Comparison of characteristics and enzymatic products of leukocytes in the skin and laminar tissues of horses administered black walnut heartwood extract or lipopolysaccharide

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Objective—To compare characteristics and enzymatic products of leukocytes detected in the skin and laminar tissues of horses administered black walnut heartwood extract (BWHE) and horses administered purified lipopolysaccharide (LPS).

Animals—25 healthy 5- to 15-year-old horses.

Procedures—Horses were randomly assigned to receive LPS (20 ng of O55:B5 Escherichia coli endotoxin/kg; n = 5) IV or 6 L of BWHE (10) or water (control group; 10) via nasogastric intubation. Horses were euthanatized 12 hours after treatment or at onset of Obel grade 1 lameness. Laminar tissue samples and skin samples from the middle region of the neck were harvested at the time of euthanasia. Leukocyte emigration (determined via CD13 immunohistochemical analysis) and matrix metalloproteinase (MMP)-2 and MMP-9 gene expressions and activities (determined via reverse transcription PCR assay and gelatin zymography, respectively) were measured in skin and laminar tissue samples.

Results—Tissues of horses receiving BWHE contained significantly higher numbers of CD13-positive cells and increased MMP-9 gene expression and activity, compared with findings in the other 2 groups. Values for laminar tissue and skin from LPS-treated horses were not increased, compared with findings in the control group, in any experiment.

Conclusions and Clinical Relevance—Results indicated that BWHE administration causes increases in CD13-positive leukocyte numbers and MMP-9 expression and activity in laminar tissue and skin in horses; similar effects were not detected following LPS administration. Leukocyte emigration in horses with experimentally induced endotoxemia and in horses administered BWHE differed markedly, thereby providing additional evidence that the development of laminitis involves more complex mechanisms than endotoxemia-induced leukocyte activation alone. (Am J Vet Res 2009;70:1383–1390)

The integument, as an organ system, is composed of the avascular epidermis and the vascular dermis, which are intimately connected at the basement membrane. The hoof of horses is a specialized modification of the integumentary system and includes interdigitations of the dermis and epidermis that are termed laminae. These interdigitations dramatically increase surface area, dissipate the load, and increase the weight-bearing strength of the hoof.1,2 The findings of previous studies3-6 of acute laminitis in horses indicate that enzymatic degradation of the basement membrane of the hoof results in dermal-epidermal separation and the subsequent loss of the integrity of laminar interdigitations, which consequently compromises weight bearing.

Proteases such as MMP-2 and MMP-9 have been implicated in the disruption of the basement membrane that occurs during episodes of laminitis in horses. The MMPs are secreted in a latent (pro-MMP) form and require an activation step before they gain full enzymatic activity. Matrix metalloproteinase-2 is normally present in laminar tissue (constitutively produced by cells such as keratinocytes and fibroblasts) and is involved in growth and remodeling of the hoof. Matrix metalloproteinase-9 is produced by neutrophils, stored in tertiary granules, and can be activated in response to inflammatory stimuli. When stimulated, neutrophils release matrix metalloproteinase-9, which contributes to the degradation of the laminar basement membrane. This degradation facilitates leukocyte emigration and facilitates the development of laminitis.

Abbreviations

BWHE Black walnut heartwood extract
LPS Lipopolysaccharide
MMP Matrix metalloproteinase
RT Reverse transcription

