using a cross sectional study.

**Methods**

Data were obtained from The Donkey Sanctuary database. The Donkey Sanctuary is a UK charity that rescues donkeys and mules from neglect and abuse and provides them with lifelong care. Some of the donkeys are kept at their eleven sanctuary sites across the South West of England, whilst others are fostered by private homes across the UK. In total, the Donkey Sanctuary cares for about 7,000 donkeys in the UK and across Europe. All donkeys resident at any of the eleven sanctuary sites across the South West of England at any time between 1st October 2015 and 29th March 2019 were included in the study. Laminitic animals were defined as any donkey having one or more episodes of veterinary diagnosed laminitis within this time period. All other animals were included in the study as controls.

For each laminitis episode, the age of the donkey at the onset of the episode, sex, and the date of onset, laminitis type and whether it was the first episode during the study period or a repeat episode during the study period were recorded. The laminitis episode type was categorised at the time of occurrence as acute or chronic by the attending veterinarian. Acute laminitis was defined as having clinical signs of laminitis in two or more limbs such as pain and presence of increased digital pulses, but no radiographic changes, and other causes of lameness ruled out [5]. Chronic laminitis was defined as having radiographic changes interpreted by the attending veterinarian at the time as being consistent with chronic laminitis.[5] The specific donkey sanctuary site at which the donkey was kept and the management group within that site were noted. The management data including feed (forage and concentrates) and bedding and body condition score (BCS) at the time of the laminitis episode were recorded. The BCS was determined by the attending veterinarian using a 5 point donkey-specific scale [6]. The last foot trimming date and foot lesion score (determined at the time of trimming for seedy toe, bruising, stretched white line and thrush based on a standardised scoring system routinely used by the Donkey Sanctuary) prior to the laminitis episode were recorded. The last body weight measured on a calibrated weigh bridge within the month prior to the laminitis episode was recorded and percentage weight change since the previous similarly obtained measurement calculated. The basal adrenocorticotropic hormone (ACTH) and insulin concentrations within the 3 months prior to the laminitis episode were noted if measured. Finally, any other medical conditions (excluding endocrinopathies) and surgical procedures, dental work, diagnostic imaging, behavioural modification therapy or movement between farms occurring in the month prior to the laminitis episode were noted. The Obel grading of each laminitis episode was not recorded in the clinical record and so could not be included in the study. Based on the inclusion criteria used in previous studies [7; 8], laminitis episodes in an individual animal were considered as different episodes if there was an interval of more than eight weeks between the episodes.

Identical information relating to age, sex, management, weight, BCS, foot trimming, foot lesion score, medical conditions, surgical procedures, behavioural modification therapy or movement between farms were recorded for control animals. The data was collected from the last entry for each control animal within the study period. The timing of this last data entry was variable in relation to the end of the study and these dates spanned the study period with each control data period overlapping with the period for which exposure data was collected for at least one laminitic animal resident at the same site.

**Data analysis**

Statistical analyses were performed using SPSS Statistics V.23 (IBM) and Prism version 8 (GraphPad). The period prevalence of laminitis for the entire study period calculated.

Continuous data (age, body weight, percentage weight change, foot lesion score) were tested for normality using the Shapiro-Wilk test and described as mean (±SD) or median (interquartile range [IQR]) as appropriate. The BCS was used to categorise an animal as normal/underweight (BCS <4/5) or overweight/obese (BCS ≥4/5). Basal ACTH concentration above the seasonally adjusted reference range for normal donkeys was used to categorise an animal as having PPID [9], whilst a basal non-fasted serum insulin concentration of >20iu/ml [10] was used to categorise an animal as having basal hyperinsulinaemia. Most donkeys received only grass and forage (combinations of hay, haylage and straw), however a small number were fed concentrates (high fibre cubes, donkey cubes or chaff-based products) and so were classified as receiving extra feed. Missing data were left as missing.

Risk factors were assessed for four laminitis outcomes: first episode of laminitis vs controls, all laminitis vs controls, acute laminitis vs controls and chronic laminitis vs controls. The same general statistical approach was applied for all four laminitis outcome models.