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ules, and released upon cellular activation. In recent studies of acute laminitis in horses, the presence of MMP-2 and MMP-9 in laminar tissue varies depending on whether the disease is naturally occurring or experimentally induced by means of carbohydrate overload or BWHE administration. Compared with findings in horses without laminitis, increases in MMP-2 and MMP-9 activities were detected in laminar tissue obtained from several horses with naturally occurring acute or chronic laminitis and in 2 horses with carbohydrate overload–induced laminitis. Tissues obtained from the latter 2 horses also had increased expression of MMP-2, although the authors did not indicate the Obel grade of lameness in the horses at the time that the samples were collected. In contrast, laminar tissue obtained from horses after administration of BWHE had increased expression and activity of MMP-9 but not MMP-2, compared with controls. Recent studies have revealed that activation of peripheral blood leukocytes is involved in the development of acute laminitis. Experimental induction of laminitis in horses via administration of BWHE has been associated with peripheral leukopenia followed by increases in myeloperoxidase and MMP-9 activities in both skin and laminar tissue. Both enzymes are produced in neutrophils, stored in cytoplasmic granules within those cells, and released upon cellular activation. Although changes in activities of those enzymes have been detected in horses following experimental induction of laminitis, changes associated with endotoxia in horses have not been assessed, to our knowledge. Endotoxemia, a condition that is commonly associated with the development of laminitis under clinical conditions, is characterized by activation and emigration of leukocytes from the peripheral circulation. Because horses with diseases that are characterized by clinical evidence of endotoxia (eg, colitis, enteritis of the proximal portion of the small intestine, and intestinal strangulation obstruction) appear to be at increased risk for development of laminitis and endotoxin has been detected in horses that develop laminitis subsequent to carbohydrate overload, there has been considerable interest in defining the relationship, if any, between endotoxia and the development of laminitis. The purpose of the study reported here was to compare the characteristics and enzymatic products of leukocytes detected in the skin and laminar tissues of horses administered BWHE and horses administered purified LPS (endotoxin). Because experimentally induced endotoxia and administration of BWHE result in leukopenia of similar severity and duration but only the latter induces clinical signs of laminitis, we hypothesized that there would be significant differences in the distribution and enzyme products of activated leukocytes in horses treated with BWHE or LPS. Consequently, we compared leukocyte emigration and MMP-2 and MMP-9 gene expressions and activities during the period in which horses developed Obel grade 1 lameness following administration of BWHE or LPS.

**Materials and Methods**

**Animals**— Twenty-five healthy horses (age, 5 to 15 years old) were used in the study. All horses were apparently not lame and lacked clinical evidence of systemic inflammatory disease. No evidence of preexisting laminitis was evident on lateral or dorsopalmar radiographic views of the forelimb digits. Prior to the start of the study, the hair on the middle region of the neck was clipped and an IV catheter was placed in the left jugular vein of each horse. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

**BWHE preparation**—For each treatment, a BWHE was prepared as described previously. Briefly, 1 kg of black walnut heartwood shavings was digested in 7 L of water at room temperature (approx 22°C) for 24 hours. An aqueous filtrate was then obtained by filtering the solution through cheesecloth. Six liters of the resulting extract was administered via nasogastric intubation to a horse that was assigned to the BWHE treatment group.

**Experimental design**—Horses were randomly assigned to a control group, a BWHE treatment group, and an LPS treatment group. Horses in the control group (n = 10) received 6 L of water via nasogastric intubation. Horses in the BWHE treatment group (n = 10) received 6 L of BWHE via nasogastric intubation. Horses in the LPS treatment group (n = 5) received 20 ng of O55:B5 Escherichia coli endotoxin/kg in 500 mL of saline (0.9% NaCl) solution via the jugular vein catheter over a 30-minute period. The time of treatment administration was designated as 0 hours.

Each horse was evaluated immediately prior to the start of the study (baseline assessment) and every hour thereafter for attitude, heart rate, respiratory rate, capillary refill time, hoof temperature, digital pulse presence, and evidence of Obel grade 1 laminitis. Horses were considered to have Obel grade 1 lameness when ≥ 2 of the following were detected: weight shifting, increased warmth of the hooves, and strong digital pulses without evidence of lameness while walking. Blood samples were collected at 2-hour intervals for WBC count determination.

Horses in the control group were euthanatized 12 hours after treatment. Horses in the BWHE treatment group were euthanatized at the onset of Obel grade 1 lameness or 12 hours after administration of BWHE if signs of Obel grade 1 lameness had not developed by that time. Horses in the LPS treatment group were euthanatized 12 hours after administration of LPS. All horses were euthanatized by use of a penetrating captive bolt.

**Collection of laminar tissue and skin samples**—Both forelimbs of each horse were disarticulated at the level of the metacarpophalangeal joint, and the hooves were cut into sections with a band saw. During the procedure, thermal damage was minimized by constant irrigation of the tissues with ice-cold physiologic saline solution (118 mM NaCl, 24 mM NaHCO3, 1 mM MgSO4, 0.435 mM NaH2PO4, 5.56 mM glucose, 1.8 mM CaCl2, and 4 mM KCl). Two full-thickness segments from each forelimb foot were then placed in the ice-cold physiologic saline solution. The hard keratinized portion of the hoof and the distal phalanx of each forelimb were removed, and specimens of laminar tissue were col-
lected via sharp dissection. Concurrently, full-thickness sections (6 × 6 cm) of skin from the middle region of the neck were collected. These specimens were also placed in the ice-cold physiologic saline solution. Half of the laminar and skin tissue samples were rapidly frozen in liquid nitrogen and stored at −80°C until assayed; the remaining tissue samples were fixed in neutral-buffered 10% formalin for immunohistochemical analysis.

**Real-time PCR assay**—To prepare the samples, the frozen tissues were first shaved with a razor blade and then homogenized by use of a motorized mortar and pestle.° Gene expression was quantified (on the basis of mRNA concentration) in a 2-step RT PCR assay. Total RNA extraction was performed by use of a total RNA purification kit according to the manufacturer’s protocol. Reverse transcription for production of cDNA was performed by use of a high-capacity cDNA RT kit. Oligo nucleotide primers and probes were designed by use of computer software,° and equine MMP-2 and MMP-9 were sequences obtained from GenBank.° The quantitative real-time PCR assay was performed in duplicate for each sample by use of a sequence detection system; the reference gene 18s rRNA was used as an endogenous control.

**Gelatin zymography**—Frozen tissue samples were homogenized by use of a tissue homogenizer in western lysis buffer (50mM HEPES, 150mM NaCl, 1% Triton X-100, 1mM egtazic acid, 6mM sodium deoxycholate, 1mM Na₂VO₄, 1mM NaF; 1mM phenylmethylsulphonyl fluoride, aprotinin [20 mg/mL], and leupeptin [20 mg/mL]). After homogenization, the samples were sonicated and centrifuged at 14,000 × g for 15 minutes at 4°C. A colorimetric protein assay (Bradford assay) was performed on the supernatant; aliquots of the supernatant were stored frozen at −80°C.

By use of a 4% stacking gel, 8% SDS-PAGE gels containing 0.1% gelatin were prepared. Samples were loaded onto the gels (20 µg of protein/lane) and electrophoresed at 100 V for 120 minutes. An MMP-2–MMP-9 positive control sample was included on each gel. After electrophoresis, each gel was washed twice for 30 minutes in 2.5% Triton X-100 on a rocking platform to remove the SDS. Gels were then incubated for 16 hours in 100mM Tris-HCl (pH, 8.0), 5mM CaCl₂, 0.005% Brij-35, and 0.001% NaN₃, at 37°C and subsequently stained by centering the field of view (40× objective lens) on a dermal vessel in the area of the primary dermal lamina and counting all CD13-positive cells in that field. One sample of both laminar tissue and skin was evaluated from each horse. For each tissue sample, 5 fields were evaluated and the mean number of CD13-positive cells was calculated.

**Data and statistical analyses**—Initially, the BWHE-treated horses were subdivided into 2 groups on the basis of whether they were euthanatized at the onset of Obel grade 1 lameness (n = 8) or at 12 hours after administration of the extract (without development of Obel grade 1 lameness; 2). Statistical analysis of data from these 2 subsets revealed no significant differences; furthermore, data from each subset did not differ from the control group data. Therefore, the subsets were re-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
<th>Laminar tissue</th>
<th>Skin</th>
</tr>
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<tbody>
<tr>
<td>MMP-2</td>
<td>LPS</td>
<td>− 6.67*</td>
<td>−10.0*</td>
</tr>
<tr>
<td></td>
<td>BWHE</td>
<td>+ 5.54*</td>
<td></td>
</tr>
<tr>
<td>MMP-9</td>
<td>LPS</td>
<td>− 2.94*</td>
<td>−3.34*</td>
</tr>
<tr>
<td></td>
<td>BWHE</td>
<td>+ 46.3*</td>
<td>2.3*</td>
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Samples were collected after horses were euthanatized at 12 hours after treatment or at onset of Obel grade 1 lameness. Fold changes representing increased and decreased concentrations, compared with the control group findings, are designated as + and − values, respectively.

*Concentration was significantly (P < 0.05) different from the corresponding control group value.
NS = Fold change not reported because MMP concentration was not significantly different from the control group value.
combined and were designated as the BWHE treatment group.

Gene expression data for MMP-2 and MMP-9 were normalized to 18S rRNA expression and analyzed relative to control tissue findings by use of the 2^(-ΔΔCT) method. For RT PCR data, a 2-tailed t test was performed to analyze data from each treatment group for MMP-2 and MMP-9 expressions in skin and laminar tissues. For all comparisons, a value of P ≤ 0.05 was considered significant.