Firstly, individual factors (sanctuary site [1-9], sanctuary group [1-48], age, body weight, percentage weight change, foot lesion score, sex, receiving extra feed, body condition score, normal/underweight or overweight/obese, other lameness, foot trimming in the two weeks before laminitis, presence of an endocrinopathy [PPID and/or basal hyperinsulinaemia], presence of another medical condition, and behavioural modification, surgery, movement, dental work or diagnostic imaging [radiography, ultrasonography and/or endoscopy for reasons unrelated to laminitis] occurring in the month before the laminitis episode) significantly associated with each of three of the laminitis outcome groups (all laminitis, acute laminitis, chronic laminitis) were determined by entering them individually into a generalised estimating equation with a binary logistic model. Donkey ID was entered into the subject box to account for the repeated measures (where appropriate) and laminitis status was the response. In order to determine the likelihood of these individual factors being associated with laminitis, all variables with P≤0.1 and their two-way interactions were entered into a multivariable model with backwards-stepwise removal until only variables with Wald P≤0.05 were retained in the final model. Confounding was checked for by trying to force back, one at a time, variables that fell out during the modelling process and assessing whether this resulted in a significant change in the model estimate parameters. The odds ratios (ORs), 95 per cent confidence intervals (CI) and Standard Error were calculated for retained variables. Secondly, individual factors significantly associated with the fourth laminitis outcome group (first laminitis episode) were identically determined using binary logistic regression.

**Results**

A flow chart to show recruitment of donkeys into the study and their subsequent categorisation and comparison is shown in figure 1.

In total, 707 animals were included in the study of which 243 (34.4%; 95% confidence interval [CI] 30.9%, 37.9%) were female and 464 (65.6%; 95% CI 62.1%, 69.1%) were male (all geldings). Overall, 364 were control animals; and 343 donkeys had a first episode episode of laminitis during the study period, of which 200/343 (58.3%; 95% CI 53.1%, 63.5%) had no further episodes and 143/343 (41.7%; 95% CI 35.5%, 46.9%) had recurrent episodes within the study period resulting in a total of 512 episodes of laminitis over the 42-month study period. The median number of episodes experienced by an individual donkey was one; 200/343 (58.3%; 95% CI 53.1%, 63.5%) had one episode, 104/343 (30.3%; 95% CI 25.5%, 35.2%) had two episodes, 33/343 (8.7%; 95% CI 6.5%, 12.7%) had three episodes, 4/343 (1.2%; 95% CI 0%, 2.3%) had four episodes and 2/343 (0.6%; 95% CI -0.2%, 1.4%) had six episodes. The period prevalence of laminitis was 48.5% (95% CI 44.8%, 52.2%) over the 42-month study period. Laminitis episodes (all and first) most commonly occurred in January, February, October and November (Figure 2).

Of the 512 laminitic episodes, 180 (35%; 95% CI 31%, 39.3%) were categorised as acute laminitis and 332 (65%; 95% CI 60.7%, 69.0%) as chronic laminitis.; 312 (61%; 95% CI 56.7%, 65.2%) occurred in geldings and 200 (39%; 95% CI 34.8%, 43.3%) in females. Both acute and chronic laminitis episodes most commonly occurred in January, February, October and November (Figure 2).

By the end of the study period, 196 animals were alive and 511 deceased; of these deaths 44 (8.6%; 95% CI 6.2%, 11.0%) were due to laminitis and the remainder were due to other musculoskeletal diseases (144; 28.1%, 95% CI 24.3%, 32.1%; predominantly osteoarthritis), gastrointestinal disease (76; 14.9%, 95% CI 11.8%, 18.0%), liver disease (44; 8.6%, 95% CI 6.2%, 11.0%), sarcoids (29; 5.7%, 95% CI 3.7%, 7.7%), poor quality of life (7; 1.4%, 95% CI 0.4%, 2.4%) and a range of other diseases (167; 32.7%, 95% CI 28.6%, 36.7%).