Following gelatin zymography, MMP-2 and MMP-9 bands were analyzed by use of quantitation software. Volume intensity was measured for the MMP-2 zymogen band (72 kDa) and the MMP-9 zymogen band (92 kDa), adjusted for background stain, and normalized to findings obtained for an MMP-2–MMP-9 positive control sample that was included on each gel. Results were reported as arbitrary units of optical density. The MMP-2 and MMP-9 data for skin and laminar tissues from control, BWHE-treated, and LPS-treated horses were compared by use of a 1-way ANOVA with a Tukey-Kramer posttest. A value of P ≤ 0.05 was considered significant. Correlation between skin and laminar tissue data was determined by use of the Pearson product-moment correlation coefficient.

The numbers of CD13-positive cells in skin and laminar tissue samples from control, BWHE-treated, and LPS-treated horses were normally distributed, as determined by use of a D’Agostino-Pearson normality test. The data from each group were compared by use of a 1-way ANOVA with a Tukey-Kramer posttest. A value of P ≤ 0.05 was considered significant.

**Results**

None of the horses in the control or LPS treatment groups developed signs of acute laminitis. Eight of the 10 horses in the BWHE treatment group developed clinical signs consistent with Obel grade 1 lameness and were euthanatized at the time that these signs were detected (9 to 12 hours after administration of the extract); the other 2 BWHE-treated horses were euthanatized 12 hours after administration of the extract. White blood cell counts in blood samples from horses in the LPS and BWHE treatment groups revealed similar decreases (> 30%) in total leukocyte counts. Leukocyte counts were lowest in the LPS- and BWHE-treated horses at the 2- and 4-hour time points, respectively. Circulating leukocyte counts returned to values indistinguishable from baseline values at the time of euthanasia in both groups.

**Figure 1**—Mean ± SEM intensity of MMP-2 (A and B) and MMP-9 (C and D) zymogen bands (determined by use of SDS-PAGE gelatin zymography) in laminar tissue (A and C) and skin (B and D) samples obtained from horses administered 6 L of water via nasogastric intubation (control group [Con]; n = 10), LPS (20 ng of endotoxin/kg IV), or 6 L of BWHE (10) via nasogastric intubation. Samples were collected after horses were euthanatized at 12 hours after treatment or at onset of Obel grade 1 lameness. *Value is significantly (P < 0.05) higher than the corresponding value for the control group. ODU = Arbitrary units of optical density.

**Figure 2**—Representative results of western blot analysis of CD13 expression in laminar tissue and skin samples obtained from 3 horses administered either 6 L of water via nasogastric intubation (control treatment [Con]), LPS (20 ng of endotoxin/kg IV), or 6 L of BWHE (10) via nasogastric intubation. Samples were collected after horses were euthanatized at 12 hours after treatment or at onset of Obel grade 1 lameness. The intensity of CD13 expression in the BWHE-treated horse is greater than that detected in the control or LPS-treated horse. + = Positive control sample.
MMP-2 and MMP-9 mRNA RT PCR assays—On the basis of detected MMP mRNA concentrations, gene expression of MMP-9 was significantly increased in both laminar tissue and skin from BWHE-treated horses, compared with findings in the control horses ($P < 0.001$ and $P = 0.034$, respectively; Table 1). A signifi-
cant increase in MMP-2 gene expression was detected in the laminar tissue samples ($P < 0.001$) but not in the skin samples from horses in the BWHE treatment group ($P = 0.096$), compared with findings in the corresponding tissue samples obtained from horses in the control group. Gene expressions of MMP-2 and MMP-9 were significantly less in the laminar tissue and skin samples from the BWHE treatment group, compared with control group data (all values of $P < 0.001$).

**Gelatin zymography**—Mean MMP-2 zymogen activity in laminar and skin samples among the control, LPS treatment, and BWHE treatment groups did not differ significantly ($P = 0.068$ and $P = 0.193$, respectively; Figure 1). There was a very weak correlation ($r^2 = 0.18$) for MMP-2 zymogen activity between skin and laminar samples from individual animals.

Mean MMP-9 zymogen activities in laminar tissue and skin samples from horses in the control group were not significantly ($P = 0.337$) different (Figure 1). However, there was a significant increase in MMP-9 zymogen activity in the laminar and skin samples from the BWHE-treated horses, compared with the corresponding activities of the control horses (both $P < 0.001$). For the laminar tissue and skin samples, mean MMP-9 zymogen activity was not significantly ($P = 0.347$ and $P = 0.980$, respectively) increased in the LPS treatment group, compared with control group data. A weak correlation ($r^2 = 0.42$) was identified between skin and laminar samples for MMP-9 zymogen activity from individual animals.

**CD13 western immunoblot analysis**—In all tissues from all treatment groups, CD13 was present at visibly detectable levels (Figure 2). A single band consistent in molecular weight (150 kDa) with that identified for leukocyte-rich equine plasma (positive control sample) was present for each individual horse sample. Band intensity was subjectively increased in skin and laminar tissue samples from the BWHE treatment group, compared with findings for samples from the LPS treatment and control groups.

**CD13 immunohistochemical analysis**—Analysis revealed that CD13-positive cells were present in sections of laminar tissue and skin samples obtained from horses in all 3 treatment groups. The CD13-positive cells in laminar tissue appeared to be exclusively of myeloid origin and were present only in the laminar dermis; the laminar epidermis contained no CD13-positive cells. The skin samples, like the laminar tissues, contained CD13-positive myeloid cells as well as CD13-positive cells within the sebaceous glands, dermal root sheath of hair follicles, and stratum basale of the epidermis. The distribution of CD13-positive nonmyeloid cells did not vary among treatment groups, whereas the distribution of CD13-positive myeloid cells within the tissues varied between intravascular and extravascular locations depending on the treatment group (Figure 3). The CD13-positive leukocytes were consistent with a mixed population of neutrophils and monocytes. All skin sections evaluated contained both intra- and extravascularly located CD13-positive myeloid cells, but the number of those cells varied depending on treatment group. Similarly, CD13-positive cells were detected in intra- and extravascular locations in laminar tissue sections, but such cells were rarely seen in the control group samples.

In laminar tissue samples obtained from the control horses, CD13-positive myeloid cells were evident within the dermal vasculature and rarely in extravascular locations (approx 0 to 2 cells/tissue section). In skin samples, CD13-positive cells were present in low numbers; these cells were located in the superficial dermis at the level of sebaceous glands and hair follicles along with intravascular myeloid cells.

In laminar tissue samples obtained from the LPS-treated horses, occasional intravascular CD13-positive myeloid cells were detected; CD13-positive cells were rarely identified in extravascular locations (approx 0 to 2 cells/tissue section). Skin samples from the LPS-treated horses also contained low numbers of CD13-positive cells, which were similar in distribution and number to those identified in skin samples from the control horses. The numbers of CD13-positive cells in skin samples from LPS-treated horses were not significantly ($P = 0.497$) greater than the numbers of those cells in the corresponding tissue samples from control horses (Figure 4).
In laminar tissue and skin samples obtained from the BWHE-treated horses, large numbers of CD13-positive myeloid cells were evident both within and surrounding the dermal vessels. In laminar tissue sections, CD13-positive cells were located predominately along the vessels of the primary dermal laminae and in deeper sections of the dermis. Similar to findings in skin samples from horses in the other 2 groups, CD13-positive myeloid cells (along with intravascular myeloid cells) were located in the superficial dermis at the level of sebaceous glands and hair follicles in skin samples from BWHE-treated horses. The number of CD13-positive cells in tissues from horses in the BWHE treatment group was significantly increased, compared with numbers of cells in both the control and LPS treatment groups (both $P < 0.001$; Figure 4).

**Discussion**

Results of the present study have provided evidence that biochemical abnormalities develop in integumentary tissues, specifically, both the skin and laminar tissues of horses during the developmental phase of BWHE-induced laminitis. Several studies\(^{8,11,21}\) have revealed that MMP-9 expression and activity in laminar tissue of horses increase during development of laminitis. In the present study, MMP-9 gene expression and zymogen protein concentration were also increased in skin samples obtained from horses with experimentally induced laminitis. Unlike the results of another study\(^{22,23}\) in which MMP-9 zymogen was not present in laminar tissues from horses that were administered water, a low basal concentration of MMP-9 zymogen was detected in both skin and laminar tissues of healthy control horses in the present study. The difference between results of these 2 studies may be attributable to slight differences in zymography protocols that may have resulted in increased sensitivity for MMP-9 zymogen detection in our investigation.