*Comparison between first laminitis episodes and control animals*

The continuous data and the number of animals within each of the categories for each categorical variable is shown in Table 1. Individual variables with a P value <0.1 on univariable analyses of risk factors associated with first laminitis were age, sex, weight, percentage weight change, receiving extra feed, body condition score, having another medical condition and undergoing behavioural modification, surgery, movement, dental work, or diagnostic imaging in the month before the laminitis episode (Table 1). Of these, age. receiving extra feed, having another medical condition, and undergoing surgery, movement, dental work, or diagnostic imaging in the month before the laminitis episode were retained (P≤0.05) on multivariable analysis (Table 2). The first laminitis group were significantly more likely to be younger, less likely to get extra feed or have another medical condition and less likely to have undergone surgery, movement, dental work or diagnostic imaging in the month preceding the laminitic episode compared to the control group. There were no significant interactions between terms.

*Comparison between all laminitis episodes and control animals*

The continuous data and the number of animals within each of the categories for each categorical variable is shown in Table 3.

Individual variables with a P value of <0.1 on univariable analyses were receiving extra feed and undergoing surgery, movement, dental work, or diagnostic imaging in the month before the laminitis episode (Table 3). All of these were retained (P≤0.05) on multivariable analysis (Table 4). The all laminitis group were significantly less likely to get extra feed, to have another medical condition or to have undergone dental work, movement or surgery in the month preceding the laminitic episode compared to the control group. Additionally, there was a significant interaction between donkeys undergoing dental work and movement in the month prior.

*Comparison between acute laminitis episodes and control animals*

The continuous data and the number of animals within each category for the categorical variables is shown in Table 5.

Individual variables with a P value of <0.1 on univariable analysis comparing acute laminitis episodes and control animals included sex, receiving extra feed, body condition score, having another medical condition. and undergoing movement, behavioural modification therapy, surgery, dental work or diagnostic imaging within one month of the laminitis episode (Table 5). All variables apart from sex, body condition score and undergoing behavioural modification were retained (P<0.05) on multivariable analysis (Table 6). Animals with acute laminitis were significantly less likely to get extra feed, to have another medical condition or to have undergone dental work, movement or diagnostic imaging in the month preceding the laminitic episode compared to the control group. Additionally, there was a significant interaction between undergoing dental work and movement in the month prior.

*Comparison between chronic laminitis episodes and control animals*

The continuous data and the number of animals within each category for the categorical variables is shown in Table 5.

Individual variables with a P value <0.1 on univariable analysis comparing chronic laminitis episodes with control animals included sex, receiving extra feed, having another medical condition. and undergoing movement, surgery, dental work or diagnostic imaging within one month of the laminitis episode (Table 5). All variables were retained (P<0.05) on multivariable analysis (Table 6). Animals with chronic laminitis were significantly less likely to get extra feed, to have another medical condition or to have undergone dental work, movement or diagnostic imaging in the month preceding the laminitic episode compared to the control group. There were no significant interactions between terms.

**Discussion**

This is the first study to report the period prevalence of and factors associated with laminitis in a single large UK donkey population. Whilst it should be acknowledged that all the animals were owned by a single charity, the Donkey Sanctuary owns a large number of animals which equates to 35% of the UK donkey population. This provides the opportunity to include a large number of animals that have regular, dedicated veterinary care for which accurate clinical records are available within a single study. This population has been used to generate meaningful data for several publications previously [1-3; 10; 11] and a similar population of horses and ponies owned by a single UK charity has been used previously for clinical laminitis research [12-14]. In addition, the animals were geographically distributed across the Donkey Sanctuary’s eleven sites in the South West of the UK. Thus, this study included a large number of animals that should be representative of the UK donkey population. However, regardless of the condition being studied, care should always be taken when translating the conclusions from one population to another.

The period prevalence of laminitis was 48.5% over the 42-month study period. There are no other similar studies in donkeys for comparison, however annual figures reported for laminitis in horses and ponies range from 0.5% to 34% [15-18]. In addition, the laminitis was frequently recurrent with 41.7% of animals having one or more episodes of recurrence during the 3.5-year study period. This is similar to the recurrence rates reported in horses and ponies of 33.7% over 6 years [13], 34.1% over 2 years [19], 53.3% over the time the animal was known to the owner [18] and 72% in a retrospective database review spanning 26 years [20].