Gene expressions and activities of MMP-9 in skin and laminar tissue samples obtained from horses in the control group in the present study were low, which is consistent with other data\(^{12,21}\) that indicate that few neutrophils are present in these tissues from healthy horses. The paucity of neutrophils in normal integument is also consistent with the low numbers of CD13-positive cells identified in tissue samples from horses in the control group. Although there was only a moderate correlation ($r^2 = 0.420$) of MMP-9 zymogen concentration between skin and laminar tissue samples, there does appear to be a prominent difference in MMP-9 zymogen concentrations in both skin and laminar tissues from control and BWHE-treated horses.

Horses in the developmental phase of laminitis become leukopenic at the same time that an increase in neutrophils can be detected in the integument.\(^{11,14,21}\) Metalloproteinase-9, myeloperoxidase, and other tissue-degrading enzymes are produced by and stored in neutrophils. These enzymes are markers of neutrophil presence and may contribute to the development of laminitis. Although these enzymes are present at increased concentrations in the skin of horses at the time of onset of laminitis, there is no clinically apparent evidence of disease in these tissues. The reason that pathological changes occur selectively in the laminar tissues, and not in the skin, most likely relates to key differences between these types of integument. For example, unlike the skin, laminar tissue is subjected to extreme biomechanical loads. As an adaptation to those stresses, laminar tissue has developed complex dermal-epidermal interdigitations that are not present in skin.\(^{22,23}\) Moreover, the vasoactive properties of the laminar microvasculature appear to be unique; the predisposition of laminar veins to vasoconstriction and the increase in digital postcapillary resistance that develops after administration of BWHE are strongly suggestive of the contribution of venous disturbances to the disease process.\(^{26–29}\)

The importance of the changes in the integumentary system that occur during the development of laminitis is highlighted by the data obtained from the LPS-treated horses in the present study. In a recent multivariate analysis of clinical risk factors for development of acute laminitis in horses,\(^{13}\) endotoxemia was the only factor significantly associated with the disease. This association was identified despite the fact that IV administration of LPS at doses 20 to 60 times as great as the dose used in the present study did not induce signs of laminitis, even when the horses were monitored for days to weeks.\(^{16}\) In the present study, LPS was administered at a dose of 20 ng of E. coli endotoxin/kg. This dose consistently resulted in the degree of leukopenia evident following BWHE administration\(^{20,31}\); also, at this dose, the clinical signs associated with administration of higher doses of LPS were minimized because those effects do not develop following BWHE administration.\(^{14}\) The administration of LPS in the present study caused leukopenia but did not result in emigration of leukocytes into the integument, which did occur following BWHE administration. The large numbers of CD13-positive myeloid cells surrounding dermal vessels in the skin and laminar tissue samples obtained from BWHE-treated horses were not detected in the samples obtained from control or LPS-treated horses. One possible reason for the lack of emigration of leukocytes into the tissues of horses administered LPS may be that the delivery of leukocytes to the laminar capillary beds was impeded as a result of the increased precapillary resistance that develops in LPS-treated horses.\(^{31,32}\)

Alternatively, the chemoattractants (eg, complement factor 5a, leukotriene B4, platelet-activating factor, and chemokines) that induce leukocytes to move from the vasculature and into the tissues\(^2\) may not be present in similar amounts in tissues of horses administered LPS or BWHE. Thus, it appears that the emigration of CD13-positive leukocytes and the increases in MMP-9 concentration and activity in horses with BWHE-induced laminitis are events that are distinct from those associated with acute endotoxemia.

Data from the present study and previous investigations\(^{11,22}\) support the concept that a generalized disruption of physiologic processes in the integument of horses occurs during the developmental phase of laminitis. To understand the naturally occurring disease process, it will be essential to determine which early pathologic changes are related to the development of laminitis...
rather than the development of endotoxemia. This information and the ease by which skin biopsy samples can be collected from horses suggest that histologic evaluation of skin samples may become a useful tool for identifying generalized alterations in the integument of horses at risk for the development of naturally occurring laminitis. The early identification of at-risk patients would allow more accurate prognosis and timely administration of aggressive interventional treatments. Furthermore, these samples may provide a means with which the progression of changes in the integument of horses with acute laminitis can be evaluated.

References