Laminitis episodes most commonly occurred in January, February, October and November. This is in contrast to some studies in horses and ponies in which laminitis was most frequently reported in the spring and summer [13], but similar to others that reported an increased risk in the winter as well as the summer and associated the risk with climatic stress influencing pasture carbohydrate content [21] . However, in the current study, this coincided with the time of year when most sites bring their donkeys in off pasture (October and November) resulting in an obvious dietary change from pasture to haylage which might trigger the laminitis and encompassing a time when the donkeys will spend more time standing on concrete in yards. Possible mechanisms by which a dietary change might trigger laminitis include changes in the gastrointestinal microflora [22; 23] and spending more time on a hard surface might be associated with concussion and altered digital perfusion [24]. Alternatively, despite the fact that the donkeys were cared for by experienced staff, it is possible that the hard surface may have made a subtle lameness more apparent. In addition, this increase in laminitis episodes coincides with the seasonal rise in circulating ACTH concentration that occurs in normal equines and in those with PPID [9; 25]. However, there is no published evidence that this seasonal rise itself is associated with an increased risk of laminitis in horses, ponies or donkeys and the presence of increased basal insulin and/or ACTH concentrations was not associated with laminitis in the current study. Finally, it coincided with the time of the year when the donkeys receive routine anthelmintic therapy (January). It has previously been reported that increasing time since anthelmintic therapy was associated with an increased risk of laminitis in horses and ponies [21]. The authors of that study made several suggestions to account for this association, including a direct effect of increased worm burden or indirect gastrointestinal damage that results in a systemic disturbance, anthelmintic drugs may exert currently unrecognised pharmaceutical effects or the use of anthelmintic drugs may be a proxy measure for the level of horse care [26]. In the current study, it is possible that anthelmintic therapy also contributed to alterations in the gastrointestinal microbiota [27] and to changes in the nutritional environment for gut bacteria [28], which in turn may increase the risk of laminitis. A lower frequency of cases in the spring and summer may be a consequence of careful management of access to pasture by the Donkey Sanctuary during this period to minimise weight gain and the risk of laminitis. Additional studies evaluating donkeys kept under different management regimens are required to confirm these findings.

There was no significant effect of age when animals experiencing an episode of laminitis and the control group were compared when all laminitis episodes were included. However, the odds of a first episode of laminitis were greater in younger animals. Whilst some studies found a significant association between increasing age and laminitis in horses and ponies [29], some did not [21], and others only found such an association in chronic cases [30; 31]. It should be acknowledged that the animals included in the current study represent an older population and so the ability to accurately detect age effects may have been reduced. Interestingly, the median ages of the all laminitis and first episode of laminitis groups were greater than the mean age of owner-reported cases of laminitis in UK horses and ponies (14.7 years) [32].

Sex has been investigated as a risk factor for laminitis in horses and ponies with varying results. In some studies, females were found to be at an increased risk [13; 29] and castration may lower the risk of laminitis [33]; whereas other studies have failed to find gender to be a significant risk factor [34]. In the present study, sex was not retained in any of the final multivariable models; however, it should be acknowledged that no stallions were available for inclusion.

Studies in horses and ponies have demonstrated that animal and management-related factors associated with laminitis include obesity [29], weight gain in the previous three months, new access to grass in the previous four weeks, box rest in the previous week, lameness or foot-soreness after shoeing/trimming, existing endocrinopathic (PPID and equine metabolic syndrome [EMS]) disease and increasing time since the last anthelmintic treatment [21]. Factors associated with a decreased risk of laminitis were feeding of additional supplements and transportation in the previous week [21]. Thus, these parameters were evaluated in the current study.

Obesity is common in donkeys in developed countries [3; 35] and over 40% of the donkeys were overweight or obese in the present study. Body condition score and being overweight/obese were not retained in any of the multivariable models. Similarly, weight and the percentage weight change were not retained; however, it should be acknowledged that the median weight change was minimal in both groups suggesting that weight was consistent over time, and there was no obvious seasonal pattern to the weight change. New access to grass in the previous four weeks or box rest in the previous week were not evaluated in the current study, However, the presence of lameness unrelated to laminitis or being foot sore after trimming were not associated with an increased odds of laminitis.

Surprisingly the presence of PPID or basal hyperinsulinaemia was not associated with increased odds of laminitis in donkeys in the current study. Similar to horses, a link between obesity, insulin dysregulation (ID) and recurrent laminitis has been reported in donkeys [10]. However, information on metabolic syndrome and ID in donkeys is limited and, unlike horses [36], a direct link between hyperinsulinemia and laminitis has not yet been demonstrated [35] through the use of the hyperinsulinemia model of laminitis [36; 37]. Previously, insulin concentrations over 20 IU/mL in donkeys were correlated with the presence of laminitis [10]; thus, this value was used as a cut-off for a diagnosis of basal hyperinsulinaemia in the current study. However, it should be acknowledged that dynamic testing for recognition of the other two manifestations of insulin dysregulation was not performed and that this cut-off value has yet to be fully validated. As a result, a number of donkeys within all groups that did actually have ID are likely to have been missed. Future studies should ideally include only animals that have had dynamic as well as basal testing for ID. Although PPID is suspected to be frequent in donkeys, epidemiological information on PPID in donkeys is lacking [35] and whilst laminitis is reported to occur in association with PPID in donkeys [38], the frequency at which it occurs is unknown. In the present study, 20% of all donkeys and 22% of all laminitic donkeys had PPID based on basal ACTH concentrations. It should be acknowledged that dynamic tests were not performed in any animals and so some control and laminitic animals may have been misclassified as non PPID. PPID diagnosis in donkeys is commonly empirically extrapolated from protocols oriented to horses [38], using primarily a basal ACTH concentration determination and performing a dynamic test in equivocal cases [39]. Whilst the seasonal variation of ACTH concentrations in healthy donkeys has been recently published [26] and was used in the current study to categorise animals as having PPID or not, dynamic tests have only been validated in a single study that included only six animals [39]. Well-designed studies investigating cut off values for ACTH in this species that consider seasonal and geographic variations, as well as additional validation of dynamic tests in a larger number of animals are needed [35].

The donkeys included in the study were kept at eleven different sites and within each site, donkeys were kept in groups which had similar management, such that animals that had suffered from laminitis previously tended to be kept together. However, neither sanctuary site nor group were significantly associated with laminitis regardless of which laminitic animals were included in the comparison with the control animals. Thus, whilst the possibility that the laminitic groups were consistently less likely to get extra feed, to have another medical condition or to have undergone dental work, movement, diagnostic imaging or surgery in the month preceding the laminitic episode may be due to the effect of management changes implemented by the Donkey Sanctuary to avoid any further laminitis risk in animals with repeated episodes should be considered, laminitis prevention interventions cannot explain the similar findings in animals experiencing their first episode. Instead, it suggests that these factors were associated with an increased odds of disease. There was a significant interaction between undergoing dental work and movement, suggesting that the effect of dental work on laminitis odds is different in those donkeys that moved compared to those that did not. The explanation for this interaction remains unclear.

It should be acknowledged that horses and donkeys differ in their expression of pain-related behaviour [40]. Pain is potentially more easily recognised in horses whereas behaviours described in horses are not reported for the donkey [40]. Donkeys may not demonstrate clear source-specific behavioural indicators of pain, but instead exhibit a non-specific general change in demeanour which cannot be easily linked to particular pathologies [41]. Thus, despite the donkeys included in the current study being cared for by staff experienced in donkey health and welfare, it is possible that some cases of laminitis were missed, resulting in these animals being classified as controls.

In conclusion, this is the first study to report the period prevalence of and investigate factors associated with laminitis in a single large population of donkeys in the South West of the UK. The period prevalence of laminitis was similar to figures reported for laminitis in horses and ponies and the laminitis was similarly frequently recurrent. The laminitic groups were significantly less likely to get extra concentrate feed, to have another medical condition or to have undergone dental work, movement diagnostic imaging or surgery in the month preceding the laminitic episode. Thus, this study highlights that factors associated with laminitis in donkeys cannot necessarily be extrapolated from horse and pony studies. However, the significance of these findings requires further investigation in a more diverse population of animals kept under different management conditions in order to determine causality.

**Figure Legends**

Figure 1: Flow chart to show recruitment of donkeys into the study and their subsequent categorisation and comparison. Groups with the same letter were compared using multivariable random-effects logistic regression modelling.

Figure 2: Seasonal occurrence of laminitis episodes in donkeys at a UK Sanctuary.

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